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A Comprehensive Study on the Need for Potential Alternatives to Antibiotic Growth Promoters: Current Strategies, Mechanisms and Significance

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Abstract

Antibiotics utilization in livestock has always been a debatable topic. They have been used in animal feed not only as antimicrobial agents but also for promoting growth and to improve their performance. Nevertheless, it has become a major concern in terms of food safety and human health. Antibiotics added to animal feed to treat infections and to improve their health have led to the emergence of antibiotic-resistance bacterial variants. Although in animal feed, antibiotics have been a regular supplement to improve their productivity and overall health of the livestock, the over utilization of these drugs for animals have led to multidrug resistance in both livestock and human health which cannot be treated using conventional clinical treatments. This has created a major economic and health concern about the antibiotic use in animal feed. The microbial cells become resistant to antibiotics through several methods, like cell membrane modification to inhibit drugs, alteration of the drug binding and recognition site, enzymatic alteration, chemical modification of the antibiotic, etc. These drug-resistant variants proliferate in the animals and are transmitted to the other animals and to humans primarily through practices like exposure to them in the farm houses, slaughter houses and meat consumption. This review summarizes the details of antibiotics, their use and over exploitation in animal nutrition. The study also focuses on the mechanism of drug resistance by bacteria, its economic impact, and various challenges to mankind. Significant emphasis on the possible solutions to combat the issue has also been provided in this review.

Keywords: Animal Feed, Antibiotics, Antimicrobial Resistance, Food Safety, Human Health, Livestock.

Introduction

The tremendous growth of animal agriculture in recent decades has significantly depended on antibiotic growth promoters (AGPs) to improve feed efficiency, stimulate growth, and avert disease outbreaks in livestock and poultry production systems. The antimicrobial or antibiotic growth promoters (AGPs) are the chemicals which destroy or inhibit the intestinal bacteria, when administrated even at low subtherapeutic dose. These help animals to efficiently digest their food and to reach maximal growth. Whenever, farm animals are exposed to pathogenic microorganism, they defend by the production of cytokines. This reaction stimulates catabolic hormones, which can reduce the muscle mass (1). Antimicrobial growth promoters (AGPs), generally provided at sub-therapeutic levels in feed or water, have demonstrated efficacy in enhancing weight gain and feed conversion ratios by influencing gut microbiota, mitigating subclinical infections, and decreasing intestinal inflammation. This approach has substantially aided in fulfilling the increasing global demand for animal protein. Nonetheless, the non-therapeutic and prolonged administration of antibiotics has concurrently expedited the establishment and spread of antimicrobial resistance (AMR) genes among pathogenic and commensal bacteria. Resistant strains may be disseminated via the food chain, the environment, and direct human-animal interaction, presenting a significant risk to public health.

Feeding antimicrobials to farming animals with the intent of meta phylaxis, prophylaxis, growth promotion can leave the residues into eco system and in their end products i.e., meat, egg, milk etc. Consumption of these residues cause the development of antimicrobial resistance, carcinogenicity, hypersensitivity reactions, teratogenicity, mutagenicity, depression in born marrow and changes in the normal microflora in

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humans (2). The antibiotics are reported to be detected in manure, soil, water bodies through the agriculture waste with nontoxic concentration to human (3). But this is a potential threat to society of developing antimicrobial resistance to human pathogens. Globally, serious attention has been given to decrease the use of AGPs in food animal production (4). The regulatory authorities like European Medicines Agency (EMA) and Food and Drug Administration (FDA) instruct farmers to focus on best farm management practice, use of natural antimicrobial in feed to compensate production loss. In 2014, Indian department of animal husbandry, dairying and fisheries posed the set of regulations to use antibiotics judiciously for treatment or feeding to food animals. In 2017, Food safety and standard authority of India amended regulations for the tolerance limit of antibiotics, pharmacologically active toxin, residues for animal-based food products. These amendments prescribes that a list of 21 antibiotics, 77 veterinary drugs should not exceed the concentration of 0.01mg per kg on the food types namely all edible tissues, fat derived from animal tissue and milk (5). However, the reduction of AGP in animal farming was partially effective and increased the financial burden to farmers. The nil use of AGP in animal feed reduced the final productivity.

A meta-analysis reports with 174 scientific articles containing 183 experiments, totalling 121,643 broiler birds contained major population as Ross breed demonstrated the economic impact without AGPs. This analysis clearly shows the antibiotics like avilamycin, flavomycin, virginiamycin significantly increased the feed conversion and the body weight than the birds fed without AGPs. In the total rearing period, the economic impact was $0.3 per bird and annual loss is to be $ 183,560,232 (6). Moreover, the withdrawal of antibiotics caused highest suffering of animals, despite improving the other animal husbandry aspects. The veterinary use antibiotics to treat the animals with infection. Now, this is another major theoretical human health hazard with respect to specific pathogens like *Salmonella, Campylobacter, E. Coli* and other zoonotic strains (7). This paper reviews the global need of finding out potential alternatives with less opportunity of resistance development to eradicate AGP in animal farming by retaining economic benefits.

Search Strategy and Highlights of the Study

A thorough literature review was performed to collect pertinent information regarding the application of antibiotic growth promoters (AGPs) in animal production and the novel alternatives being investigated to improve growth and health. This technique guaranteed that the evaluation included the latest, high-caliber, and thematically pertinent material to deliver a fair and thorough comprehension of AGPs and their possible alternatives.

The presented review aims in emphasising the use of natural alternatives for AGP. It provides an -in-depth comprehensive detailing of the various mechanisms of action across diverse classes of AGP alternatives and the identification of critical knowledge gaps that necessitate additional translational research. In particular, we have incorporated various categories of AGP alternatives—including probiotics, prebiotics, phytogenics, organic acids, antimicrobial peptides, bacteriophages, and immune modulators into a detailed comparative framework that outlines their mechanisms of action, target sites, and impacts on gut health and growth performance. The review proposes mechanistic understanding across these alternatives by emphasizing both shared and unique mechanisms, such as modulation of gut microbiota, enhancement of immunity, competitive exclusion of pathogens, and anti-inflammatory effects, a compilation not previously undertaken.

Additionally, we identify domains necessitating translational research, including the progression from experimental models to field validation, along with the necessity for combinatorial or synergistic approaches to improve efficacy while minimizing resistance risk.

Hence, when compared to the earlier reports, our presented review offers a practical approach and translational roadmap that can assist both academia and industry in creating realistic, sustainable alternatives to AGPs, although it does not introduce an entirely new classification method. Our review's originality consists on proposing mechanisms from numerous alternatives to antibiotic growth promoters and identifying critical gaps that necessitate future translational study. We incorporate many categories of alternatives (probiotics, prebiotics, phytogenics, organic acids, antimicrobial peptides, etc.) into a comparative framework, delineating their modes of action and impacts on gut health and performance. Although we do not provide a completely novel classification system, our review offers a mechanistic synthesis and a translational framework to direct future research and practical implementation.

Findings and Their Impact

The current scenario of AGP application in animals, their potential in animal growth, various antibiotics in animal feed, their results and implications in animal health and human as well are analysed along with key findings that are presented below.

Effect of Antibiotics in Livestock – Recent Developments and Consequences: Antibiotics are widely used to increase the growth rate and conversion of animal feed to animal products. Apart from improved growth rate and efficiency, treatment of sick animals and preventing the occurrence of diseases are some of the other benefits of these antibiotics being used in feed. For example, antibiotics like penicillin, chloro-tetracycline, oxytetracycline, tetracycline, are used majorly for increasing the efficiency and growth rate in terms of hatchability, increased egg production and feed efficiency than their utilization for disease control and prevention in poultry (8).

It is accepted that the need of antimicrobial drugs and antibiotics in animals is essential for promoting animal health, productivity and welfare. Although this has led to enhanced animal health and derived food, it is also raising alarming global concern due to its contribution to the prevalence of resistant variants of microbes by increasing their resistance to antimicrobial drugs. This in turn reverses all these benefits it offers and creates a health compromising scenario which is highly undesirable. The various advantages of antibiotics in animal agriculture and their potential threats are represented in Figure 1. The figure shows that the application of antibiotics as growth -promoters provides benefits like improved feed conversion, prevention from infections and diseases. It also highlights that it may lead to the consequences of antibiotic-resistant strains, dominant resistant gene and it's transfer to humans, animals and environment (9). It overally increases the cost of production too. Earlier reports have indicated the evidences of antibiotic-resistant bacterial strains that have been isolated from animals. This has definitely fuelled the debate further in its application in animal feed (10). Research has also shown that it is not possible to replace antibiotics in humans with their analogy in the animal antibiotics, thereby clearly showing that humans can also have the possibility of encountering bacterial strains that are resistant to the therapeutic antibiotics. For instance, avoparcin is a glycopeptide antibiotic. This is not used in humans. Whereas when used in animal feed it is known to be associated with the occurrence of avoparcin resistant strains. These strains are in turn cross-tolerant to another glycopeptide antibiotic vancomycin which finds use in human. It is also evident from the research demonstrated in animal feed that the resistance causing genes from the bacteria of animal origin can be transferred to the bacteria of human origin. It was also demonstrated that sepiolite, a non-antibiotic feed additive facilitates the horizontal transfer of resistance gene in the animal digestive tract (9).

There are also certain important antibiotics like the third generation of cephalosporins which are avoided in the application for animal agriculture rather used in humans. It is significant to understand that suitable antibiotic alternatives for the management of livestock infections have to be developed. Similarly, apart from treatment, prevention of diseases is of greater importance and has to be emphasized and research on prophylactic measures and medicines is the need of the hour. Simultaneous vigilance and control of unregulated sale and easy availability of the various antibiotics can further help in curbing the issue significantly especially in developing countries (11).

It is also argued that antibiotics have to be continually added to the animal feed for promoting their growth (12). Growth in animals may be hindered due to several reasons prevailing in their health or their environment of growth. Such reasons for occurrence of diseases in animals, retardation in their growth have to be analysed and these incidences have to be reduced or prevented. This prevention would lead to the non-dependence of antibiotics that are intended for promoting growth in animals. The unhygienic condition in the various farm houses and the poultries exposes the animals to infections and thus requiring antibiotic supplementation.

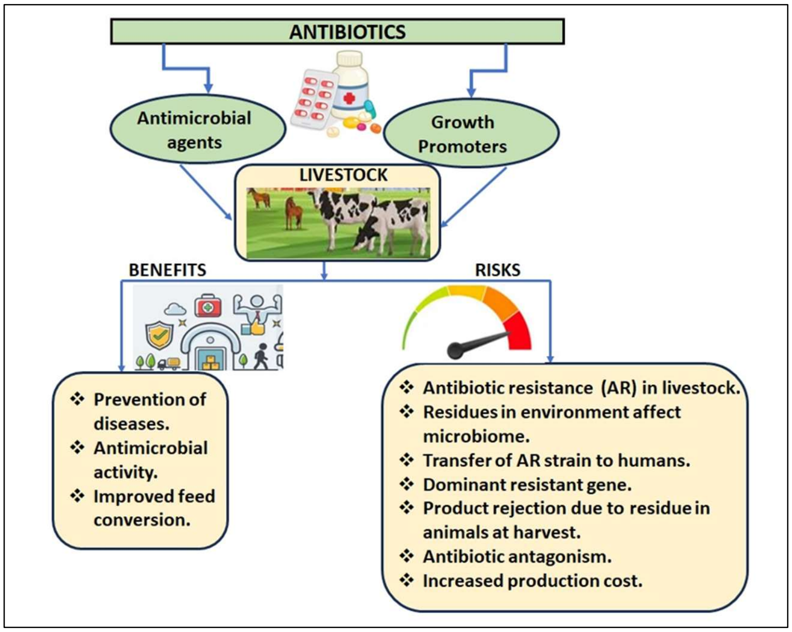

Figure 1: Advantages and Disadvantages of Antibiotics in Animal Feed

Therefore, it is necessary to provide a hygienic environment for the poultry and farm houses. Although it is impossible and has regulatory challenges to entirely impose a ban on antibiotic application in animals globally, nevertheless monitoring and controlling its use for optimum utilization averting emergence of resistance strains is extremely vital. Certain regulations can also help and be effective on implementation. For example, the "principle of proof" i.e. the evidence gathered prior to a compound's ban adopted by FDA is a practical approach to control the situation. FDA has also implemented some regulation on the labelling of antibiotics to contain the problem. The antibiotic manufacturers have to voluntarily mention in the relabelling clearly about the disapproval for use as growth promoters in animals and notify that this be used only under the supervision of a veterinarian. The regulation also plans to put an end to the application of medically useful antibiotics as growth promoters and prevent the prophylactic use of antibiotics against pathogens in animals. Whereas, it does not mandate the restriction of use of non-human antibiotics in animals as growth promoters. It is imperative to understand that antibiotics in animal feed is a highly debatable concern requiring strong awareness among people.

Various Antibiotics and their Role in Animal Feed: Antibiotics basically produce either microorganism for competitive exclusion for another microorganism or their synthesized chemicals. Antibiotics are classified by either their chemical structure or mechanisms of action. They are often complex structures and a single molecule possess different functionalities. Many antibiotics are currently in use as additives in animal feed for both nutrition, therapeutic and animal production purposes. These antibiotics are anionic, cationic, neutral or zwitterionic under different pH condition. Table 1 gives the details of various antibiotics commonly used as supplements in animal feeds along with their impacts on animal health and production. It highlights antibiotic application in cattle, chicken, swine and turkey. The consequences of their utilization showed antibiotic residues in most of the cases and identification of antibiotic-resistant microbial strains.

Table 1: Antibiotics Supplemented in Animal Feed

Antibiotic Used	Animal Feed used	Results and Implications	References
Penicillin	Chicken and Calves (meat)	Detection of antibiotic residues.	(13)
Tetracyclines	Chicken (Breast, Thigh, Livers)	Prevalence of Tetracycline Residues in Chicken Meat and Liver Sold to Consumers.	(14)
Oxytetracycline	Cow (Raw milk)	Antibiotic Residue Persistence in Milk in India's Bihar Region.	(15)
Sulfonamides	Cow (Raw milk)	Main antibiotic residues in milk.	(16)
Quinolones	Chicken, Beef	Quinolone Antibiotic Residues in Chicken Meat and Beef.	(17)
Amoxicillin	Eggs	Antibiotic Residues in Milk and Eggs.	(18)
Sulphamethazine	Beef Cattle	Antibiotic Residues in marketed beef.	(19)
Enrofloxacin	Chicken Tissues	Detection of antibiotic residues.	(13)
Ciprofloxacin	Spanish chicken	According to RAPD and PFGE studies, several resistant human isolates were quite similar to chicken isolates.	(20)
MDR	Greece raw milk, cattle, sheep and goat	The existence of mec Increased intensity of animal contact was substantially correlated with a gene in the farmer.	(21)
Apramycin	Chinese swine, Chicken	Human apramycin resistance gene aac(3)-IV was shown to have 99.3% similarity to animal strains.	(22)
Cefuroxime, streptomycin, nalidixic acid	Turkey chicken, and beef retail meats	ESBL strains that pose a major risk to human health were found in food.	(23)
MDR	Chinese pork	Pork strains and human mcr-1-positive strains were grouped together.	(24)
Ciprofloxacin	Cauca chicken	The link between food and bacteria obtained from patients was validated by phenotypic and genotypic analysis.	(25)

Tetracycline	U.S. chickens	Antibiotic-resistant strains of E. coli possessing transferable plasmids were identified in the gut microbiota of caregivers following the introduction of tetracycline on the farm.	(26)
Roxarsone	Chickens and turkeys	Antibiotic Effects on Food Animal Microbiomes.	(11)
Virginiamycin	Beef cattle, chickens, swine, turkeys	Antibiotic Effects on Food Animal Microbiomes.	(11)
Colistin	Swine	"How China is getting its farmers to kick their antibiotics habit".	(27)
Bacitracin	Beef cattle, chickens, swine, and turkeys	Antimicrobial effect in animal due to feed Additives	(28)
Neomycin	Beef cattle, chickens, swine, and turkeys	Changes in intestinal flora due to animal feed.	(26)
Chlorotetracycline	Dairy Cattle	Minimal quantities of chlorotetracycline were detected in the milk of cows subjected to elevated feeding levels, and these trace amounts were eradicated from all cows' milk within 48 hours after the cessation of antibiotic administration.	(27)

Discussion

The bacteria exposed to antimicrobials are forced to develop the strategies for their survival with the classic concept of survival of fittest (29). In this natural phenomenon, the bacterium evolves as a resistant strain against antimicrobials. The drug resistance develops because of genetic changes which is obtained through mutation on the locus of microbial chromosome that controls susceptibility or the transfer of gene from one to another bacteria. Microorganism develops resistance against different class of antimicrobials with the same mechanism of action or molecules with closely resembling structure. This relationship is known as co resistance, eg., neomycin, kanamycin co resistant, erythromycin, lincosamide co resistant etc.

Note: Ciprofloxacin: *Citrobacter Spp., E.Coli, Klebsiella Spp., Enterobacter Spp., Pseudomonas Spp., and Acinetobacter Spp., Enterococcus*, Methicillin resistant *S.aureus* (MRSA) (30).
Deoxycycline: *Klebsiella* Spp., *Pseudomonas, Enterobacter, citroacter, Acinetobacter,* Methicillin resistant *S.aureus* (MRSA) (30).

Different Modes of Action of Antibiotic Resistance

Around one percent cells in total population which are in stationary phase or dormant stage or not active at growth stage will not be susceptible to any antimicrobials; this characteristic is known as persistence (29). There are two types of resistance 1) Natural or inducible resistance, 2) Acquired resistance. The natural resistance mechanism involves intrinsically as a trait or induced for mutational changes in gene during the exposure to antibiotics, eg. lipopolysaccharide in gram negative bacteria and multidrug efflux pump. The transfer of genetic material occurs vertically and this is the most common type of resistance appeared in most of the bacteria. Acquired resistance mechanism is the one where the genetic material gets exchanged between two bacterial cells through any of the reproduction process such as transformation, transduction or conjugation. The gene transfer will be in horizontal line. Plasmid mediated transmission of resistance gene is the most common route for gene transfer (29). The bacteria undergo mutation of every 106 to 109 cell division cycles. There are four main categories of antibiotics resistance mechanism. 1) Limiting the uptake of drug 2) Modification of the drug target site 3) Drug Inactivation 4) Active drug efflux.

Intrinsic resistance mostly follows the mechanism of limiting uptake, drug inactivation and drug efflux. The acquired resistance mechanism follows drug target modification, drug inactivation and drug efflux. Gram negative bacterial cells use all three mechanisms, whereas gram positive use limiting the uptake, efflux pump, as they do not have liposaccharide membrane and certain types of drug efflux pump (4). Many bacteria produce enzymes to irreversibly modify or inactivate the antibiotics, eg. β lactamase, aminoglycoside modifying enzymes, chloramphenicol acetyltransferase. In other mechanism, bacteria reduce the intracellular drug accumulation by diminishing the protein channels (porin) or by the efflux pumps. Porins are the protein channels which allow the passage of many hydrophilic substances. The efflux pumps involve in the removal of antibiotics from the intracellular compartment or intermembrane spaces through membrane proteins (exporters). Most efflux pumps transport multiple drug molecules and this mechanism is the key to drug resistance mechanism (31). The various mechanisms used by microorganisms in resisting the drugs are represented in Figure 2. The figure highlights some of the important mechanisms like enzymatic degradation of the drug molecules, resistance mediated by cell wall protein modification and drug inhibition. Other mechanisms include presence of antibiotic resistance gene in plasmids and in some cases chromosomal mutation causes antibiotic resistance. The presence of efflux pumps in cell membrane leads to the release of these drugs from the cell. So far, there are five super families of efflux identified detailed below,

- ATP Binding Cassette (ABC) transport: This pump utilizes energy derived from ATP hydrolysis to transport amino acids, drugs, ions, polysaccharides, protein, sugar etc. It performs uptake on efflux transport.
- Multidrug and toxic compound extrusion (MATE): Na^+ gradient is used as energy source to efflux cationic dye and fluoroquinolone dyes.
- Small multidrug resistance (SMR) family: These are hydrophobic, energized by the proton motive force (H^+) and mainly efflux lipophilic cations.
- Major facilitator (MFS) family: These efflux pumps get energy from many ways via H^+ symport, H^+ antiport and solute. They can transport anions, drugs (e.g., macrolides, tetracycline), metabolites (e.g., bile salt), sugar etc.
- Resistance nodulation cell division (RND) family: This family catalyzes efflux via substrate/H^+ antiport. These mechanisms are found majorly in gram-negative bacteria. They are involved in the efflux of antibiotics, detergents, dye, heavy metals, solvents etc. (31).

Biofilm formation is yet another mechanism, by which bacteria potentially create resistance to antibiotics. Biofilm is the matrix formed by extracellular polymeric substance produced by bacteria. This comprises of polysaccharide, protein, lipids and extracellular DNA. Biofilm provides both chemical and mechanical protection to bacterial. So, the action of conventional antibiotics will not be sufficient to kill bacteria. Also, the nutrient scarcity could make bacteria become resistant to antibiotics.

Non-Antimicrobial Action of Growth Promoters - Benefits and Risks

In 1940, the growth-promoting properties of antibiotics were first discovered when chlortetracycline fermentation waste was used as a vitamin source for animals. Later, it was confirmed that growth promotion activity occurred by Chlorotetracycline (CTC). Thereafter, addition of antibiotics in animal feed started practiced. AGPs improve the animal production both quantitatively and qualitatively. Primarily, antibiotics reduce the intestinal microbial load which directly causes more energy utilization towards animal growth. Antibiotics play many other indirect roles in animals' metabolism and physiology. Antibiotics have effect on gut integrity, gut functioning, immune mechanism, antioxidant response, alleviating stress etc (4). Less utilization of nutrients by intestinal microbes makes the nutrient more available for intestinal absorption. The use of antibiotics improves the feed conversion with better use of expensive nutrients like vitamins, protein etc. (32).

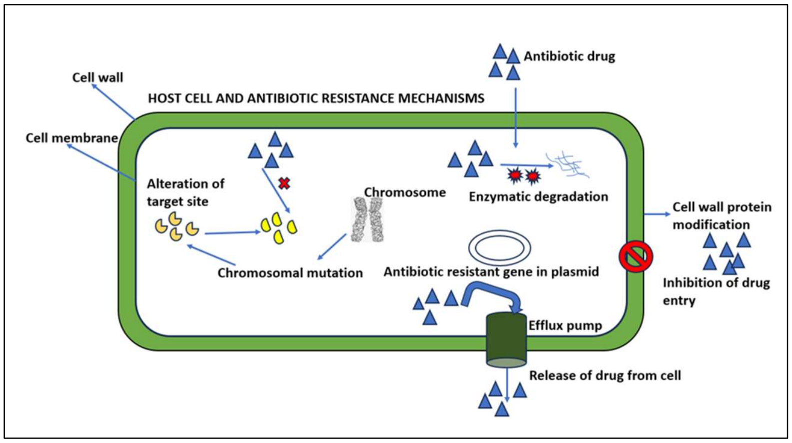

Figure 2: Modes of Antibiotic Resistance in Host Cell

The antibiotics reduce mucosal cell proliferation, decrease the mucosal turnover reducing the intestinal motility. It is reported that AGP effect in germ free animals closed the mucosal cell proliferation in the absence of luminal short chain fatty acids, which is usually derived from microbial fermentation in gut. Studies have reported that the use of AGP in germ-free animals suppresses mucosal cell proliferation when luminal short-chain fatty acids (typically derived from microbial fermentation in the gut, are absent. The gut size, including thinner intestinal villi, experienced a decrease in the total gut wall. This reduction in gut wall thickness and the lamina propria of the villi has been associated with enhanced nutrient absorption. Erythromycin was found to have improved gut motility (33).

The nutrient availability and absorption were improved by AGP in animal feed. AGP stimulates the vitamin synthesis of intestinal bacteria, reduces the harmful bacterial enzymes like urease and taurocholic acid. The AGP supplement improves vitamin absorption, fatty acid and glucose absorption increases the plasma nutrients (4). Pigs supplemented with chlorotetracyclin, pencillin, sulfamethazine were found to produce high serum, IGF1 (34). Ionophores enhanced absorption of nitrogen, magnesium, phosphorous, zinc and selenium with inconsistent effect on calcium, potassium, sodium. In cattles, ionophores reduces ketosis, bloat, acidosis, eg virginiamycin (34). AGP supplementation reduces oxidation and inflammation reaction whenever animals are at stress condition. Broilers received 1g/kg of flavophospholipol that had higher lymphocyte, monocyte and immunoglobulin G. Avilamycin supplementation to 10mg/kg of feed to broilers improved the immunity level of proinflammatory particles in serum (13). Reactive oxygen species in physiology is capable to react with DNA, polyunsaturated fatty acids (PUFAs) in cell membrane and initiate lipid peroxidation. This reaction releases several toxic metabolites like melanoldehyde (MDA). These react with free amino acid, phospholipid, nucleic acid and leads to cell death. Moreover, the presence of ROS further induces inflammation reaction. During stress condition, the animals utilize more energy to manage ROS and inflammation response due to challenging condition. Some antibiotics such as quinolones, macrolide, tetracyclin and β lactam reported to reduce proinflammatory cytokines in lipopolysaccharide stimulated human monocyte *invitro* condition (8). One milligram of Avilamycin was able to scavenge 132.0 nmoles of hydroxyl radicals (34) and decreased lipid peroxidation in terms of thiobarbutyric acid and malonaldehyde production (35). Besides antibiotics, many hormones are being used extensively for the promotion of growth in animals. Regulation of hormones in the circulatory system governs the deposition of protein in an animal due to which they have become vital in promoting growth and development. Hormones act in many ways in contributing to animal growth:

- Improved quality of meat by reducing fat deposition in animals producing lean meat.
- Enhancement in feed efficiency leading to more growth with lesser feed.
- Administration of bovine growth hormone (rBGM) to lactating cow increases the milk production and thereby the lactating period.
- Similarly, growth promoting hormones increase the endogenous estrogen production and growth.

Overally, the use of growth promoting hormones has helped animals grow larger in size, quicker on less feed. This drastically reduces the feed costs and lowers the cost of production. Some of the natural hormones like progesterone, estrogen and testosterone have been replaced by their synthetic alternatives like melengestrol acetate, zeronol, and trenbolone. These have been approved by the Veterinary Drugs Directorate (VDD) for use in beef production in Canada and other countries (36).

Risks Associated with the Prolonged Use of Growth Promoters: Utilizing growth promoters for animal health, their production and development has led to many concerns. Apart from antibiotic resistance, their application in animal production has led to many health concerns like hormonal residues in meat leading to androgenic and estrogenic effects, carcinogenic effects in humans. Producers use growth promoters to increase the growth rates in animal, improve product quality and overall efficiency (37). Whereas the prolonged use of these growth promoting substances has led to the increased risk of antibiotic resistance in human, developmental, neurobiological, genotoxic and also carcinogenic effects (38). There are always chances of existence of these antibiotic doses in the animal products too. Exceeding the minimum residue levels (MSLs) of drugs administered to animals poses a huge risk of generation of bacterial resistance in humans due to residues of antimicrobial drugs in food from these animal sources.

Some of these studies are represented in Table 2. The table provides details of the growth hormones in animal feed and its impact. For example, the natural hormone 17β-ostradiol is used in animal production for growth. Whereas it has been found to cause tumour promoting effects It binds to the estrogen receptors, regulates the transcriptional genes causing changes in cell proliferation and finally leads to cancer cell growth. Similarly, hormones like progesterone, zeronol, testosterone, melengestrol acetate, trenbolone used in dairy production for cattle is linked to causing genotoxic and carcinogenic effects on consumers through the products that can be the parent compound or their metabolites. Certain steroid hormones, beta-agonists and somatotropins used as growth promoters have raised questions on animal health, their welfare and led to ethical concerns in their application for animal health (39). In milk production, growth promoters are immensely used to cater to the high energy requirements of cattle and lactating cows to improve milk productivity and stimulate muscle formation in animals. Antibiotics like monensin, flavophospholipol and virginiamycin used in United states in cattle industry have shown several harmful effects in them. The antibiotics were known to cause a condition called as lactic acidosis that weakened the animal and affected their milk production. A combination of lasalocid and monesin antibiotic was then administered that inhibited the lactate producing bacteria. Post 2000, the use of glycopeptide was banned. Several antibiotics have been no longer permitted for use after realizing their harmful effects in the animal and their prolonged effect in human after consumption. Use of pristinamycin and quinupristin have also been banned after 2000 (33).

Table 2: List of Some Growth Promoters Used in Animal Feed and Their Impact

Growth promoters	Use in Animal feed	Consequences	References
Progesterone	Meat/milk production-Chicken/Cattle	Hormonal residues in meat and products-Antibiotic resistance.	(40)

Testosterone	Meat and milk-Cattle/Poultry	Androgenic effects in humans	(41)
Zeranol,	Increases milk production-Cattle	Estrogenic effect in humans	(42)
Trenbolone	milk production/meat-Cattle/beef	Carcinogenic, risk of virilization in humans.	(43)
Recombinant Bovine growth hormone (rBGH)	Increases milk production-Cattle	Presence of bovine IGF-1 in milk-Consumption by human increases risk of cancer-like prostate, breast, lung and colon-rectum.	(44)
Trenbolone acetate (TBA)	Meat production-Cattle	Reproduction of both male and female mammals of various species	(45)
Etradiol	Increasing feed efficiency and weight gain - Beef cattle	Antibiotic resistance in human.	(44)
Monensin	Muscle growth and milk production-Cattle	Skeletal and/or cardiac muscle issues in target species	(45)

AGP Alternatives and their Significant Characteristics

The continuous utilization of antimicrobials in food animals for promoting their growth and development has lead to detrimental public health problems. Moreover, whenever the same type of antimicrobials is utilized the condition of resistance is even stronger and serious health issues occur on contracting such microbes. Antimicrobial or antibiotic growth promoters prepare the resistance in bacteria and transfer them from food to humans. Due to the inappropriate, excessive use and constant exposure to antibiotics the microbes can adapt to their surrounding environment and become resistant (46).

The first step to prevent the health hazards in humans is to reduce the use of these antimicrobials in animal production. Regulations that advices the use of antibiotics in the presence of a public health safety assessment should be followed. More beneficial and effective would be to develop alternatives to these antibiotics which can work in the similar mechanisms to promote yield and increase production. Alternatives that are natural, cost effective and enhance the efficiency of feed conversion for promoting animal health and welfare could be a boon (47). Phytogenic derivatives, antimicrobial peptides, probiotics, prebiotics, enzymes, phage therapy and organic acids can be a great boon to fetch profits and success in replacing antibiotics in animal feed (48). They can be highly promising in promoting the growth, preventing and contain diseases and improving the overall yield and production (35). On the other hand, these sources cannot be considered as a complete replacement in efficiently increasing the production at a very large-scale farming level. That can only complement and compensate for the complete exclusion of antibiotics to a certain level in the animal feed. Hence it is usually recommended that these can be added or blended to improve the production and gain more profit to the farmers. These natural alternatives for antibiotics ensure better production, food safety, biocompatibility and prove to be eco-friendly too. Although, these alternatives provide a non-toxic, better and satisfactory impact on the growth of animals with better nutrient absorption along with factors for stimulating better health in the livestock, their effective implementation as alternatives to antibiotic growth promoters (AGPs) necessitates demonstrable efficacy, thorough assessment of toxicological safety, residue elimination, and customer acceptance. These elements are essential for obtaining regulatory authorization and public confidence. Probiotics, prebiotics, synbiotics, and postbiotics are generally recognized as safe (GRAS) for application in livestock and poultry. They infrequently cause systemic infections, do not generate harmful metabolites at prescribed levels, and are swiftly expelled from the gastrointestinal tract. Due to their composition of living or inactivated bacteria and dietary fibers, they do not leave chemical residues in meat, milk, or eggs. Their inherent image enhances consumer adoption, as they are frequently seen as advantageous and sustainable feed constituents (49). Phytogenic feed additives, including essential oils, plant extracts, tannins, saponins, and flavonoids, often exhibit low to moderate toxicity, accompanied by well-defined safe inclusion thresholds. At elevated concentrations, certain bioactive constituents may provoke cytotoxicity or hepatic enzyme activity, therefore necessitating dose adjustment. The majority of phytochemicals are swiftly digested and eliminated, leading to little tissue residues, but formal withdrawal periods are not consistently defined. Consumers typically see these additives favorably because of their botanical source and potential for "clean-label" manufacture, while their intense aroma or flavor may sometimes influence feed palatability (50).

Organic acids (formic, propionic, butyric, lactic acids) possess a long-standing record of safe utilization. At advised quantities, they exhibit minimal systemic toxicity and are completely converted to CO_2 and water, resulting in no residues in animal products. They are broadly recognized by authorities and consumers as safe preservatives and growth-enhancing feed acids (51). Enzymes, including xylanases, proteases, and phytases, are often harmless, exhibiting minimal toxicity and no residue accumulation due to their degradation by digestive processes. Although there is a danger of occupational allergenicity during handling, it does not compromise the safety of animal products. Enzymes are widely accepted by consumers as natural performance enhancers (52). Antibacterial peptides (AMPs) exhibit potential antibacterial and immunomodulatory properties with minimal systemic toxicity and are swiftly destroyed by proteases, leading to insignificant residues. Nonetheless, due to its recent introduction, extensive long-term toxicity and tissue retention investigations are scarce. Consumer acceptance is moderate, with some individuals viewing AMPs as synthetic or resembling drugs (53). Bacteriophages exhibit excellent specificity for target pathogens, are non-toxic to eukaryotic cells, and do not leave residues in animal products as they diminish following the eradication of their bacterial hosts. They are deemed biologically harmless; nonetheless, consumer perception continues to be wary due to their link with viruses (54).

Real World Significance and Feasibility

The shift from laboratory or controlled experimental investigations to extensive animal production entails numerous practical, economic, and regulatory obstacles. The feasibility is contingent upon various interrelated aspects.

Economic Efficiency:

- Production Costs: Alternatives such as probiotics, enzymes, or phytogenics necessitate industrial-scale manufacturing, stringent quality control, and occasionally cold-chain logistics. Initial expenses may exceed those of traditional antibiotics (55).
- Feed Conversion Efficiency (FCE): The economic advantage is associated with enhancements in growth performance, feed efficiency, and diminished illness prevalence. Cost-benefit assessments indicate that high-performing alternatives (probiotics, organic acids, enzymes) can compensate for their elevated prices by enhancing feed conversion efficiency and decreasing veterinary expenses.
- Market Variability: The prices of plant extracts, essential oils, and specialist enzymes may fluctuate seasonally or geographically, impacting cost predictability for farmers.

Acceptance by Farmers:

- Usability: Farmers favor additives that can be seamlessly integrated into current feed formulas without disrupting production practices. Powdered or premixed feed additives are more readily adopted than items necessitating specialized handling or dosing (56).
- Perceived Efficacy: Adoption is contingent upon recognized advantages, including enhanced weight gain, diminished morbidity, or decreased death. The absence of observable effects or erratic outcomes can diminish farmer confidence.
- Knowledge and Training: Effective application frequently necessitates educating farmers on dosage, storage, and the integration of biosecurity protocols.

Regulatory Frameworks:

- United States (Food and Drug Administration and United States Department of Agriculture): The FDA oversees feed additives in accordance with the Food, Drug, and Cosmetic Act (FD and C Act). Probiotics, enzymes, organic acids, and phytogenics often either possess Generally Recognized as Safe (GRAS) status or necessitate New Animal Drug Applications (NADAs) for therapeutic assertions. Documentation of safety, residue, and efficacy data is required for approval (57).

- European Union (EFSA and EU Prohibition on Antibiotic Growth Promoters): Since 2006, the European Union has prohibited the use of antibiotics as growth enhancers. Alternatives must adhere to EFSA rules for feed additives, encompassing safety, efficacy, and environmental risk assessment. The EU prioritizes consumer protection and the utilization of natural additives, hence promoting the adoption of probiotics, phytogenics, and organic acids.
- Global Variation: Regulatory frameworks vary significantly between nations, impacting international commerce and adoption. Certain places possess definitive approval routes, whilst others encounter ambiguity, potentially hindering expansion.

Consumer Perception:

- Demand for "Antibiotic-Free" Products: Heightened consumer knowledge of antimicrobial resistance stimulates demand for livestock products produced without antibiotics. This generates market incentives for the adoption of alternatives (58).
- Product Quality and Safety: Consumers anticipate the absence of any undesirable flavor or odor alterations in meat, milk, or eggs. Robust plant-derived additions or innovative molecules must be meticulously designed to preserve organoleptic characteristics.
- Trust and Labelling: Transparent labelling and reliable certification (e.g., organic, antibiotic-free) increase acceptance and the propensity to pay a premium.
- Stability and Shelf Life: Numerous alternatives, particularly probiotics and enzymes, necessitate meticulous storage to preserve viability and effectiveness. Industrial feed systems must meet these specifications.
- Integration with Biosecurity and Health Programs: Alternatives are most efficacious when coupled with appropriate hygiene, vaccination, and nutrition. Scaling up necessitates a comprehensive farm management strategy.
- Supply Chain and Manufacturing Capacity: Industrial-scale production must provide uniform quality, strain specificity, and reproducibility across batches.

Practical Considerations for Prevention of Antibiotic Resistance

Another step to ensuring the reduced effect of the antibiotics to humans is to follow measures for minimizing antimicrobial use as given below in Figure 3. It details some of the practical measures and practices that can be followed to prevent antibiotic resistance in animal feed. The figure emphasizes the switch over from antibiotics to its natural additives, follow good hygiene practices, implementation of biosecurity measures and general conditions on farms (56). It also highlights the importance of judicious use of antibiotics only for disease treatments under expert supervisions and regulation for its applications and creating general awareness about it.

Avoid antinutritional components (e.g., lectins, protease inhibitors); ¬ Integrate natural feed ingredients or additives that improve feed conversion efficiency (e.g., in-feed enzymes, competitive exclusion products, probiotics, prebiotics, acidifiers, plant extracts, nutraceuticals, essential oils,). The global shift away from antibiotic growth promoters (AGPs) in poultry and livestock production has led to a surge in research exploring natural and functional alternatives. A wide array of strategies—including probiotics, prebiotics, phytobiotics, postbiotics, essential oils, and nanoformulations—have demonstrated promising effects on growth performance, gut morphology, immune modulation, and microbial balance. Notably, combinations such as *Lactobacillus* spp. with phytobiotics, or multi-strain probiotics like *Pediococcus pentosaceus* and *Saccharomyces boulardii*, have shown synergistic benefits in enhancing intestinal health and reducing pathogenic bacteria. Nanoencapsulated essential oils and chitosan-based delivery systems further improved bioavailability and efficacy, offering innovative delivery platforms for these alternatives.

Essential oils influence gut microbiota by selectively inhibiting harmful bacteria like *E. coli* and *Clostridium perfringens*, while sometimes supporting beneficial microbes, depending on the type and dose. They also affect microbial metabolite production, such as short-chain fatty acids, which are vital for gut health. Probiotics, on the other hand, enhance host immunity by strengthening gut barrier function, modulating cytokine responses, and increasing immunoglobulin levels. They interact with gut-associated lymphoid tissue (GALT) to stimulate both innate and adaptive immune responses. Together, these effects contribute to improved disease resistance and overall health in poultry and livestock.

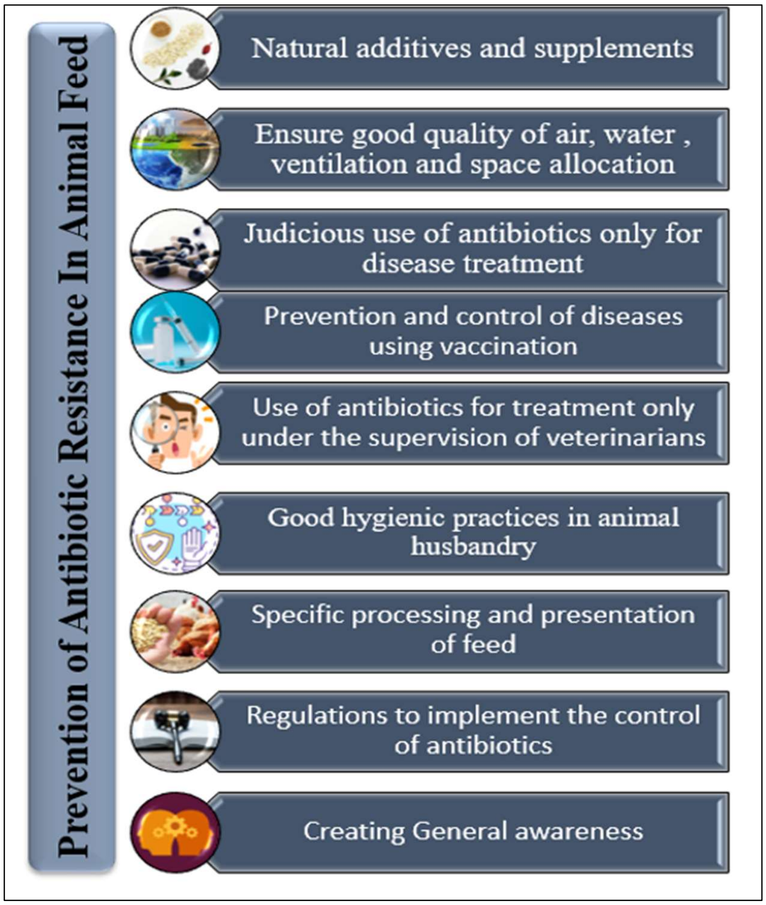

Figure 3: Role of Nanotechnology in Controlling Microbial Resistance Development

Several studies emphasized the immunomodulatory potential of these alternatives, particularly in response to bacterial challenges like *Clostridium perfringens* and avian pathogenic *E. coli*. Probiotics such as *Bacillus licheniformis*, *L. rhamnosus* GG, and *E. faecalis* strai ns were shown to upregulate tight junction proteins, anti-inflammatory cytokines, and antioxidant enzymes, while reducing lesion scores and mortality. Postbiotics also emerged as a viable option, capable of modulating innate immune responses and restoring gut homeostasis during infection. Additionally, some alternatives, including garlic extract and *Bacillus*-based probiotics, enhanced vaccine responses against Newcastle disease virus (NDV), indicating their potential to support both innate and adaptive immunity.

Alternatives to antibiotic growth promoters (AGPs) like essential oils positively influence the gut microbiota by selectively inhibiting pathogenic bacteria such as E. coli, Clostridium perfringens, and Salmonella spp., while fostering the proliferation of beneficial species like Lactobacillus and Bifidobacterium. This modulation enhances microbial equilibrium, diminishes detrimental fermentation byproducts, and fortifies gut barrier integrity. Probiotics similarly promote host health by augmenting innate and adaptive immune responses, activating macrophages and dendritic cells, modulating cytokine production, and strengthening mucosal barrier function. Probiotics facilitate the maintenance of immunological homeostasis and promote immune tolerance, thereby enhancing the host's resistance to infections. Both essential oils and probiotics collaboratively enhance gut health and immunity, positioning them as viable alternatives to antibiotic growth boosters. Probiotics closely interact with the host's immune system by augmenting defense systems, mitigating inflammation, and preserving mucosal barrier integrity.

Alternatives to antibiotic growth promoters (AGPs) improve gut health by both modifying microbiota and fortifying the intestinal epithelial barrier. Probiotics and synbiotics enhance the expression of tight junction proteins and stimulate the formation of short-chain fatty acids, hence boosting enterocyte health. Phytogenics and essential oils mitigate inflammation, fortify tight junctions, and augment mucus secretion. Organic acids reduce intestinal pH, suppress infections, and butyrate directly sustains enterocytes. Enzymes enhance nutrition digestion, hence alleviating epithelial stress. Antimicrobial peptides (AMPs) and bacteriophages specifically target infections while safeguarding commensal organisms, so indirectly reinforcing barrier integrity. Certain nanoparticles may mitigate oxidative stress and pathogen burden; nonetheless, elevated amounts can be harmful. These alternatives support intestinal permeability, bolster mucosal immunity, and boost nutrient absorption, so promoting improved growth performance and systemic health.

The following table depicts the proven effects of essential oils, probiotics in terms of antibiotics replacement in animal feed (Table 3). The table lists their use in combination with organic substances or as standalone for improving animal productivity. They have been successful in replacing antibiotics like ciprofloxacin, avilamycin, bacitracin methylene disalicylate, enramycin, monensin and tylosin. It gives an overall idea of the positive impact of natural alternatives to antibiotics.

Table 3: The Effect of Essential Oils, Probiotics in Antibiotic Replacement in Animal Feed

Feed additives	Productivity	Animal	Replaced Antibiotic	Reference
Thyme Oil (TO)	Moderate improvement in performance and bacterial count	Broiler chickens	Ciprofloxacin	(59)
TO-loaded-CS-NPs	Comparable to Ciprofloxacin; improved weight and reduced bacterial count	Broiler chickens	Ciprofloxacin	(59)
Carvacrol + Cinnamaldehyde	Improved TWG (↑1.67%), FCR (↑5.7%), gut morphology	Broiler chickens	Avilamycin	(60)
EOA1 (EO + Organic Acids)	Reduced C. perfringens load, improved microbiota diversity	Broiler chickens	Bacitracin Methylene Disalicylate (BMD)	(61)
EOA2 (EO + Organic Acids)	Reduced gut inflammation, improved mucin expression and gut health	Broiler chickens	Bacitracin Methylene Disalicylate (BMD)	(61)
Essential Oil Blend (EOB)	Improved early ADG and dressing percentage	Feedlot steers	Monensin + Tylosin	(62)
Nanoprotected REO	Improved lipid profile, immunity, antioxidant	Broiler chickens	Enramycin	(63)

	status, gut morphology			
Nanoencapsulated REO	Improved BWG, FCR, digestibility, carcass traits, meat quality, gene expression	Broiler chickens	Enramycin	(63)
Microencapsulated essential oils and organic acids	Improved early growth, gut morphology, enzyme activity, immune genes	Broiler chickens	Bacitracin Methylene Disalicylate (BMD)	(64)
Enterococcus faecalis	Reduced NE lesions, improved immunity and gut barrier	Broiler chickens	General antibiotic alternative	(65)
Pediococcus pentosaceus + *Saccharomyces boulardii*	Improved carcass traits, reduced fat, modulated microbiota	Post-weaning pigs	Non-therapeutic antibiotics	(66)
Spirulina platensis and *Chlorella vulgaris*, Rose mary extract (REO), *Multispecies probiotics*	Improved growth, antioxidant status, gut morphology	Broiler chickens	Avilamycin	(67)
Pseudomonas monteilii	Improved growth, immunity, antioxidant status, gut microbiome stability	Grass carp	General antibiotic alternative	(68)
Paenibacillus ehimensis	Enhanced metabolism, growth, immunity, reduced mortality	Zebrafish	General antibiotic alternative	(69)
Lacticaseibacillus rhamnosus and *Bifidobacterium lactis*	Inhibited APEC, reduced colonization, modulated microbiota	Broiler chickens	Antibiotics for APEC	(70)
Lactobacillus spp. (meta-analysis)	Improved ADG, ADFI, G/F, VH, V/C ratio	Piglets	AGPs (general)	(71)

Mechanism of Action of the Alternatives

Essential oils and phytogenics are plant-derived compounds with broad-spectrum antimicrobial properties. They disrupt bacterial cell membranes, causing cell death and disrupting quorum sensing, reducing virulence and inhibiting biofilm formation. Essential oils, like thymol and carvacrol, also exhibit anti-inflammatory and antioxidant activities by modulating inflammatory signaling pathways and scavenging free radicals. Some EOs stimulate cytokine production, contributing to immunomodulatory effects. Phytogenics, on the other hand, are diverse plant-derived bioactive compounds that have beneficial effects on animal health and performance. They modulate gut microbiota, promote beneficial bacteria, and suppress pathogenic strains. They enhance intestinal barrier integrity, reducing intestinal permeability or "leaky gut." Phytogenics possess strong anti-inflammatory and antioxidant properties, helping to mitigate oxidative stress and inflammation in gut tissues. The combined use of these bioactive compounds often results in synergistic effects, enhancing their individual benefits. For example, probiotics and prebiotics form synbiotics, improving colonization and metabolic activity of beneficial microbes. Postbiotics, due to their stability and consistent efficacy, complement these strategies by delivering reliable immunomodulatory and barrier-enhancing effects without the need for live microbial cultures. This synergy makes them powerful tools in the replacement of antibiotic growth promoters in animal nutrition. Essential oils and phytogenics are powerful tools in the fight against gut health issues. They provide both antimicrobial and anti-inflammatory effects, offering a holistic approach to gut health management. Postbiotics, due to their stability and consistent efficacy, complement these strategies by delivering immunomodulatory and barrier-enhancing effects without the need for live microbial cultures. This synergy makes them powerful tools in the replacement of antibiotic growth promoters in animal nutrition. The combined use of these bioactive compounds often results in synergistic effects that enhance their individual benefits. For example, probiotics and prebiotics form synbiotics, which improve the colonization and metabolic activity of beneficial microbes. This synergy makes them powerful tools in the replacement of antibiotic growth promoters in animal nutrition.

The various alternatives and their mechanisms of action have been reported in Table 4. The table shows the use of AGP alternatives in livestock by highlighting the several key mechanisms for microbial inhibitions by lowering of gut pH, gut microbiota modulation, immune system stimulation, nutrient absorption and reduced inflammation. The table also summarizes that the essential oils and phytogenics work together to provide both antimicrobial and anti-inflammatory effects, offering a holistic approach to gut health management. Postbiotics, due to their stability and consistent efficacy, complement these strategies by delivering immunomodulatory and barrier-enhancing effects without the need for live microbial cultures. This synergy makes them powerful tools in the fight against gut health issues in animal nutrition. The combination of bioactive compounds, such as probiotics, prebiotics, essential oils, and phytogenics, can significantly replace antibiotic growth promoters in animal nutrition. These compounds work together to improve the colonization and metabolic activity of beneficial microbes, providing both antimicrobial and anti-inflammatory effects. Essential oils and phytogenics work together to provide both antimicrobial and anti-inflammatory effects, offering a holistic approach to gut health management. Postbiotics, due to their stability and consistent efficacy, complement these strategies by delivering reliable immunomodulatory and barrier-enhancing effects without the need for live microbial cultures. The synergy between these bioactive compounds makes them powerful tools in the replacement of antibiotic growth promoters in animal nutrition. Probiotics and prebiotics form synbiotics, which improve the colonization and metabolic activity of beneficial microbes. Essential oils and phytogenics work together to provide both antimicrobial and anti-inflammatory effects, offering a holistic approach to gut health management. Postbiotics, due to their stability and consistent efficacy, complement these strategies by delivering reliable immunomodulatory and barrier-enhancing effects without the need for live microbial cultures. In conclusion, the combination of these bioactive compounds offers a more effective and sustainable alternative to antibiotic growth promoters in animal nutrition.

Table 4: Recent Studies on the Mechanism of Action of AGP Alternatives

Alternative	**Livestock**	**Key Findings**	**Mechanism of Action**	**Reference**
Essential Oils	Poultry (*Salmonella* inhibition in feed)	Strong antimicrobial activity against *Salmonella*; effective MIC levels established	Lowering gut pH, inhibiting pathogens, promoting beneficial bacteria	(72)
Phytogenics	Slow-growing chickens (KUB breed)	Improved feed conversion ratio, reduced abdominal fat, comparable to AGPs	Antibacterial compounds (phenol, eugenol, α-mangostin) enhance gut health	(73)

Alternative	Livestock	Key Findings	Mechanism of Action	Reference
Probiotics	Male ISA Brown layer chickens	Lactic acid bacteria enhanced growth, carcass traits, immune organ development; better than AGPs	Gut microbiota modulation, immune stimulation, pathogen inhibition	(74)
Prebiotics	Poultry (general application)	Stimulated beneficial bacteria, enhanced SCFA production, improved gut health	Substrate for probiotics, pathogen exclusion, microbiota modulation	(75)
Postbiotics	General (clinical and livestock applications)	Safe, stable, effective in gut health and immunity; applicable in clinical trials	Immune modulation, antioxidant effects, gut barrier enhancement	(76)
Postbiotics	General (food safety and livestock)	Antibacterial, anti-inflammatory, effective in obesity and disease prevention	Bioactive metabolites regulate microbiota, reduce inflammation	(77)
Prebiotics	Broiler chickens	MOS with probiotics improved weight gain, FCR, and intestinal health	Enhanced microbiota balance, SCFA production, immune response	(78)
Postbiotics	Broiler chickens (Cobb 500)	Improved weight, feed efficiency, mucin production, antioxidant enzymes	Gut barrier strengthening, nutrient absorption, immune modulation	(79)
Postbiotics	Broiler chickens	Improved growth, carcass traits, intestinal morphology, immune status	Enhanced intestinal integrity, immune modulation, growth gene expression	(80)

Despite the diversity of promising results, the effectiveness of these alternatives is often strain-specific and context-dependent, influenced by factors such as dosage, delivery method, animal species, and environmental conditions. While some interventions showed limited or inconsistent outcomes such as in ovo probiotic administration or onion-based prebiotics—others consistently improved gut health, performance, and disease resilience. Collectively, these findings support the integration of well-characterized, evidence-based antibiotic alternatives into animal nutrition programs to promote sustainable, health-oriented production systems. Continued research is essential to optimize formulations, understand mechanisms of action, and validate efficacy under commercial conditions.

Promote animal welfare by ensuring high-quality air and water supply, adequate ventilation rates, and appropriate space allocation throughout all phases, including production, transport, and slaughter.

Implement stringent protocols for disease control, such as vaccination.

Utilizing particular processing and presentation techniques for feed that enhance its conversion into animal products, hence improving growth rates and output yields.

Implementation of effective husbandry and hygienic techniques inside animal production facilities.

Specific instances in which antibiotic use cannot be compromised should be limited to those antibiotics that (i) result in a substantial improvement in animal husbandry for livestock, (ii) have minimal or no therapeutic application in animals and humans, and (iii) do not contribute to the emergence of resistant strains or diminish the effectiveness of prescribed medications.

Role of Next Generation Sequencing and Synthetic Biology in Tailoring Antimicrobials and Probiotic Application

Next generation sequencing enables the discovery of new probiotic strains by providing high-resolution insights into the composition and functional potential of microbial communities in the gut or fermented environments. Through metagenomic and 16S rRNA sequencing, researchers can identify uncultured or low-abundance microbes, analyze their genomes for probiotic traits (e.g., adhesion, stress tolerance, antimicrobial production), and select promising candidates for further validation. This approach accelerates the identification of host-specific, niche-adapted strains with targeted health benefits.

Synthetic biology on the other hand, facilitates the development of tailored antimicrobials by engineering microbial systems to produce customized antimicrobial peptides, bacteriocins, or enzymes that target specific pathogens without disrupting beneficial microbiota. Using gene editing tools like CRISPR and modular genetic circuits, scientists can design microbes or cell-free systems that respond to infection signals, produce therapeutic molecules on demand, or deliver precision treatments. This allows for highly specific, programmable alternatives to broad-spectrum antibiotics, reducing resistance risks and improving host compatibility.

To reduce antibiotic use in animal feed, strategies include adopting precision nutrition, improving farm biosecurity, and integrating natural alternatives like probiotics, essential oils, and phytobiotics. These alternatives support gut health and immunity, reducing the need for antibiotics. However, to prevent resistance development even with natural additives, it's crucial to rotate feed components, avoid overuse, and monitor microbial shifts through metagenomics. Essential oils and probiotics should be strain-specific, dose-optimized, and used in combination with other functional ingredients to minimize selective pressure. Future research should focus on understanding host–microbiota–immune interactions, developing targeted delivery systems, and validating efficacy under commercial farm conditions to ensure sustainable and safe antibiotic-free production

Nanotechnology offers a promising approach to reduce microbial resistance development in animal feed additives. Through nanoencapsulation, essential oils and probiotics can be delivered directly to the gut, enhancing their stability and effectiveness at lower doses. This targeted delivery minimizes exposure to non-target microbes, reducing selective pressure. Nanocarriers also improve bioavailability, allowing for more consistent therapeutic effects. Multifunctional nanoparticles like silver or zinc oxide can be engineered to act synergistically with natural compounds, offering broad-spectrum antimicrobial action. Smart-release systems further refine this by activating only in response to pathogens or specific gut conditions. Some of the characteristics of nano-based alternatives that can be beneficial in controlling antibiotic resistance are controlled and targeted delivery, enhanced bioavailability and stability, smart release systems. Future research should focus on designing species-specific nano delivery systems, evaluating long-term safety, and integrating microbiome profiling to personalize feed strategies.

Recent advances in nano-based and phytogenic feed additives offer promising alternatives to antibiotics in livestock and aquaculture. The application of nano-based alternatives and their effect on animal production is given in Table 5. The table lists some of the nano-based feed like insect meal altered gut microbiota in catfish, cinnamon nanoparticles, essential oil nano emulsions, manganese and cobalt oxide nanoparticles, thymoquinone nano emulsions that have proved to enhance the growth, immunity, and pathogen resistance. Nano emulsions of peppermint oil and Nigella sativa-chitosan provided antibacterial protection in broilers, and selenium nanoparticles combined with cinnamon oil effectively inhibited diarrhea-causing pathogens in buffaloes. Nano-zinc oxide and symbiotic composites improved gut health and performance in piglets, supporting their role in replacing banned therapeutic zinc oxide.

These innovations highlight the potential of integrated strategies to combat antimicrobial resistance and improve animal productivity. Nevertheless, nanoparticles remain in the experimental phase and elicit significant safety concerns. Evidence indicates possible cytotoxicity, oxidative stress, and bioaccumulation contingent upon particle type, size, and dosage. Data on residue depletion and long-term toxicity are scarce, and consumer adoption is minimal due to uncertainties over their environmental and food safety implications.

Table 5: Nano-Based Alternatives to Antibiotics

Nano Molecule / Feed	Dose	Animal	Antibiotic Replaced	Effect	Reference
Black Soldier-Fly Insect Meal	30% of diet	African Catfish Juveniles	None	Altered gut microbiota; increased Gram-positive bacteria	(81)
Cinnamon Nanoparticles (CNPs)	Not specified (group-based)	White New Zealand Rabbits	Streptomycin	Improved growth, immunity, antioxidant activity; reduced pathological lesions	(82)
Peppermint Essential Oil NE (PEONE)	25–100 µl per bird/day	Broiler Chicks	Enrofloxacin	Antibacterial effect; improved BWG, FCR, gut health, and meat quality	(83)
Mn_2O_3 and Co_3O_4 Ultrafine Particles	Mn_2O_3: $\leq 4.9\times10^{-4}$ mol/L Co_3O_4: $\leq 1.5\times10^{-5}$ mol/L	*E. coli* (in vitro)	None	Strong bactericidal effect; cell wall damage and cytoplasmic leakage	(84)
Sodium Alginate-Coated Nano-ZnO (saZnO)	500 mg Zn/kg diet	Weaned Piglets	High-dose ZnO	Improved growth, immunity, antioxidant status; reduced Zn excretion	(85)
Nigella sativa-Chitosan Nanoparticles (CNP-NS)	Not specified (prophylactic)	Broiler Chicks	None	Complete protection against *Salmonella*; enhanced mucosal immunity	(86)
Alginate Nanoencapsulated Synbiotic	Not specified	In vitro (multiple pathogens)	Gentamicin (comparison)	Strong antimicrobial and antioxidant activity; effective against bacteria and fungi	(87)
Thymoquinone Nanoemulsion (TQN)	Graded concentrations	Rabbits	None	Enhanced growth, immunity, antioxidant status; protection against *P. multocida*	(88)

Nigella sativa Extract (NSE)	Up to 500 mg/kg diet	Rabbits	MRSA-targeted antibiotics	Antimicrobial, growth-promoting, immunomodulatory; restored antibiotic sensitivity	(89)

Many recent studies have substantiated that the use of antibiotics for growth promotion does not provide significant benefits to agricultural applications or animal feed and should be altered with a natural way that acts both against the microbes and also supplements the agricultural requirements or animal nutrition. Decreasing the dependence of antimicrobial growth promoters can prevent bacterial resistance in food, animal feed and thereby in humans. The necessity for growth promoters can be eliminated, with the improvement of animal production, transport environment and conditions by reducing stock density and stress, by increasing hygiene and by introducing disease control techniques.

Several governments across the world have already started implementing regulations and legal authority to limit and even ban the use of antibiotics for non-therapeutic use (49). Hence in the food production and related activities, it is extremely important and need of the hour to make better future-oriented choices without compromising on the safety aspects of animals, human and the environment.

Conclusion

The negative impacts of antibiotics in animal feed have become a major universal concern. Several evidence have proven the over exploitation of antibiotics in various animals causing antimicrobial resistance and eventually transfer of the resistance variants of micro-organisms to humans. Though discovery of antibiotics or antimicrobial drugs are a major scientific advancement, however the dark side of their use in the live stocks seems to have generated antibiotic-resistant bacteria posing a major threat to both the livestock and human. The occurrence of highly strong antibiotic-resistant bacteria is linked to the application of these drugs as the higher use of antibiotics parallels the emergence of a greater antimicrobial resistant strain. In certain food animals also, these resistant strains are being generated. These antibiotic-resistant bacteria and their corresponding resistant genes from the animal sources can be very easily transferred to human by several means majorly being consumption.

Since antibiotics are majorly used in food animal production, better alternative that are non-toxic and natural must be replaced in animal feed. Their use in animal feed must be stringently regulated especially in developing countries. It is also emphasized that antibiotics be used only for the treatment of diseases in animals and over exploitation of these antibiotics in animal feed for promoting their growth can turn to be fatal for the livestock and human as well. Extensive research should follow up for developing and utilizing potent antibiotics for disease control and treatment. In addition to it, widespread awareness on the irrational use of antibiotics in animal feed has to be created and regulations to implement the control of antibiotic use have to be developed. Global implementation of these measures is also very necessary in creating a better understanding among the producers and consumers on the use of antibiotics in livestock. Future research must urgently focus on developing nano-based alternatives to antibiotic growth promoters, particularly using essential oils or probiotics tailored to specific microbial strains. Emphasis should be placed on optimizing dosage, targeted delivery, and achieving cost-effective commercial scale-up to reduce antibiotic use in livestock farming and mitigate the risk of antimicrobial resistance.

Abbreviations

AGPs: Antibiotic Growth Promoters, FDA: Food and Drug Administration, ABC Transport: ATP Binding Cassette Transport, MATE: Multidrug And Toxic Compound Extrusion, SMR: Small Multidrug Resistance, MFS: Major Facilitator Superfamily, RND: Resistance Nodulation Cell Division, PPB: Penicillin Binding Protein, CAT: Chloramphenicol Acetyl Transferase, PUFAs: Polyunsaturated Fatty Acids.

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Author Contributions

All the authors equally contributed to the study's conceptualization, original draft writing, review, editing, visualization and formal analysis.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Artificial Intelligence (AI) Assistance

The authors declare no use of Artificial intelligence (AI) for the write-up of the manuscript.

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