International Research Journal of Pharmacy and Medical Sciences
ISSN (Online): 2581-3277

Antibiotics and Probiotics Uses in Veterinary Medicine and the Presence of Coumarin in it

Shams Sabeeh Abdulraheem
National Center of Drug Control and Research, Baghdad, Iraq
Email address: shams.rahim1202@copharm.uobaghdad.edu.iq

***Abstract*—** *Despite the fact that the production of safe food for human consumption is the primary purpose for animal husbandry, the habitat as well as well-being of the animals must be taken into consideration. Based on microbiological attitude, the production of healthy food from animals includes the considering of foodborne pathogens, on one side and on the other side, the methods used to fight against pathogens during breeding. The conventional method used to control or prevent bacterial infections in farming is the actively using of antibiotics. Despite the fact that the banning of these compounds as growth promoters can cause different changes in animal breeding and their use has afterward been limited to the treatment and prevention of bacterial infections. Unfortunately, the excessive and abusive use of these compounds have led to multiple problems which can cause harmful consequences on the consumer health which includes: Resistance to many antibiotics and the existence of antibiotic residues in the food. The effective use of probiotics seems to be an acceptable alternative to overcome these problems because of their capability to modulate the immune system and the intestinal microflora, and additionally considering their antagonistic contribution against definite pathogenic bacteria and their ability to play a part as growth factor (sometimes correlated with prebiotics) when it has been used as feed additives. This review aims to apotheosis that some of the effects for the use of antibiotics in the animal rearing as well as point up the present knowledge on the use of the probiotics as a feed additive, their impact on animal production and their potential efficacy as an alternative to common antibiotics. Also the presence and uses of coumarin in some of these products*.

***Keywords*—** *Antibiotics, Growth promoters, public health, animal nutrition, feed additive, probiotic, coumarin*.

I. INTRODUCTION

Veterinary medicines, especially antibiotics, are among the most important components related to the animal feed production. Generally, the main use of antibiotics in animals is for treatment and prevention of diseases and the growth promotion [1].

A major concern has been that repeatedly exposing these animals to a small doses of antibiotics contributes significantly to antimicrobial resistance, Studies over decades shown an explicit relationship between antimicrobial use and antimicrobial resistance in the veterinary science, the use of antibiotics in food animal production constitutes a major contributing factor to current antimicrobial resistance crisis and that antibiotics should only be used for the treatment of sick animals based on prior diagnosis of the disease [2].

Antibiotics kill the bacteria or prevent proliferation and growth of bacteria by inhibiting cell wall synthesis, hinder protein synthesis, inhibiting nucleic acid production, altering cell membrane properties, and impeding metabolites' function. Bacteria can escape antibiotics actions by creating a new resistance mechanism through gene mutations. Misuse of the antibiotics in humans and animals can accelerate antibiotic resistance, antimicrobials are frequently used in food animal production. Therefore, food animal producers are important participants to prevent the overuse and misuse of antimicrobials [3].

Various antimicrobials have made a significant contribution for the prevention, control, and treatment of infectious diseases in animals since 1940s, Low and sub-therapeutic dose of the antimicrobials plays very important role for the improvement of feed efficiency, promotion of animal growth, and the prevention and control of diseases, It is undeniable that rational use of antimicrobials plays a vital role in production of food animals and the protecting of public health, while the irrational and irresponsible use may cause antimicrobial resistance. Benefits and risks of antimicrobial drugs, used in food-producing animals, continue to be a complex and disputable issues. The risks of antimicrobial drugs to the public health associated with antimicrobial resistance raised great concern lately, while the benefits of antimicrobial drugs; such as the prevention and treatment of animal diseases, protection of public health, increase animal production, and development of the environment, were ignored most of the time [4]. The potential warning to human health resulting from inappropriate use of antibiotics in the food animals is significant, as pathogenic-resistant organisms cultivated in these livestock are composed to enter food supply and could be widely diffused in the food products. Commensal bacteria present in livestock found frequently in the fresh meat products and may serve as reservoirs for the resistant genes that could be potentially transferred to pathogenic organisms in humans [5].

Recent outbreaks of the food-borne diseases highlight the need for reducing bacterial pathogens in the foods of animal origin. Animal enteric pathogens are direct source for food contamination. The modulation of the gut microbiota with a new feed additives, such as probiotics and prebiotics, towards host-protecting functions to support the animal health, is a topical issue in animal breeding and creates a fascinating possibilities [6].

Various benefits have been associated with the use of probiotics in the farm animals including: ameliorate growth and feed efficiency, enhanced the product quality and reduced mortality. While the mechanisms through which probiotics produce their effects beneficially are not well understood, their

Shams Sabeeh Abdulraheem, "Antibiotics and Probiotics Uses in Veterinary Medicine and the Presence of Coumarin in it," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 5, Issue 5, pp. 56-66, 2022.

part in the gastrointestinal microbiota modifications is believed to be the main mechanism of action. The use of probiotics in fresh and fermented meat products has been also indicate the decrease of the pathogenic and spoilage microorganisms and the development of the sensory characteristics. Although the use of probiotics has been associated with many benefits, their benefits in improving the animal performance and quality of the products is highly variable. Factors that dominate such variances are dependent on the probiotic strain being used and its stability during the administration/inoculation and storage, nutritional and health status, frequency and dosage and finally the age of the host animal [7].

II. Scope of the Document

The empirical background for the safety, effects and regulation of the antibiotics and probiotics utilized in animal feed is the focal point of this research. It is not a narrative-review for the effects of antibiotics and probiotics. Due to distinction in the species, genera and the strains of micro-organisms, husbandry practices, age, animal species, duration of application and the dose rate. It isn't possible to finish the meaningful of the narrative-review. This document supply information about the antibiotics and probiotics, their effects in different categories of livestock, their mode of action, the global regulatory situation and the safety and risk associated with their use in the animal nutrition.

III. Antibiotics: Definition and Classification

3.1 Definition of antibiotics

The word "antibiotic" was conceived from the word "antibiosis" which precisely means "against life". In the old days, antibiotics were examined to be an organic compounds created by one microorganism which are toxic to the other microorganisms. Associated with this, these (Antibiotics) are the substances that can be developed or produced by microorganism, which particularly impede the generation of or kill the microorganism (that are dangerous to the human health) at very low concentrations [8].

3.2 Classification of antibiotics

Antibiotics have been classified in a different way, but the commonest classification is based on molecular structure, mechanism of action and spectrum of activity. Antibiotics could also be classified based on route of administration and whether it kills or inhibit the growth of microorganisms [9].

3.2.1 Classification based on molecular structure

Antibiotics are categorized according to their chemical structures (Table 1) [10].

TABLE 1. Antibiotics classification according to chemical structure [10]

Group	Internal group	Representative with practical importance
Carbohydrate antibiotics	1.Aminoglycosideantibiotics 2.Other(N- and C-) glycosides	Streptomycin, Neomycin
Macro cyclic lactone (lactam) antibiotics	1.Macrolide antibiotics	Erythromycin
	2.Polyeneantibiotics 3.Macrolactam antibiotics	Amphotericin
Quinone and similar antibiotics		Oligomycin
Amino acid Peptide antibiotics		Tetracyclines Penicillins, Cephalosporins,
		Bacitracin, Polymyxins
Nitrogen-containing Heterocyclic antibiotics		
Oxygen-containing Heterocyclic antibiotics	1.Non-condensed(single) heterocycles	No practical importance
	2.Condensed (fused) heterocycles	
	1.Furan derivatives	No practical imporance
	2.Pyran derivatives	
Alicyclic antibiotics	1.Cycloalkane derivatives	
	2.Small terpenes	Streptovitacins
	3.Oligoterpene antibiotics	
Aromatic antibiotics	1.Benzene compounds	Chloramphenicol
	2.Condensedaromatic comp.	Grisefulvin
	3.Non-benzene aromatic comp.	Novobiocin
Aliphatic antibiotics	1.Alkane derivatives	Varitin
	2.Aliphatic carbocyclic acid derivatives	

Shams Sabeeh Abdulraheem, "Antibiotics and Probiotics Uses in Veterinary Medicine and the Presence of Coumarin in it," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 5, Issue 5, pp. 56-66, 2022.

3.2.2 Classification based on mechanism of action

Antibiotics can be classified according to mechanism of action in to:

1. Agents that inhibit bacterial cell wall synthesis: These comprise penicillins and cephalosporins that are structurally similar and dissimilar agents, such as vancomycin, cycloserine, bacitracin and the imidazole antifungal agents.
2. Agents that act on cell membrane of the microorganisms directly affecting the permeability and bring on intracellular compounds leakage: These comprise polyene antifungal agents, polymyxin, nystatin and the amphotericin B that bind to the sterols of the cell wall.
3. Agents that affect the 30s and 50s ribosomal subunits functions leading to reversible inhibition of the protein synthesis: These comprise erythromycins, tetracyclines, chloramphenicol and clindamycin.
4. Agents that alter the protein synthesis by binding to the 30s ribosomal subunit: These comprise aminoglycosides which cause cell deaths eventually.
5. Agents that affect the metabolism of the nucleic acid: These comprise rifamycins that inhibit the DNA dependent RNA polymerase.

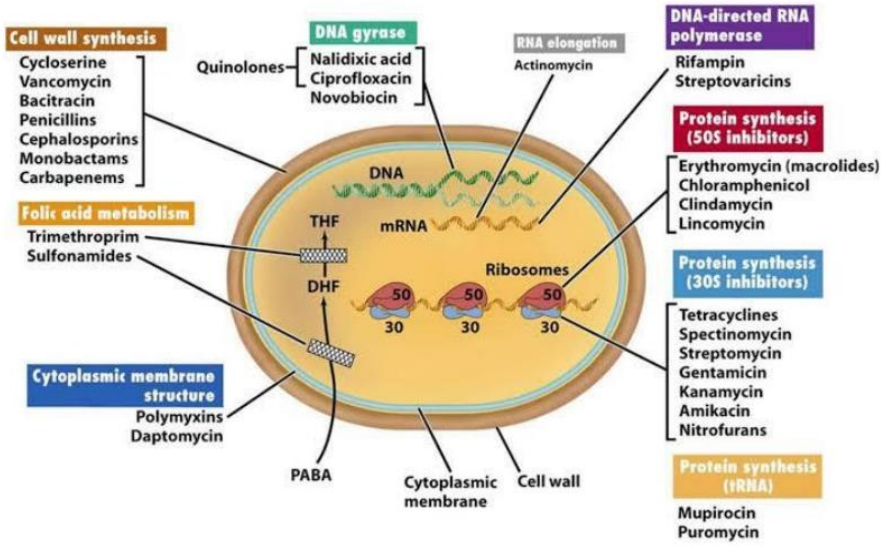

Figure 1. Classification of antibiotics depending on mechanism of action [9]

3.2.3 Classification based on spectrum of activity

Antibiotics are life-saving medications and have been the cornerstone of public health. Furthermore, they are the most common medications prescribed for the children. Antibiotics are generally categorized according to their spectrum of antimicrobial activity; the spectrum means the number of the organisms affected by the same drug, as either broad-spectrum or the narrow-spectrum antibiotics. The Broad spectrum antibiotics affect several types of bacteria and fungi and it is usually used where the specific type of the microorganism is unknown, such as doxycycline, azithromycin, amoxicillin and clavulanic acid, mupirocin, and fluoroquinolones, target a wide range of the gram-positive and gram-negative bacteria, whereas narrow-spectrum antibiotics are used only when we know the specific type of microorganism. These are more effective on specific microorganisms but less effective on others, such as vancomycin, fidaxomicin, and sarecycline, only target limited types of clinically relevant bacteria [11]

3.2.4 Classification based on route of administration

Antimicrobial agents are also classified based on the mode of administration in to:

-Oral: antibiotics that are administered through the oral cavity.
-Parenteral: antibiotics that are administered through Injection.
-Topical: antibiotics that are administered through application on body surfaces [9].

3.2.4 Classification based on killing effect

Antibiotics have been classified into: bactericidal (ie, they kill bacteria by inhibiting the cell wall synthesis), examples include: Beta-lactam antibiotics (penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems) and vancomycin. Also bactericidal are daptomycin, fluoroquinolones, metronidazole, nitrofurantoin, co-trimoxazole, telithromycin and bacteriostatic (ie, they only inhibit the growth or the proliferation of bacteria), this group includes: tetracyclines, sulfonamides, spectinomycin, trimethoprim, chloramphenicol, macrolides and lincosamides [11].

3.3 Mechanism of antibiotic resistance

Emergence of the antibiotic resistant pathogenic bacteria poses a serious public health challenge worldwide. However, antibiotic resistance genes are not confined to the clinic; instead they are widely prevalent in different bacterial populations in the environment [12]. The mechanism of antibiotic resistance can be shown in (figure 2).

Shams Sabeeh Abdulraheem, "Antibiotics and Probiotics Uses in Veterinary Medicine and the Presence of Coumarin in it," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 5, Issue 5, pp. 56-66, 2022.

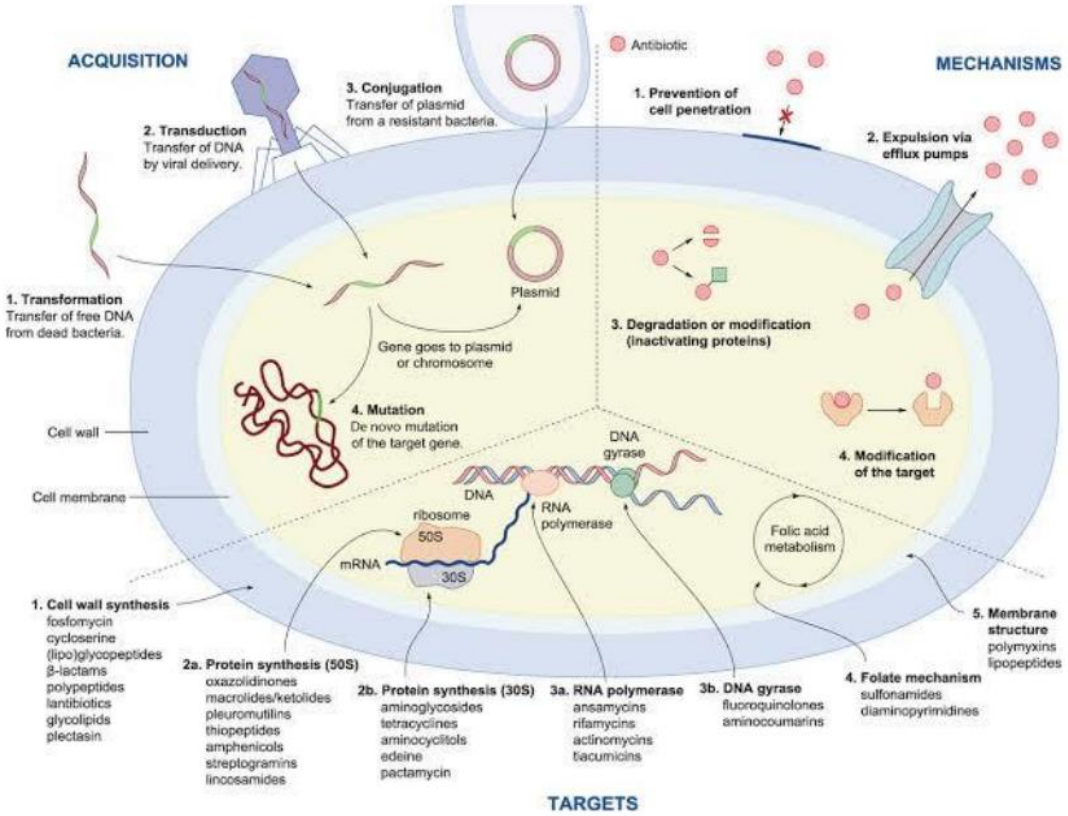

Figure 2. Mechanisms of antibiotic resistance [9]

3.4 Food animals and antibiotics

Antibiotics are delivered to the animals for a variety of purposes including: therapeutic use to treat the sick animals, prophylactic use to prevent infection in the animals and feed efficiency/growth promotion. Antimicrobial growth promotants (AGPs) were first recommended in the mid-1950s, when it was found that small and sub therapeutic amount of antibiotics like procaine penicillin and tetracycline (1/10 to 1/100 the quantity of the therapeutic dose), transfer through feed to the animals, could strengthen the feed-to-weight ratio for swine, poultry and the beef cattle [13].

Concern about antibiotics uses in animals and its possible impact on human health make up two major issues: the antibiotic agents which have been used and the way of using it. There is a prospect that the antibiotics (the once that are important in human medicine) should not be used therapeutically in food-producing animals, specifically for a mass medication. The prophylactic use of them confer a problem in two ways: the antibiotic agents used and the absence of definition of what is a suitable duration of the prophylactic use of them. The use of the Growth-promotant is possibly the area of the highest study, as some of the antibiotics that have been used are now considered as compromising some key human antibiotics efficacy and the treatment duration may be for the entire life of treated animals [14].

3.4.1 Growth promoters

Growth promoters are the substances that are included in the feeds as a supplement or given by injections as drugs to ameliorate the feed usage and the farm animal's growth. In regions where there is increasing tendency of beef and milk dictate, growth promoters are very important to serve human needs. The hormonal anabolic implants (Both estrogenic and androgenic) are the most extensively appeal growth promoters, bovine somatotropin (BST), Repartitioning agents (Beta-agonists), feed additives and probiotics. All non-nutrient feed additives including exogenous enzymes that improve the animal growth and antibiotics can be mainly reported as growth promoters [15].

3.4.2 History of the use of growth-promotant antibiotics

The antibiotics usage as growth promoters in animal feeds has been authorized in the member rank of the European Union at the end of 50 years. However, concerns about the antimicrobial resistance development and the antibiotic resistance genes transformation from animal to human microbiota, give raise to withdraw approval for the antibiotics as growth promoters in the European Union in January 1, 2006.

The antibiotics growth promoter effect was discovered since 1940s, when it was observed that the animals fed dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues improved their growth [16].

TABLE 2. Summary of antibiotics registered for use as growth promotants in Australia, the EU and the USA (adapted from World Health Organization, 1997; Joint Expert Technical Advisory Committee on Antibiotic Resistance, 1998) [14]

Class	Antibiotic	Australia	EU	USA
Arsenicals	3-Nitro-arsonic acid and others	Pigs, poultry		Pigs, poultry
β-Lactams	Penicillin			Pigs
Glycopeptides	Avoparcin	Pigs, meat poultry, cattle	Suspended 1996	
Lincosamides	Lincomycin			Pigs
Macrolides	Erythromycin			Pigs
	Kitasamycin	Pigs		
	Oleandomycin	Cattle		
	Tylosin	Pigs	Suspended 1999	
	Spiramycin		Suspended 1999	
Oligosaccharides	Avilamycin		Pigs, meat poultry	
Pleuromutilins	Tiamulin			Pigs
Polyethers (ionophores)	Lasolocid	Cattle		
	Monensin	Cattle	Beef cattle	Cattle
	Narasin	Cattle		
	Salinomycin	Cattle, pigs	Pigs	
Polypeptides	Bacitracin	Meat poultry	Suspended 1999	Pigs, poultry, cattle
Quinoxalones	Carbadox			Pigs
	Olaquindox	Pigs		
Streptogramins	Virginiamycin	Pigs, meat poultry	Suspended 1999	Pigs, poultry, cattle
Tetracyclines	Tetracycline			Pigs, poultry, cattle
Bambermycins	Flavophospholipol	Pigs, poultry, cattle	Poultry	

3.4.3 Category of growth promoters

Growth promoters of Beef Cattle are classified into five groups; feed additives, hormonal implants, growth hormone (Somatotropins), beta-agonists and probiotics. The feature of each growth promoter are as follows [17]:

- Feed additives: It is a substance included in feed to accomplish a particular need of the animals. The additive may supply the needed nutrient or rise an animal's resistance to disease. various feed additives were accessible including organic acids, antibiotics and an exogenous enzyme.
- Hormonal implants: Hormonal implants may enhance the growth during suckling, growing and finishing stages of meat production. Types of hormones most widely used in the cattle production in the form of implant include natural hormones, (estradiol, testosterone and progesterone) and the synthetic ones (trenbolone acetate and zeranol).
- Growth hormones (GHs): Is a single polypeptide chain consisting of 191 amino acids differing between species significantly. By stimulating the metabolism and protein accretion concurrent with a reduction in the fat deposition it raises weight gain. Bovine Somatotrophine (BST) which is a bovine growth hormone produced by pituitary gland of the cow is one of these (GHs).
- Repartitioning agents (β-adrenergic agonists): These agents raise the growth efficiency by stimulation beta-adrenergic receptors on cell surfaces. They act as a repartitioning agents to alter carcass conformation by modifying nutrient partitioning to decrease fat deposition up to 40% and raise muscle protein content up to 40%. A wide range of compounds has been investigated as a beta-agonists including cimaterol, clenbuterol, fenoterol, isoprenaline, mabuterol, ractopamine, salbutamol, terbutaline and zilpaterol.
- Probiotics: These compounds are mono or mixed cultivation of the living microorganisms, which by ameliorating properties of the indigenous microflora it convinces beneficial effect onto the host. Diverse microorganisms have been examined as probiotics these are; bacteria, fungi particularly yeast and mushroom and mixed cultures involve different microbes. Bacteria are more often reported as a probiotic than fungi. The microorganisms used as probiotics are advisable in Genera Lactobacillus and Bifidobacteria are frequently reported.

3.4.4 Mode of action of growth-promoting antibiotics

The term growth promotion applies to increase in the performance or productivity achieved in food producing animals following the addition to their diet of the feed antibiotics or growth promoters. Numerous methods are available for different animal species. Growth promotion mechanisms are only poorly understood but it is now mostly accepted that any development in the performance is related directly to diversity of direct effects on gut microbial flora and indirect effects on intestinal tissues.

-The direct effects include: intervention with bacterial cell wall development, inhibition of bacterial growth, initiation of filament development and interference with the intestinal bacterial metabolism.

-The indirect effects include: a depletion of the intestinal mucosal layer thickness and a decrease in certain mucosal cell enzymes production. Other effects noticed in the gut of growth promoted animals and birds include a decrease in the popularity of the antibiotic resistance plasmids and decrease the frequency of the transference of the plasmid in enteric coliforms [18].

3.4.5 Problems associated with antibiotic use in animals

The use of antibiotics in food animals selects for bacterial resistant to the antibiotics used in humans, and these might spread via food to the humans and cause human infection. By transformation from other bacteria, all antibiotics can select spontaneous resistant mutants and bacteria that have acquired resistance. These resistant variation, in addition species that are inherently resistant become dominant and spread in the host-animal inhabitant. The excessive antibiotic usage, the more likely are resistant inhabitant to progress between pathogens and commensal bacteria of an increasing animals number in an exposed inhabitant. Nevertheless, there is great contrast: in treated individuals some bacteria very rapidly evolve resistance, others remain susceptible. Antibiotic resistance explained in this way as a microbiological phenomenon, which may or may not have a clinical implications depending on the pharmacokinetic and pharmacodynamics parameters as they appeal to definite antibiotics. Low-level resistance (diminished antibiotic potency within the clinically susceptible range) is noticeable, it may be the first step towards clinical resistance. These examinations have always been important in the rational antimicrobial therapy terminologies, and by a final appeal for prudent therapy in the human and veterinary medicine they have been recall [19].

3.5 Coumarin in veterinary medicine

The nucleus of coumarin is the basis of different compounds possessing different pharmacological and physiological activities. Plant extracts possessing these compounds have been applied against the gastrointestinal diseases, paralysis, typhus, for the cure of leucoderma, and as anticoagulants. In veterinary medicine, they have been used against trichomonas infection or used as diuretics. They have also been appealed in industry, principally as food flavors [20]. Coumarins (1,2-benzopyrones or 2H-1-benzopyran-2-ones) constitute a crucial family of the naturally occurring benzopyrone compounds, all of them consist of a benzene ring linked to a pyrone ring (figure 3). Natural coumarins can be sort into six main groups as following: simple coumarins, bisoumarins, furanocumarins (linear type and angular type), benzocoumarins, pyranocoumarins (linear type and angular type) and coumestans. They are regarded as a secondary plant metabolite that keep the plant secure from infections, with its basic role in the plant biochemistry and physiology; they serve as enzyme inhibitors, antioxidants and precursors of toxic substances. Specifically, these compounds presume in the activity of the plant growth hormones and growth regulators, photosynthesis and respiration control [21].

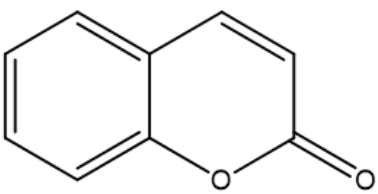

Figure 3. Chemical structure of coumarin

Heterocyclic compounds have reward excessively to the community in the form of variety of drugs for the treatment of different diseases and hold an obvious part in the medicinal chemistry due to their diverse biological activities. The combination of heterocycles affords a chance to develop novel multicyclic compounds with an enhance biological activities. In the recent years, these natural chemicals have gotten much curiosity from the scientific community due to their wide variety of biological actions (including the capability to interact with the biological systems). The role of heterocycles in the biological system is very important. Biochemical processes of the elements of the living organisms like RNA, DNA etc., are depend on heterocycles. Privileged structures are regularly used as a template for investigating and discovering a very significant compounds. Coumarins are the main scaffold present in different plants and some bacteria and fungi (figure 4) [22].

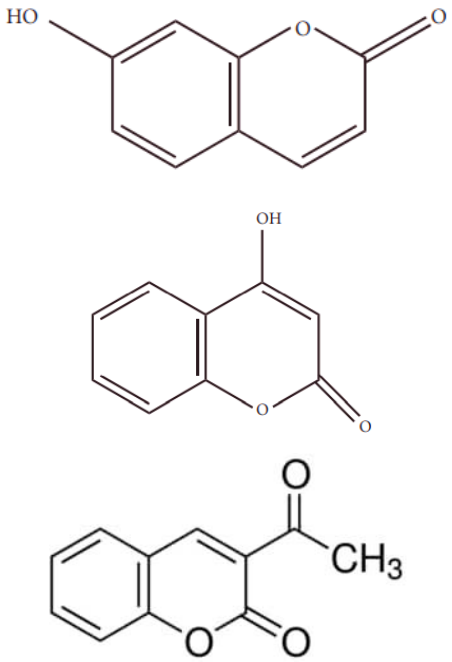

Figure 4. Structure of some biologically active coumarins

Coumarins are natural compounds that were noticed in 80 species of the plants. They have diverse utilizations including the medical, tobacco, food, perfumery, and spirit industries. Also, anti-swelling and diastolic effects. Even so, excess utilization of coumarins and its derivatives may adversely influence our health, by causing cirrhosis of the liver through absorption from the intestines into the lymph and blood [23]. The presence of coumarin in food is regulated in the EU by the Regulation (EC) No 1334/2008 of the European Parliament and of the Council 16 December 2008, according to the Annex III of this regulation, coumarin shouldn't be included as a substance into the food, in spite of the fact that the maximum level for coumarin is laid down for a certain compound food to which cinnamon is included as a flavoring and/or food ingredient with a flavoring characteristics (Table 3) [21].

Shams Sabeeh Abdulraheem, "Antibiotics and Probiotics Uses in Veterinary Medicine and the Presence of Coumarin in it," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 5, Issue 5, pp. 56-66, 2022.

TABLE 3. The food compound in which the existence of the coumarin is limited [21]

Compound Food	Maximum Level of Coumarin (mg/kg)
Traditional and/or seasonal bakery wares containing a reference to cinnamon in the labeling	50
Breakfast cereals including muesli	20
Fine bakery ware, with the exception of traditional and/or seasonal bakery wares containing a reference to cinnamon in the labeling	15
Desserts	5

Coumarin is a flavoring which can cause hepatotoxicity in the experimental animal in a proportion of the human population. The tolerable daily intake (TDI) may be exceeded in consumers with high intake of the cinnamon containing high levels of coumarin [24].

IV. PROBIOTICS: DEFINITION AND CLASSIFICATION

4.1 Definition of probiotics

The global validate definition of the probiotics is live microorganisms that confer a health benefit onto the host when administered in adequate quantity. The further definitions that advanced through the years have been prohibitive by order of the mechanism of actions, delivery format, site of action, methods, or the host. The US Food and Drug Administration (FDA) utilize another expression for the live microbes for regulatory objectives these includes: 1- live microbes used in the animal feeds are called "direct-fed microbial", 2- they are classified as "live bio-therapeutics" when intended for use as human drugs. Even though, no legal definition of the probiotics prevails in the United States or in other nation, which permit the marketing of products categorize as "probiotics" that don't meet an elementary criteria required in the scientific descriptions [25]. Because of health benefits probiotics confer to the host, they are considered a beneficial components of the microbiota that have been utilized for centuries. Only recently, probiotics have a contribution in: modulation of respiratory, immunological and gastrointestinal functions established to be scientifically evaluated and completely appreciated. Probiotics including *Escherichia coli* Nissle 1917 and lactic acid bacteria have been assessed for the use to treat or prevent a wide range of intestinal maladies including constipation, inflammatory bowel disease and the colon cancer [26].

4.2 Taxonomy of probiotics

The taxonomy of probiotic lactic acid bacteria was formed according to the morphological, biochemical and physiological characteristics with molecular-based phenotypic and genomic techniques. The most studied are genera Lactobacillus, Bifidobacterium and Enterococcus (Table 4) [27].

TABLE 4. Microorganisms used as probiotic agents [27]

Lactobacillus species	Bifidobacterium species	Others
L. acidophilus	*B. bifidum*	*Bacillus cereus*
L. casei (rhamnosus)	*B. longum*	*Escherichia coli*
L. reuteri	*B. breve*	*Saccharomyces cerevisiae*
L. bulgaricus	*B. infantis*	*Enterococcus faecalis*
L. plantarum	*B. lactis*	*Streptococcus thermophilus*
L. johnsonii	*B. adolescentis*	
L. lactis		

Lactic acid bacteria are among the most important probiotic microorganisms typically associated with human gastrointestinal tract. Recent molecular techniques including several genotyping methods and the polymerase chain reaction-based, have become progressively important for the differentiation of probiotic strains or for the species discovery. On the basis of particular physiologic and the functional properties, probiotic strains are selected for potential application, some of which may be set on in vitro. The identification and classification of a probiotic strain may give a strong evidence of its typical habitat and the origin. The species or the genus name, may indicate the safety of the strain and technical applicability for use in the probiotic products. Molecular typing methods including pulsed-field gel electrophoresis, the restriction fragment length polymorphism and the repetitive polymerase chain reaction are tremendously valuable for the particular detection and characterization of such strains selected for the application as probiotics [28].

4.3 Safety of probiotics

There is a substantial regard in the probiotics for a diversity of medical conditions and millions of population all over the world overwhelm probiotics daily to recognize health benefits. Bifidobacteria, lactobacilli and lactococci have mostly been evaluated as safe. Regarding the safety of the probiotics, there are three theoretical concerns: (1) the incident of the disease, such as endocarditis or bacteremia, (2) the transfer of antibiotic resistance in the gastrointestinal flora, and (3) metabolic or toxic effects on the gastrointestinal tract [29]. Hundred years ago, probiotics have been used properly in foods and the dairy products. Newly, there has been increasing regards in the usage of probiotics to prevent mitigate or to treat some significant diseases. Abundance clinical trials have investigated probiotics usage for the diseases ranging from necrotizing colitis in premature infants to hypertension in adults [30]. There is great experimental verification that support the health benefits of the probiotics including: relieve of the lactose intolerance symptoms, enhancement of the intestinal microbial balance by antimicrobial activity, prevention of food allergies, anti-tumorigenic activities, enhancement of the immune potency, anti-oxidative and anti-atherogenic effects, and a hypo-cholesterolemic property. The bulk of the probiotics and its products are marketed as foodstuffs, with the profess GRAS (generally regarded as safe) status [31]. It was examined that it was important to evolve a science-driven, because there are published reports of infrequent infections involving lactobacilli or bifidobacteria, including two cases recommended to be associated with probiotic strains, evidence-based survey of lactobacilli and bifidobacteria safety used as probiotics in the foods [32].

4.4 Mechanism of action of probiotics

Probiotic mechanisms of action include: *1*) normalization and colonization of perturbed intestinal microbial communities in the children and adults, *2*) enzymatic activity and the production of volatile fatty acids, 3) competitive exclusion of pathogens and bacteriocin production, 4) cell

antagonism, cell adhesion, and mucin production, *5*) modulation of the immune system, and finally *6*) interaction with the brain-gut axis [33].

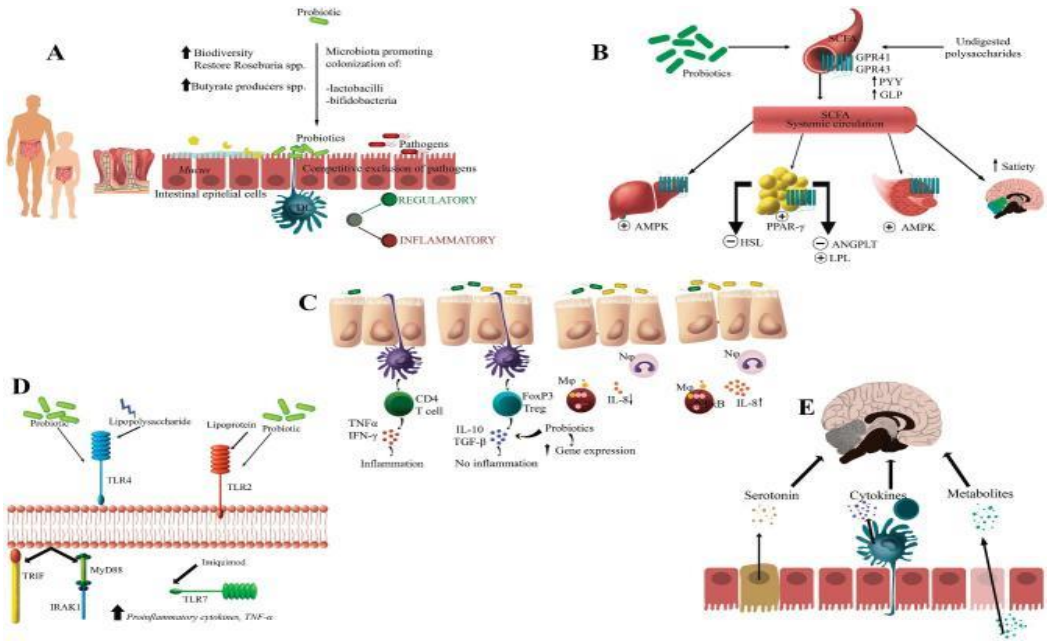

Figure 5. Summarizes of the mechanisms of action of probiotics [33]

TABLE 5. Mechanisms of Action of Probiotics [34]

Antimicrobial Activity
Decrease luminal pH
Secrete antimicrobial peptides
Inhibit bacterial invasion
Block bacterial adhesion to epithelial cells
Enhancement of Barrier Function
Increase mucus production
Enhance barrier integrity
Immunomodulation
Effects on epithelial cells
Effects on dendritic cells
Effects on monocytes/macrophage
Effects on lymphocytes
- B lymphocytes
- NK cells
- T cells
- T cell redistribution

The definite mechanism of action that influence the crosstalk between the microbe and the host remain vague but there is a growing verifications proposed that the function of "both systemic and a mucosal" immune system can be moderate by bacteria in the gut. The modern enthralling evidence has demonstrated that manipulating the microbiota can influence the host. Newly mechanism of actions by which the probiotics exhibit their beneficial effects have been recognized and now it is clear that notable variances exist between different probiotic bacterial strains and species. In order to successfully treat the disease, the microorganisms needed to be selected in a more logical manner [34].

Probiotic lactic acid bacteria (LAB) have possible potential as alternatives to the antibiotics, both in therapeutic and prophylactic applications. Several examinations have documented the prevention or the reduction of GI diseases by probiotic bacteria. After all, the activities of probiotic bacteria are closely linked with the situation in the GI-tract (GIT) of the host and changes in the enteric microorganism's population, a deeper understanding of gut-microbial interactions is required in the selection of most suitable probiotic [35].

4.5 Probiotics in veterinary medicine

Probiotic therapy is becoming increasingly popular in the veterinary medicine, both for therapeutic uses and for the growth promotion. Probiotics may be used in food animal species to prevent or treat diseases. Other objectives include increasing the growth rate, improving feed conversion, stimulating the immune system, and decreasing shedding of zoonotic pathogens. Accessible probiotics can be categorized into two main categories; One category is the colonizing species such as *Lactococci*, *Lactobacillus,* and *Enterococcus*, and the other one is the free-flowing non colonizing species which include both *Saccharomyces cerevisiae and Bacillus*. These beneficial microbes are able to ameliorate the animal overall health by upgrade the gut microbial balance. Probiotics are also effective in helping boost weight gain and feed conversion rates in the newborn animals [36]. Probiotics becoming a common means of combating the intestinal diseases of various origins: chronic inflammation, infectious pathologies, and autoimmune disorders. Its complex action

Shams Sabeeh Abdulraheem, "Antibiotics and Probiotics Uses in Veterinary Medicine and the Presence of Coumarin in it," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 5, Issue 5, pp. 56-66, 2022.

combined with low side effects makes them the promising drugs particularly in veterinary medicine; with an increasing trend as regards to the inefficient use of the antibiotics in the livestock industry. One of the principal mechanisms of actions of the probiotics is "action - modulation of host immunity" is perhaps the most difficult and the most actively studied by reason of that it is crucial for therapy. "Immunobiotics" which are probiotics that modulate the host's immune response, interact with the various innate and adaptive immune cells changing the expression of pro- and anti-inflammatory cytokines. This action is provided by both cellular components of probiotic microorganisms and their metabolites, and is essentially associated with host's immunocompetent cells' pattern-recognition receptors [37]. Probiotics could represent an effective alternative to the use of synthetic substances in nutrition and in medicine. The efficacy of the probiotics may be potentiated by several methods: the selection of more efficient strains; gene manipulation; the combination of several strains; and the combination of the probiotics and synergistically acting components [38]. Use of beneficial microorganisms, probiotics, is becoming interesting for the prophylactic or therapeutic application against several diseases including helminths. "Zoonoses" are diseases that can be transmitted from the animals to humans in a reversible way, despite zoonotic helminth diseases being still a big problem to the general health and the agriculture industries globally; they were still neglected in both the veterinary medicine and the human [39]. The probiotics were improved feed conversion for the target species, reduced morbidity or mortality and benefits to the consumer through improved product quality, probiotics combinations with a variety of mechanisms of action could magnify the protective scope of bio-therapeutic preparations and enhance probiotics making them more effective than their components individually. Bacterial probiotics were effective in pigs, chickens, and pre-ruminant calves; whereas the fungal and yeasts probiotics were given better calculations in adult ruminants. Probiotics protected the animals against pathogens, enhanced immune response, reduced antibiotic use and shows a high index of safety [40]. Probiotics usage in the animal feeding is related with the confirm effectiveness of them in the modulation of the intestinal microbiota. Probiotic strains administration of both individual and combined once may have a remarkable effect on the daily increase of body weight and the total body weight of various animals, utilization and absorption of feed. Probiotic microorganism's addition to the feed results in better quality and quantity of meat, milk, and eggs. Moreover, the probiotics decrease the weak limbs effects in broiler chicken. In the case of piglets, the probiotics main expected effect is a reduction of frequency of diarrhea, constitute a problem in the initial post-weaning weeks. The probiotics efficacy in fighting diarrhea is one of the most commonly measured aspect. Re-combined probiotics are one of the novelest biomedical applying of genetically modified organisms (GMO). the clinical side effects absence is one of the most important benefit of the probiotics [41].

4.6 Coumarin in probiotics

The most important role of Coumarins as an alternative therapeutic procedures depending on their ability to slow down the biofilm development of the clinically significant pathogens and to block quorum sensing signaling systems has been emphasized. The appearance of CO in the food isn't considered to give rise to the risk to humans. CO up to date is an agricultural antibacterial substance against *Ralstonia solanacearum*. In addition, advanced derivatives of CO including those from the bark of *Zanthoxylum avicennae*, may have a variety of effects [42]. The presence of the synergistic mechanisms of Radix Angelicae containing COs and ligustrazin has been discovered in the treatment of migraines [43]. In the plant extracts, Coumarin derivatives may be an optimistic substance. Thus, modern studies have released the ability of coumarins to decrease the virulence and biofilm formation in the Pseudomonas aeruginosa, suppress pathogenic plant bacteria by; acting on the quorum of bacteria, its effectiveness against aflatoxicosis in the animals, low cytotoxic activity and reduces oxidative stress. At the same time, there is an insufficient information for the effectiveness of coumarin derivatives on the productivity, immunity and ecology of the gastrointestinal tract microorganisms of the farm animals [44]. Metabolite fingerprinting revealed the antimicrobial coumarin scopoletin as a dominant metabolite that is produced in the roots and excreted to the rhizosphere in a MYB72- and BGLU42-dependent manner, scopoletin selectively impacts the assembly of microbial community in the rhizosphere [45]. Coumarin, which is a part of citrus waste, can have a positive effect on the biochemical profile of the laying hens, in particular the level of gamma-glutamyltransferase, thereby indicating the activity of enzymes such as ALT and AST. The additional inclusion of a coumarin derivative in the diet of broiler chickens helps to reduce aspartate aminotransferase as well as the level of the alanine aminotransferase [46].

V. CONCLUSION

Antibiotic-resistant human pathogens are a major challenge in the human medicine. There is some risk that excessive attention to the transfer of antibiotic-resistant bacteria or genes from animals to man will divert attention from the need to address problems inherent in the medical use of antibiotics. Human enteric infections with salmonella and campylobacter are seldom cured with the antibiotics, "antibiotic resistance" is hence, less important than it is with a few of other more consequential infections. However, it is still not allowable for individuals to be unessentially exposed to the resistant strains of bacteria in their food. It is clear that the antibiotic resistance is a point at issue with animal strains of salmonella, campylobacter and E. coli; and that these microorganisms contaminate individuals as a consequence of the contamination of animal carcasses and meat. Controlling the antibiotic resistance itself is necessary. Farmers as the end-users of antibiotics must be more careful of antibiotic-resistance complications, and it is critical that on-farm; The Quality Assurance programs mark the responsible uses of the

antibiotics on the whole than by solely trying to decrease the incidence of residues in the tissues. Distinctly, on farms there is a case for the reducing antibiotic usage. It is difficult to justify the use of potentially-valuable antibiotics as a growth-promoting agents, and long-term use of antibiotics for the prevention of disease clearly contributes to the antibiotic-resistance problem. It is important to estimate these approaches to make sure, for example that the probiotics and competitive exclusion products don't carry antibiotic-resistance genes themselves. However, for the therapeutic use in animals, it will always be necessary to have obtainable range of antibiotics. Consumers are concerned about the safety of foods and the health-giving. The farming industries should be alert to the requirements to ensure that animal products are healthy and safe for consumers, therefore, increasing litigation based on the premise that the individuals shouldn't suffer any harm from food. So, the aim should be; "to produce a product with no residues and with minimum levels of bacteria that have no acquired antibiotic resistance". Animal usage controls will not resolve the current problems in the human medicine, but may help to lengthen the healthy life of any new classes of antibiotics when they are established. The beneficial properties of the probiotics when used as a feed additive are very encouraging for animal breeding. The study of existing literature on this subject allowed us to highlight their potential use as an alternative for conventional antibiotics.

Conflict of Interest

The authors declare no conflict of interest.

Notes/Thanks/Other declarations

The author declare that she has no competing interests.

Appendices and Nomenclature

Abbreviations	Explaination
DNA	Deoxyribo Nucleic Acid
RNA	Ribonucleic Acid
AGPs	Antimicrobial growth promotants
BST	Bovine somatotropin
GH	Growth hormone
TDI	Tolerable daily intake
FDA	The US food and drug administration
GRAS	Generally regarded as safe
LAB	Lactic acid bacteria
GI	gastrointestinal
GIT	Gastrointestinal tract
GMO	Genetically modified organisms
CO	coumarin
EU	European union
USA	United States of America

REFERENCES

1. Bacanlı M, Başaran N. Importance of antibiotic residues in animal food. Food and Chemical Toxicology. 2019;125:462-466.
2. Van T, Yidana Z, Smooker P, Coloe P. Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. Journal of Global Antimicrobial Resistance. 2020;20:170-177.
3. Kasimanickam V, Kasimanickam M, Kasimanickam R. Antibiotics Use in Food Animal Production: Escalation of Antimicrobial Resistance: Where Are We Now in Combating AMR?. Medical Sciences. 2021;9(1):14.
4. Hao H, Cheng G, Iqbal Z, Ai X, Hussain H, Huang L et al. Benefits and risks of antimicrobial use in food-producing animals. Frontiers in Microbiology. 2014;5.
5. Landers T, Cohen B, Wittum T, Larson E. A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. Public Health Reports. 2012;127(1):4-22.
6. Gaggìa F, Mattarelli P, Biavati B. Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology. 2010;141:S15-S28.
7. Al-Shawi S, Dang D, Yousif A, Al-Younis Z, Najm T, Matarneh S. The Potential Use of Probiotics to Improve Animal Health, Efficiency, and Meat Quality: A Review. Agriculture. 2020;10(10):452.
8. Khan F. Antibiotics Classification and Visual Target Sites for Bacterial Inhibition. Advances in Pharmacology & Clinical Trials. 2018;3(3).
9. Fomnya H, Ngulde S, Amshi K, Bilbonga G. Antibiotics: Classifications and mechanism of resistance. International Journal of Applied Microbiology and Biotechnology Research. 2021;9:38-50.
10. Kebede G, Zenebe T, Disassa H, Tolosa T. Review on Detection of Antimicrobial Residues in Raw Bulk Milk in Dairy Farms. African Journal of Basic & Applied Sciences. 2014;6(4):87-97.
11. Grada A, Bunick C. Spectrum of Antibiotic Activity and Its Relevance to the Microbiome. JAMA Network Open. 2021;4(4):e215357.
12. Peterson E, Kaur P. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. Frontiers in Microbiology. 2018;9.
13. Marshall B, Levy S. Food Animals and Antimicrobials: Impacts on Human Health. Clinical Microbiology Reviews. 2011;24(4):718-733.
14. Barton M. Antibiotic use in animal feed and its impact on human healt. Nutrition Research Reviews. 2000;13(2):279-299.
15. Herago T, Agonafir A. Growth Promoters in Cattle. Advances in Biological Research. 2017;11(1):24-34.
16. Castanon J. History of the Use of Antibiotic as Growth Promoters in European Poultry Feeds. Poultry Science. 2007;86(11):2466-2471.
17. Getabalew M, Alemneh T, Zewdie D. Types and Uses of Growth Promoters in Beef Cattle. Journal of Veterinary Medicine and Animal Sciences. 2020;3(1):1027.
18. Walton J. Modes of action of growth promoting agents. Veterinary Research Communications. 1983;7(1):1-7.
19. Phillips I. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. Journal of Antimicrobial Chemotherapy. 2003;53(1):28-52.
20. Feuer G., Med. C. 3 The Metabolism and Biological Actions of Coumarins. Progress in Medicinal Chemistry. 1974;10:85-158.
21. Lončar M, Jakovljević M, Šubarić D, Pavlić M, Buzjak Služek V, Cindrić I et al. Coumarins in Food and Methods of Their Determination. Foods. 2020;9(5):645.
22. Abdulraheem SS, Hadi MK. Synthesis and Characterization of New Coumarin Derivatives as Possible Antimicrobial Agents. International Journal of Drug Delivery Technology. 2021;11(4):1484-1490.
23. Kowalczyk P, Madej A, Paprocki D, Szymczak M, Ostaszewski R. Coumarin Derivatives as New Toxic Compounds to Selected K12, R1–R4 E. coli Strains. Materials. 2020;13(11):2499.
24. Woehrlin F, Fry H, Abraham K, Preiss-Weigert A. Quantification of Flavoring Constituents in Cinnamon: High Variation of Coumarin in Cassia Bark from the German Retail Market and in Authentic Samples from Indonesia. Journal of Agricultural and Food Chemistry. 2010;58(19):10568-10575.
25. Sanders M. Probiotics: Definition, Sources, Selection, and Uses. Clinical Infectious Diseases. 2008;46(s2):S58-S61.
26. Behnsen J, Deriu E, Sassone-Corsi M, Raffatellu M. Probiotics: Properties, Examples, and Specific Applications. Cold Spring Harbor Perspectives in Medicine. 2013;3(3):a010074-a010074.
27. Georgieva M, Andonova L, Peikova L, Zlatkov A. Probiotics – Health Benefits, Classification, Quality Assurance And Quality Control – Review. Pharmacia. 2014;61(4).
28. Holzapfel W, Haberer P, Geisen R, Björkroth J, Schillinger U. Taxonomy and important features of probiotic microorganisms in food and nutrition. The American Journal of Clinical Nutrition. 2001;73(2):365s-373s.
29. Snydman D. The Safety of Probiotics. Clinical Infectious Diseases. 2008;46(s2):S104-S111.

30. Doron S, Snydman D. Risk and Safety of Probiotics. Clinical Infectious Diseases. 2015;60(suppl_2):S129-S134.
31. Liong M. Safety of probiotics: translocation and infection. Nutrition Reviews. 2008;66(4):192-202.
32. Borriello S, Hammes W, Holzapfel W, Marteau P, Schrezenmeir J, Vaara M et al. Safety of Probiotics That Contain Lactobacilli or Bifidobacteria. Clinical Infectious Diseases. 2003;36(6):775-780.
33. Plaza-Diaz J, Ruiz-Ojeda F, Gil-Campos M, Gil A. Mechanisms of Action of Probiotics. Advances in Nutrition. 2019;10(suppl_1):S49-S66.
34. Ng S, Hart A, Kamm M, Stagg A, Knight S. Mechanisms of action of probiotics: Recent advances. Inflammatory Bowel Diseases. 2009;15(2):300-310.
35. van Zyl W, Deane S, Dicks L. Molecular insights into probiotic mechanisms of action employed against intestinal pathogenic bacteria. Gut Microbes. 2020;12(1):1831339.
36. Peng M, Salaheen S, Biswas D. Animal Health: Global Antibiotic Issues. Encyclopedia of Agriculture and Food Systems. 2014:346–57. doi: 10.1016/B978-0-444-52512-3.00187-X. Epub 2014 Aug 21. PMCID: PMC7151998.
37. Refeld A, Bogdanova A, Prazdnova E, Beskopylny A, Olshevskaya A, Maltseva T et al. Immunobiotics mechanisms of action and prospects of use in veterinary medicine. E3S Web of Conferences. 2020;210:06017.
38. Bomba A, Nemcová R, Mudroňová D, Guba P. The possibilities of potentiating the efficacy of probiotics. Trends in Food Science & Technology. 2002;13(4):121-126.
39. Reda A. Probiotics for the Control of Helminth Zoonosis. Journal of Veterinary Medicine. 2018;2018:1-9.
40. Musa H, Wu S, Zhu C, Seri H, Zhu G. The Potential Benefits of Probiotics in Animal Production and Health. Journal of Animal and Veterinary Advances. 2009;8(2):313-321.
41. Markowiak P, Śliżewska K. The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathogens. 2018;10(1).
42. Duskaev G, Rakhmatullin S, Kvan O. Effects of Bacillus cereus and coumarin on growth performance, blood biochemical parameters, and meat quality in broilers. November-2020. 2020;13(11):2484-2492.
43. Feng S, He X, Zhong P, Zhao J, Huang C, Hu Z. A metabolism-based synergy for total coumarin extract of radix *Angelicae dahuricae* and ligustrazine on migraine treatment in rats. Molecules. 2018;23(5):1004.
44. Duskaev G, Kvan O, Kosyan D, Rakhmatullin S, Levakhin G. Coumarin derivative and <i>Bacillus cereus</i> change live weight and cecal ecology in broilers. AIMS Agriculture and Food. 2021;6(1):360-380.
45. Stringlis I, Yu K, Feussner K, de Jonge R, Van Bentum S, Van Verk M et al. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. Proceedings of the National Academy of Sciences. 2018;115(22).
46. Kvan O, Rahmatullin S, Makaeva A, Sheida E, Duskaev G. Effect of Bacillus cereus IP 5832 and coumarin in the diet on the general state of the broilers. IOP Conference Series: Earth and Environmental Science. 2021;677(4):042074.