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Antibiotics and Probiotics in Animal Food

Impact and Regulation

Edited by Asghar Ali Kamboh

Antibiotics and Probiotics in Animal Food - Impact and Regulation

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IntechOpen Book Series

Veterinary Medicine and Science

Volume 13

Aims and Scope of the Series

Paralleling similar advances in the medical field, astounding advances occurred in Veterinary Medicine and Science in recent decades. These advances have helped foster better support for animal health, more humane animal production, and a better understanding of the physiology of endangered species to improve the assisted reproductive technologies or the pathogenesis of certain diseases, where animals can be used as models for human diseases (like cancer, degenerative diseases or fertility), and even as a guarantee of public health. Bridging Human, Animal, and Environmental health, the holistic and integrative "One Health" concept intimately associates the developments within those fields, projecting its advancements into practice. This book series aims to tackle various animal-related medicine and sciences fields, providing thematic volumes consisting of high-quality significant research directed to researchers and postgraduates. It aims to give us a glimpse into the new accomplishments in the Veterinary Medicine and Science field. By addressing hot topics in veterinary sciences, we aim to gather authoritative texts within each issue of this series, providing in-depth overviews and analysis for graduates, academics, and practitioners and foreseeing a deeper understanding of the subject. Forthcoming texts, written and edited by experienced researchers from both industry and academia, will also discuss scientific challenges faced today in Veterinary Medicine and Science. In brief, we hope that books in this series will provide accessible references for those interested or working in this field and encourage learning in a range of different topics.

Meet the Series Editor

Rita Payan Carreira earned her Veterinary Degree from the Faculty of Veterinary Medicine in Lisbon, Portugal, in 1985. She obtained her Ph.D. in Veterinary Sciences from the University of Trás-os-Montes e Alto Douro, Portugal. After almost 32 years of teaching at the University of Trás-os-Montes and Alto Douro, she recently moved to the University of Évora, Department of Veterinary Medicine, where she teaches in the field of Animal Reproduction and Clinics. Her primary research areas include the molecular markers of the endometrial cycle and the embryo–maternal interaction, including oxidative stress and the reproductive physiology and disorders of sexual development, besides the molecular determinants of male and female fertility. She often supervises students preparing their master's or doctoral theses. She is also a frequent referee for various journals.

Meet the Volume Editor

Dr. Asghar Ali Kamboh is an associate professor in the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture University, Pakistan. He obtained his Ph.D. in Veterinary Sciences from Nanjing Agricultural University, China, in 2012. He has 17 years of research experience in microbiology, particularly in the area of gut microbiota modulation for improved health, immunity, production, and meat quality in farm animals. Currently, his research interests are antimicrobial resistance, microbial contamination of animal foods, plant antioxidants, pathogens of animal origin, and modulation of immunity and gut microbiota via dietary manipulation in poultry and other farm animals. He has more than 100 publications in high-impact, peer-reviewed journals and has supervised more than twenty-five MPhil scholars. He has edited and authored numerous books and book chapters and participated in many international and national conferences and workshops. Dr. Kamboh is also an editor, editorial board member, and guest editor for many scholarly journals.

Contents

Preface **XV**

Chapter 1 **1**
Introductory Chapter: Antibiotics and Probiotics in Animal
Food – Impact and Regulation
by Asghar Ali Kamboh, Riaz Ahmed Leghari and Nazar Ali Korejo

Chapter 2 **7**
Phytochemicals as Alternatives to Antibiotics in Animal Production
by Ionela Hotea, Monica Dragomirescu, Adina Berbecea and Isidora Radulov

Chapter 3 **33**
A Review of Application Strategies and Efficacy of Probiotics in Pet Food
by Heather Acuff and Charles G. Aldrich

Chapter 4 **55**
Alternatives to the Use of Antibiotics in Animal Production
by Olusegun Oyebade Ikusika, Clyde Haruzivi and Thando Conference Mpendulo

Chapter 5 **67**
Potential Substitutes of Antibiotics for Swine and Poultry Production
by Ho Trung Thong, Le Nu Anh Thu and Ho Viet Duc

Chapter 6 **93**
Swine Production: Probiotics as an Alternative to the Use of Antibiotics
*by Alejandra Cardelle-Cobas, Lucía Coy-Girón, Alberto Cepeda
and Carolina Nebot*

Preface

Nowadays, consumers are cognizant of the quality of animal-origin foods. In developed countries, many people are foregoing buying animal foods that have been produced using antibiotics and/or other synthetic growth promoters. This is a major shift in consumer trends that has led to increased research on alternative growth promoters. In the last few decades, significant developments have been made in probiotics, making them potential unconventional growth promoters to obtain maximum production and reduce disease burden in various food-producing animals such as poultry, swine, and large and small ruminants. Moreover, attempts are being made to use probiotics in the diets of pet animals to improve their health status. This book presents comprehensive information on the use of probiotics as a substitute for in-feed antibiotics as well as their impact on consumers and farmers with reference to regulations. Thus, this book is a useful resource for a wide range of readers including nutritionists, researchers, progressive farmers, poultry/animal students, pet owners, farm managers, animal producers, and many others.

Chapter 1 summarizes the scope of the book and highlights the importance of removing antibiotics from animal production to safeguard the health and wellbeing of people.

Chapter 2 discusses substitutes for antimicrobials to use in organic farming. It focuses on phytoadditives such as cinnamon, thyme, papaya, onion, garlic, orange peel, green tea, ginseng, coriander, aloe vera, and others to modulate gut microbiota to improve health and production in poultry, pigs, and ruminants. The chapter highlights the strong potential of botanicals to kill harmful bacteria in the gut as well as boost the growth of helpful bacteria.

Chapter 3 reviews the importance and application strategies of probiotics in pet food and discusses the manufacturing process of pet foods and the challenges to keeping viable probiotic organisms in the food/food ingredients. It stresses the importance of strain selection to obtain desirable physiological characteristics (e.g., thermal resistance, acid/bile confrontation, and oxygen tolerance) and stabilization ability (e.g., encapsulation freeze drying, and sporulation) during various processing conditions (e.g., temperature, pressure, pH, moisture, etc.), application methods, packaging, and storage conditions.

Chapter 4 explores the replacement of antibiotic growth promoters (AGPs) with natural alternatives for sustainable animal production with improved consumer satisfaction. The chapter lists various options to use instead of AGPs, such as phytogenic compounds, probiotics, prebiotics, organic acids, enzymes, phage therapy, fossil shell flour, and antimicrobial peptides. The use of such alternative options in large-scale animal farming also has protective effects on the environment.

Chapter 5 describes more options to replace AGPs. It proposes the use of CRISPR-Cas9, a promising gene editing approach, as a practical option to control the prevalence of antibiotic resistance genes in bacterial populations as well as to eliminate pathogens with high precision. Moreover, the chapter suggests hyperimmune antibodies, bacteriophage therapy, synbiotics, essential oils, minerals, and recombination enzymes as useful options to replace in-feed antimicrobials.

Finally, Chapter 6 discusses various probiotic options for sustainable swine production, including *Bacillus subtilis*, *Clostridium butyricum*, *Lactobacillus acidophilus*, *Bacillus licheniformis*, *Enterococcus faecalis*, and *Saccharomyces cerevisiae*.

I would like to thank all my friends and family who supported me in this endeavor. I am grateful for the support of my wife who always encourages me to work hard for the propagation of science. I am also thankful to all the authors for their excellent chapters. Finally, I express my appreciation to the editorial staff of IntechOpen for their assistance throughout the publication of this book.

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Chapter 1

Introductory Chapter: Antibiotics and Probiotics in Animal Food – Impact and Regulation

Asghar Ali Kamboh, Riaz Ahmed Leghari and Nazar Ali Korejo

1. Introduction

In farm animals, use of antibiotics is very common since decades to control, treat, and prevent infections. The first antibiotic, i.e., penicillin, was discovered by Alexander Fleming in 1928 when he was working on Staphylococcus bacteria. Interestingly, some traces of tetracycline antibiotic were recognized in the guts of ancient mummies and skeletons from ancient Nubians (350–550 AD) showing that antibiotics were actually discovered by the peoples of old world [1]. Since discovery, penicillin was recognized as a marvel drug as it saved billions of human and animal lives. In the Second World War, penicillin was heavily used to treat troops that led to the start of antibiotic resistance due to emergence of resistant bacteria [2]. After world war, Thomas Jukes, a British-American biologist, revealed that in-feed antibiotics in poultry may help to improve performance of birds. This study makes a revolution in commercial farming and many antibiotics such as tetracycline, bacitracin, penicillin, etc., were adopted to use as growth promoters by adding a sub-therapeutic level in the feed [3]. Today, the annual business of antibiotic industry is about 25 billion USD [2].

It has been estimated that from 1961 to 2014, global meat consumption raised from 24 to 43 kg per capita. This happens due to industrialized farming practices that primarily use antibiotics to increase yields, control diseases, reduce labor costs, and contain economic risks for producers [3]. As a net result, antimicrobial resistance (AMR) is increasing day by day, and currently, it is a global threat that is recognized as a "ticking time bomb" by the researchers. Because, it is estimated that dissemination frequency of AMR and its environmental reservoirs may create the superbugs in the near future [4].

The history of probiotics is as old as the use of fermented foods in human diet. The word probiotic was first used by the Lilley and Stillwell in 1965. They adopted this term from a Greek word that means "for life." Since beginning, the word probiotic was adopted to express the microbial products (secretion) that have effect on the growth of other microorganisms. In 1974, Parker used and redefined it as "organisms and their substances which effects intestinal balance." Later in 1989, Fuller made some modifications in the definition and defined it as "live microorganisms that beneficially affects intestinal microbial balance" [5]. Currently, probiotics are used in

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humans, animals, poultry, and fish farming to optimize the gut health and to reduce the chances of disease occurrence. These are well-known alterative of antibiotic growth promoters [6].

2. Merits and demerits of antibiotics and probiotics use

There are plenty of evidences that clearly established that use of antimicrobials in farm animals for therapeutic and/or growth promotion causes the creation of antibiotic-resistant bacteria in the environment that ultimately deteriorate the therapeutic options in human medicine [7–9]. About, 7 million deaths in hospitals have been estimated due to antibiotic-resistant infection [10]. Antibiotics that are used in food-producing animals such as poultry led to transfer of resistant bacteria to human beings via animal food. In the human gut, these bacteria may further transfer resistant genes into the non-pathogenic commensal flora [11]. It is estimated that the antibiotics used in poultry are not completely metabolized in body tissues that accumulate in meat [12] and also excreted into the environment via poultry droppings [13]. The global consumption of antibiotics is around 100,000–200,000 tons per year. Among this about 46.1% use is for animals alone (with majority of use in commercial poultry). The summary of commonly used antibiotics in poultry industry is presented in **Table 1**.

When poultry droppings used as manure in agriculture fields, then these antimicrobials enter into the soil ecosystem and made significant alterations in the soil contagious communities [14]. Moreover, crops/vegetables cultivated in such fields when consumed by humans transmit antimicrobial-resistant genes to them [10, 15].

In 1981, the American Council for Agricultural Science and Technology published a report on the use of antibiotics in feed animals [16]. Though the report did not provide any data that use of antibiotics in animals causes the emergence of resistant microorganisms that may produce drug-resistant infections in human beings; however, it started a debate on the use of antibiotics in food animals [17]. In the

Antibiotic name	Class	Use in poultry*
Enrofloxacin, Sarafloxacin	Quinolones	Infections cure
Neomycin, Gentamicin	Aminoglycosides	Infections cure
Tylosin , Erythromycin	Macrolides	Infections cure
Penicillin	ß-Lactams	Infections cure, AGP
Lincomycin	Lincosamides	Infections cure, AGP
Bacitracin	Polypeptides	AGP
Monensin, bambermycin, semduramicin, salinomycin	Ionophores	AGP
Chlortetracycline, oxytetracycline, tetracycline	Tetracyclines	Infections cure

**AGP: Antibiotic growth promoter*
Adopted from Ref. [13].

Table 1.
Summary of commonly used antibiotics in poultry.

last decade of the twentieth century, several countries such as Sweden, Denmark, Namibia, and European Union Commission banned the use of antibiotics in food-producing animals and directed to adopted suitable alternative approaches to get optimal animal production [6].

By the start of the twenty-first century, pharmacists and nutritionists are trying to develop some alternatives to maintain or enhance farm animals' performance and well-being. Many substitutes were tested experimentally using in vivo and vitro approaches for their effectiveness in both animals and humans. Among those tested alternatives, one is probiotics [18, 19].

Probiotics could be defined as the live microorganisms that have useful effects on the host health when fed in suitable amount [20]. These were recognized as one of the best replacements due to their multiple useful aspects for both for humans and animals [21]. Probiotic can be used to decrease the dangerous bacteria and to increase the growth and production of animals by improving gut function [22]. Probiotics have also been recommended in mice for treatment of antibiotics-induced dysbiosis [23] and in humans [24]. Probiotics include the microorganisms of various species such as bacteria, yeast, and fungi. Some probiotics of bacterial origin (namely *Lactobacillus, Bifidobacterium, Streptococcus,* and *Bacillus subtilis*) also have antimicrobial effects against many pathogenic microbes such as *Staphylococcus aureus, E. coli, Salmonella typhimurium,* and *Clostridium perfringens,* etc. However, correct dose and proper selection of probiotics strains are important to get maximum health effects [25, 26].

3. Future perspective of antibiotics and probiotics use

It has been estimated that true survival and well-being of human being are hidden in chemical-free organic food production that is only possible via the use of good substitutes of synthetic growth promoters such as medicinal plants, prebiotics, probiotics, etc. [27]. Because the use of antibiotics in food-producing animals causes the creation of resistant microorganisms that disseminate to human beings via the food items (milk, meat, eggs, etc.), animal movements, food handlers and by other indirect mechanical means. Available data show that probiotics are good and feasible alternative of synthetic growth promoters to use in food-producing animals. These are known for their positive effects on GIT health. They protect the gut from pathogenic bacteria by producing selective antimicrobial substances and reducing toxin production and also enhance the digestion by stimulation of digestive enzyme synthesis. Probiotics help to restore gut mucosa, upregulate the intestinal motility, improve mucous production, and modulate the host innate immunity by stimulation of Th1 and Th2 immune components. Probiotics create cross-feeding between various bacterial strains of intestinal ecosystem and also reduce the blood cholesterol level via bile salt hydrolase action [28]. Keeping in view the available studies, probiotics could be used in the feed of animals to enhance their growth potential in combination of good animal husbandry practices. There is need of further studies concerning their mechanism of action, mode of delivery and to improve their in vivo efficacy.

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Chapter 2

Phytochemicals as Alternatives to Antibiotics in Animal Production

Ionela Hotea, Monica Dragomirescu, Adina Berbecea and Isidora Radulov

Abstract

Despite the continuous improvement of feed diets and recipes, animal health problems persist. For their treatment, antibiotics and chemotherapy have been shown to have side effects hard to control. The antibiotic residues in animal products may endanger human health. Since the antibiotics were restricted in animals' diets, which were previously used to keep under control digestive and respiratory pathologies, as well as allergies, so the researchers began to search for natural alternatives. Thus, it was developed the concept of phytoadditives, and these natural plant extracts are gaining ground in animal farming. Since then, more and more animal breeders and farms are willing to use various types of phytoadditives. This chapter aims to present the most widely used phytochemicals in animal nutrition, their effects on animal production and health, and to make some recommendations on the use of phytochemicals in farm animals' diets.

Keywords: phytochemicals, antibiotics, antimicrobial resistance, poultry, pigs, ruminants

1. Introduction

Antibiotics, since their discovery in the 1920s, have had a significant contribution to the economic growth of animal production. They were used as food supplements in sub-therapeutic doses in order to increase and make food conversion more efficient by preventing infections [1]. The antibiotics used as feed additives in the animal industry have contributed to the intensification of modern animal production. Starting with the intensification of animal husbandry, there is a constant concern regarding the large-scale use of food antibiotics that can lead to the development of the phenomenon of antimicrobial resistance. This represents a potential threat to human health [2, 3].

Due to the emergence of the phenomenon of antimicrobial resistance, the World Health Organization (WHO) established guidelines and recommendations to stop the use of antibiotics as growth promoters in 1997. One year later, in 1998, the EU banned the first phase for poultry, and the use of antibiotics as additives in their feed later in 2006, establishing a complete ban on the use of prophylactic antibiotics in the feed of all animals [4–6].

Consequently, various alternatives were sought to reduce the use of antibiotics in animal production, in order to maintain their health and performance. The types of additives available to increase animal productivity while maintaining the health of the human population include probiotics and prebiotics, plant extracts, essential oils, dietary fiber and enzymes, antimicrobial peptides, functional amino acids, hyperimmune antibodies from eggs, clays, and/or metals [2, 3, 7–10]. The optimal combinations of different compounds, together with good management and breeding practices, can be the key to intensifying the performance and productivity of animals with the aim of reducing and/or replacing antibiotics in the animal industry [3].

Phytochemicals have been used in the past to treat various ailments. Some compounds of plant origin, such as phenols, organosulfur compounds, terpenes, and/or aldehydes, have different properties: antimicrobial (antibacterial, antifungal, antiviral, and antiprotozoal), antioxidant, immunomodulatory, or mycotoxin detoxifying, as well as maintaining the integrity of the intestinal mucosa and maintaining the balance of the digestive microbiota [10–13]. Phytochemical substances are characterized by the fact that they have low residues, do not develop resistance or side effects, and can be used for prophylactic or therapeutic purposes against pathogenic bacteria. It has also been shown to act as functional additives by improving animal health and growth performance.

Phytochemical compounds have great potential as substitutes for classic antibiotics and can enter the structure of feed additives with a promising effect on animal production. Developing new classes of antibiotics around a phytochemical core may be the best solution to the growing antibiotic resistance crisis [10, 14].

2. The most common types of potentially pathogenic bacterial species

Several studies carried out in order to evaluate the antimicrobial activity of plants have demonstrated their effectiveness against different pathogens. The use of plant extracts aims to obtain natural additives with antimicrobial properties that could be used in the feed mixture, to determine the reduction of antibiotic consumption and the use of more natural diets for animals [15].

According to the European Food Safety Authority (EFSA) report in 2012 on zoonotic pathogens of food origin, *Campylobacter jejuni*, *Salmonella*, *Escherichia coli,* and *Listeria monocytogenes* have been described with increased incidence in animal flocks and raw animal products [16].

Campylobacter can be found in the intestinal tract of animals and in the oral cavity of humans, having the ability to cause disease in both hosts [17]. *Campylobacter* infection in human populations results from the handling or ingestion of undercooked poultry contaminated with this pathogen. In the United Kingdom, it is estimated that 80% of raw meat is contaminated with these bacteria [6, 18]. Thus, *C. jejuni* was the cause of the majority of confirmed zoonotic cases in humans in 2010, registering a significant increase in human campylobacteriosis reported by the European Union. The main reservoir for these zoonoses continues to be chicken meat, in the European Union 30% of fresh chicken meat units are positive for *Campylobacter*, with a variation between 3.1% and 90.0% [16].

Salmonella, the causative agent of the disease salmonellosis, is usually found in the intestinal tract of animals and humans, where it infects foods, such as poultry and eggs. Salmonellosis, as a disease transmitted through the food of animal origin, is known as a public health problem due to its high morbidity and mortality among humans [6]. Although in recent years, a reduction in salmonellosis cases has been observed, through good management of the control programs of this infection, salmonellosis still remains an important disease with an economic impact, by affecting the productive performance of animals and by making the human population sick due to consumption of contaminated eggs and meat [15, 19]. *Salmonella* is most often detected in the fresh carcass of broilers. In the European Union, the proportion of positive samples for *Salmonella* varies between 0.2% and 27.8%, with an average value of 1.2%. In humans, cases of *Salmonella enteritidis* disease are most commonly associated with the consumption of contaminated eggs and poultry meat, while *Salmonella typhimurium* cases are mostly associated with the consumption of contaminated meat from pork, poultry, and cattle [16].

E. coli (*E. coli*) is normally part of the natural intestinal microbiota of humans and animals, being the most dominant aerobic bacteria with 10^6–10^9 colony forming units (UFC) per cm of the intestine of poultry (chicken and turkey). This bacterium is one of the first species to colonize the human and animal intestine [6, 20]. Ingestion of animal foods containing antibiotic-resistant *E. coli* becomes a source of antimicrobial-resistant bacteria in the human gut, and this may affect the use of medicinal antibiotics or cause opportunistic diseases in the future [21]. In 2005, at the European level, 3314 cases of *E. coli* VTEC illnesses were reported, mainly associated with the consumption of fresh beef [22]. Therefore, there is a need for alternative control measures, such as the use of natural phytochemicals, that do not develop resistance [6].

The genus *Listeria* has 17 species, of which only two species are considered pathogenic, producing the disease called listeriosis. *L. monocytogenes* is considered pathogenic for humans and several animal species, while *Listeria ivanovii* is pathogenic, especially for ruminants and occasionally for humans [23, 24]. Due to the increased risk of infection with *L. monocytogenes* for the unborn, infants, and the elderly, it is considered one of the most important zoonotic agents with implications for food safety through the consumption of processed preparations of animal origin [25]. As a result of poor-quality control measures during food processing/handling and packaging, contamination with *L. monocytogenes* can occur, creating public health concerns, considering that 4.9% of pre-prepared animal products are contaminated with this bacterium [24–27].

One of the biggest challenges in the meat industry is keeping products safe without being contaminated with pathogens. Before slaughtering the animals, a number of measures are used to reduce the intestinal passage of pathogens, such as careful formulation of the diet regarding the macronutrient content, the use of antibiotics and additives that stimulate animal growth, phenolic antimicrobial compounds, organic acids, and acidifying products in animal feed, used on a large scale throughout the world [28]. It has been shown that some plants and their extracts stimulate the growth of certain bacteria, having a prebiotic effect. This effect, combined with the antimicrobial action of some extracts or essential oils, changes the intestinal microflora and reduces the microbial load by suppressing the proliferation of bacteria. There are some claims that some phytochemicals increase the turnover of the intestinal mucosa and prevent the attack of pathogenic bacteria by maintaining a healthier commensal population. In this context, it is very interesting to consider the use of natural plant extracts, essential oils, or some of their components as indispensable ingredients in the formulation of diets for animals, in order to reduce the excretion of pathogenic bacteria [6].

3. The most common plants with antimicrobial activity

The extensive, inappropriate, irregular, and indiscriminate use of antibiotics has led to the emergence of antimicrobial resistance [29]. Antibiotic resistance can lead to the inability to medically treat various infectious diseases [30, 31]. This situation is worrying and considered by the World Health Organization (WHO) as perhaps the most urgent problem facing medical science [32, 33]. Considering the lack of a new generation of antibacterials, as well as the increase in resistance of the existing generations of antibiotics, plants could represent a solution to this shortcoming [31].

According to the World Health Organization (WHO), there are more than 1340 plants with defined antimicrobial activity and more than 30,000 antimicrobial compounds that have been isolated from plants [32, 34]. Plants have the ability to develop secondary metabolites with various functions for the plant, such as a role in defending against pests, adapting to the environment, or providing the plant with a specific smell and taste. These compounds can be classified from a chemical point of view into three classes, recognized for their biological activity: terpenoids, phenolics, and alkaloids [31, 35, 36]. Thus, plants can represent an almost unlimited source of bioactive compounds and their use as antimicrobial agents can be exploited in different ways, considering that natural antimicrobial agents can act alone or in different combinations (**Table 1**) [37–39].

Currently, there is more and more research on the antimicrobial effect of plant extracts from different regions of the world. Most studies have analyzed a group of plants or even a single plant, regarding their effect on various infectious diseases in various species of animals, either with a curative or preventive effect. Further, the most common species of plants recognized as having antibacterial action are presented.

Echinacea purpurea is a plant from the daisy family, frequently used in traditional medicine for its multiple health benefits [40]. The genus *Echinacea* has medicinal value due to the contained chemical components [41]. The compounds can be isolated from the roots or aerial parts of plants and are mainly represented by volatile compounds, alkyl amides, polyphenols, caffeic acid derivatives, polysaccharides, alkaloids, and many other different structures [42–44]. Regarding the volatile compounds, the essential oils are considered as potential medicinal agents [44]. For *E. purpurea*, the main compounds found in the essential oils of leaves and roots include germacrene D (18.1% and 20.3%), naphthalene (7.8% and 6.4%), caryophyllene oxide (11.3% and 12.2%), α-phellandrene (6.9% and 6.6%), α-cadinol (9.1% and 5.9%), and caryophyllene (4.5% and 4%) [45]. It can be highlighted that the essential oils obtained from *E. purpurea* present a great variability of compounds in their chemical composition. However, the sesquiterpene germacrene D is the most abundant compound [44].

The medicinal importance of *Echinacea* derives from its antimicrobial properties against bacteria, fungi, and opportunistic diseases, so that it constitutes a valuable alternative to semisynthetic antibiotics. These properties are due to its ability to stimulate the immune system, producing more white blood cells. Echinacein, caffeic acid, and chicory are the components that produce this stimulation. It has also been proven its ability to stimulate the production of interferon, a protein that the body itself produces to neutralize viruses [46].

Echinacea has proven to be effective in treating various animal diseases. Some pathologies respond to *Echinacea* treatment, either through the direct antiviral or antibacterial effect, or through the anti-inflammatory effect. In addition, some organisms, especially bacteria, such as *Salmonella* and *Campylobacter* species, can

Common name	Scientific name	Compound	Classic	Activity
Alfalfa	*Medicago sativa*	—		Gram-positive organisms
Allspice	*Dioica allspice*	Eugenol	Essential oil	General
Aloe	*Aloe barbadensis*, *Aloe vera*	Latex	Complex mixture	*Corynebacterium, Salmonella, Streptococcus*
Apple	*Malus sylvestris*	Phloretin	Flavonoid derivatives	General
Ashwagandha	*Withania somniferum*	Withaferin A	Lactones	Bacteria, fungi
Aveloz	*Euphorbia tirucalli*	—		*S. aureus*
Bael tree	*Aegle marmelos*	Essential oil	Terpenoid	Fungous
Pear conditioner	*Bites the charantia*	—		General
Barberry	*Berberis vulgaris*	Berberine	Alkaloid	Bacteria, protozoa
Basil	*Ocimum basilicum*	Essential oils	Terpenoids	*Salmonella*
Bay	*Laurus nobilis*	Essential oils	Terpenoids	Bacteria, fungi
Betel pepper	*Betel pepper*	Catechols, eugenol	Essential oils	General
Black pepper	*Piper nigrum*	Piperine	Alkaloid	Fungi, *E. coli Lactobacillus*
Blueberries	*Vaccinium* spp.	fructo	Monosaccharides	*E. coli*
Brazilian pepper tree	*Schinus terebinthifolius*	Terebinthone	Terpenoids	General
Buch	*Barosma setulina*	Essential oil	Terpenoid	General
Burdock	*Arctium lappa*		Polyacetylenes, tannins, terpenoids	Bacteria, fungi, viruses
Buttercup	*Ranunculus bulbosus*	Protoanemonin	Lactones	General
Carraway	*Carum carvi*		Coumarins	Bacteria, fungi, viruses
Cascara Sagrada	*Rhamnus purshiana*	Tannins	Polyphenols	Viruses, bacteria, fungi
Cashews	*Anacardium pulsatilla*	Salicylic acids	Polyphenols	*Propionibacterium acnes,* Bacteria, fungi
Castor bean	*Ricinus communis*	—		General
Ceylon cinnamon	*Cinnamomum verum*	Essential oils, others	Terpenoids, tannins	General
Chamomile	*Matricaria chamomilla*	Anthemic acid	Phenolic acid	*Mycobacterium tuberculosis, S. aureus, Salmonella typhi*
		—	Coumarins	Viruses

Common name	Scientific name	Compound	Classic	Activity
Chaparral	*Larrea tridentata*	Nordihydroguaiaretic acid	Lignans	Skin bacteria
Chili peppers, paprika	*Capsicum annuum*	Capsaicin	Terpenoid	Bacteria
Cloves	*Syzygium aromaticum*	Eugenol	Terpenoid	General
Dough	*Erythroxylum coca*	Cocaine	Alkaloid	Bacteria
Cockles	*Agrostemma githago*	—		General
Coltsfoot	*Tussilago farfara*	—		General
Coriander, cilantro	*Coriandrum sativum*	—		Bacteria, fungi
Cranberries	*Vaccinium* spp.	Fructo	Monosaccharides	Bacteria
Dandelions	*Taraxacum officinale*	—		*C. albicans, Saccharomyces cerevisiae*
Dill	*Anethum graveolens*	Essential oil	Terpenoid	Bacteria
Echinacea	*Echinaceae angustifolia, E. purpurea*	—		General
Eucalyptus	*Eucalyptus globulus*	Tannin	Polyphenol	Bacteria, viruses
		—	Terpenoid	
fava bean	*Faba bean*	Fabian	Thionin	Bacteria
Gamboge	*Garcinia hanburyi*		Resin	General
Garlic	*Allium sativum*	Allicin, ajoene	Sulfoxides	General
			Sulfated terpenoids	
Ginseng	*Panax notoginseng*		Saponin	*E. coli, Sporothrix schenckii*, *Staphylococcus*,
Glory lily	*Glorious gorgeous*	Colchicine	Alkaloid	General
Goldenseal	*Hydrastis canadensis*	Berberine, hydrastine	Alkaloids	Bacteria, *Giardia duodenale,* trypanosomes, Plasmodia
gotu kola	*Centella asiatica*	Asiatocosides	Terpenoid	*Mycobacterium leprae*
Grapefruit peel	*Citrus paradise*		Terpenoid	Fungous
Green tea	*Camellia sinensis*	catechins	Flavonoids	General, *Shigella, Vibrio, S. mutans, Viruses*
Harmel, rue	*Peganum harmala*	—		Bacteria, fungi
Hemp	*Cannabis sativa*	β-Resercyclic acid	Organic acid	Bacteria and viruses
Henn	*Lawsonia inermis*	Gallic acid	Phenolic	*S. aureus*

Common name	Scientific name	Compound	Classic	Activity
Whoops	*Humulus lupulus*	Lupulonehumulone	Phenolic acids	General
		—	(Hemi)terpenoids	
Horseradish	*Rustic armor*		Terpenoids	General
Hyssopi	*Hyssopus officinalis*	—	Terpenoids	Viruses
(Japanese) herb	*Rabdosia trichocarpa*	Trichorabdal A	Terpenes	*Helicobacter pylori*
Lantana	*Lantana chamber*	—		General
—	*L.*	Lawson	Quinones	*M. tuberculosis*
Lavender-cotton	*Santolina chamae cyparissus*	—		Gram-positive bacteria, *Candida*
Lemon balm	*Melissa officinalis*	Tannins	Polyphenols	Viruses
Lemon verbena	*Aloysia triphylla*	Essential oil	Terpenoid	*E. coli, M. tuberculosis, S. aureus, Ascaris*
Licorice	*Glycyrrhiza glabra*	Glabrol	Phenolic alcohol	*S. aureus, M. tuberculosis*
Lucky nut, yellow	*Thevetia peruviana*	—		*Plasmodium*
Poppy, nutmeg	*Myristica fragrans*	—		General
Marigold	*Calendula officinalis*	—		Bacteria
Mesquite	*Prosopis juliflora*	—		General
Mountain tobacco	*Arnica montana*	Helanins	Lactones	General
Oak	*Quercus rubra*	Tannins	Polyphenols	General
		Quercetin	Flavonoids	
Olive oil	*Olea europaea*	Hexanal	Aldehydes	General
Onion	*Allium onion*	Allicin	Sulfoxides	Bacteria, *Candida*
Orange peel	*Citrus sinensis*	—	Terpenoid	Fungous
Oregon harrows	*Mahonia aquifolia*	Berberine	Alkaloid	*Plasmodium*, Trypanosomes, general
Pao d'arco	*Tabebuia*	Sesquiterpenes	Terpenoids	Fungous
Papaya	*Carica papaya*	Latex	Mix of terpenoids, organic acids, alkaloids	General
Pasque-flower	*Anemone pulsatilla*	Anemonins	Lactones	Bacteria
Peppermint	*Peppermint*	Menthol	Terpenoid	General
Periwinkle	*Vinca minor*	Reserpines	Alkaloid	General
Peyote	*Lophophora williamsii*	Mescaline	Alkaloid	General
The poinsettia	*Euphorbia pulcherrima*	—		General

Common name	Scientific name	Compound	Classic	Activity
Poppy	*Papaver somniferum*	Opium	Alkaloids and others	General
Potato	*Solanum tuberosum*	—		Bacteria, fungi
Prostrate knotweed	*Polygonum aviculare*	—		General
Purple prairie clover	*Petalostemum*	Petalostemumol	Flavonoids	Bacteria, fungi
Quinine	*Cinchona* sp.	Quinine	Alkaloid	*Plasmodium* spp.
Rauvolfia, chandra	*Rauvolfia serpentina*	Reserpines	Alkaloid	General
Rosemary	*Rosmarinus officinalis*	Essential oil	Terpenoid	General
Sainfoin	*Onobrychis viciifolia*	Tannins	Polyphenols	Ruminal bacteria
Sassafras	*Sassafras albidum*	—		Helminths
Savory	*Mountain saturation*	Carvacrol	Terpenoid	General
Senna	*Cassia angustifolia*	Rhein	Anthraquinone	*S. aureus*
Smooth hydrangea, seven barks	*Hydrangea arborescens*	—		General
Snake plant	*Rivea corymbosa*	—		General
St. John's wort	*Hypericum perforatum*	Hypericin, others	Anthraquinone	General
Sweet flag, calamus	*Acorus calamus*	—		Enteric bacteria
Tansy	*Tanacetum vulgare*	Essential oils	Terpenoid	Helminths, bacteria
Tarragon	*Artemisia dracunculus*	Caffeic acids, tannins	Terpenoid, Polyphenols	Viruses, helminths
Thyme	*Thymus vulgaris*	Caffeic acid	Terpenoid	Viruses, bacteria, fungi
		Thymol	Phenolic alcohol	
		Tannins	Polyphenols	
		—	Flavones	
Tree bard	*Podocarpus nagi*	Totarol	Flavonoids	*P. acnes*, other gram-positive bacteria
		Nagilactone	Lactones	Fungous
Tua-Tua	*Jatropha gossyphiifolia*	—		General
Turmeric	*Curcuma longa*	Curcumin	Terpenoids	Bacteria, protozoa
		Turmeric oil		
Valerian	*Valeriana officinalis*	Essential oil	Terpenoid	General

Common name	Scientific name	Compound	Classic	Activity
Willow	*Salix alba*	Salicin	Phenolic glucosides	General
		Tannins	Polyphenols	
		Essential oil	Terpenoid	
Wintergreen	*Gaultheria procumbens*	Tannins	Polyphenols	General
Woodruff	*Gallium odoratum*	—	Coumarin	General, Viruses
Yarrow	*Achillea millefolium*	—		Viruses, helminths
Yellow dock	*Rumex crispus*	—		*E. coli, Salmonella, Staphylococcus*

Selection of data from reference [39].

Table 1.
Plants with antimicrobial activity.

also be important sources of infection for humans through contaminated food. Some researchers, through numerous published studies, have emphasized the importance of evaluating herbal preparations as substitutes for antibiotics that are frequently used in farm animals [46–48]. *Echinacea* extracts have a modern tradition of veterinary applications [49, 50] existing studies similar to those described for human pathologies, or even controlled studies in animals. Thus, it was concluded that *Echinacea* treatments are safe and free of significant side effects. This conclusion is also supported by studies in mice and rats in which no toxic effects were observed [46, 51]. In addition to controlling infections in animals, herbal preparations have also proven their effectiveness in stimulating immunity, supporting growth, and improving performance [46, 52].

Ginger, the rhizome of *Zingiber officinale*, frequently used as a spice is also used to cure various diseases [53]. It plays an important role in cancer prevention by inactivating and/or activating different molecular pathways. Different studies highlight the therapeutic role of ginger in the management of infectious diseases by modulating biological activities, through anti-inflammatory and antioxidant activities [54]. Ginger contains many active ingredients, including terpenes and oleoresin, included in the generic name of ginger oil. Ginger also contains volatile oils of approximately 1–3% and non-volatile components with a pungent smell and taste—oleoresin [55]. The major components identified from the terpene category are sesquiterpene hydrocarbons and phenolic compounds, such as gingerol and shogaol. Also, lipophilic extracts of rhizomes have been isolated, with the production of potentially active gingerol, which can be converted into shogaol, zingerone, and paradol [54].

Previous research has shown that ginger and its compounds play a vital role in preventing microbial growth or acting as an antimicrobial product. The studies carried out support the antimicrobial activity of ginger against *E. coli*, *Salmonella typhi*, and *Bacillus subtilis*. It has also been proven that ginger also has antifungal properties, the ethanolic extract from a ginger powder having a pronounced inhibitory action against *Candida albicans* [54, 56, 57]. The main constituents, such as gingerol, showed antibacterial activity against oral bacteria, proving to be an active inhibitor for *Mycobacterium avium* and *Mycobacterium tuberculosis* [54, 58, 59].

There are studies on the use of natural extracts based on ginger and its derivatives in animal feed, as feed additives for their effects on growth performance, production quality, health as well as economic efficiency [60, 61]. The ginger essential oil has proven strong antimicrobial action against most pathogenic microorganisms, bacterial (*Staphylococcus aureus*, *E. coli,* and *Pseudomonas aeruginosa*) and fungal (*Aspergillus niger* and *C. albicans*) [62]. Thus, ginger and its compounds can be considered harmless because they do not present acute toxicological side effects. According to several studies, it can be concluded that feed supplements based on ginger positively influence animal growth and carcass development, with a reduction in the amount of abdominal fat. It can also be emphasized that food supplements with ginger have a positive influence on immune and antioxidant function in animals [60, 61].

Oregano (*Origanum vulgare* subsp. *hirtum*) is a plant widely used in cooking, as an aromatic plant, and also frequently used in traditional medicine. The chemical analysis of oregano essential oil highlighted the presence of several ingredients, most of them proving important antioxidant and antimicrobial properties [63]. Carvacrol and thymol, the two main phenols that make up about 78–85% of oregano essential oil, are mainly responsible for antimicrobial activity. Other minor constituents, such as the monoterpene hydrocarbons γ-terpinene and p-cymene, further contribute to the antibacterial activity of the oil [64]. In the scientific literature, there are many publications related to the chemical composition and antimicrobial properties of the essential oil obtained from different species of oregano and their use in different commercial preparations as antibiotics and antioxidants [65–67].

The different species of oregano are one of the most studied herbs used for their antimicrobial activity- antibacterial, antifungal, and antiviral. Among the activities and applications of oregano essential oil reported in the livestock industry and meat production are antioxidant, preservative, antimicrobial, and anticoccidial effects, as well as improving the production of digestive enzymes, stimulating digestion and blood circulation, and improving immune status [68–70]. The improvement of feed utilization efficiency and animal rearing performance could be determined by changes in intestinal morphology, such as the increase in the height of intestinal villi or the intensification of enzyme activity, with the improvement of protein digestibility due to the intervention of chymotrypsin and by the prevention of parasitosis [70–72]. Thus, it can be concluded that oregano essential oil used as a feed additive has beneficial effects on animal health and production.

Rosmarinus officinalis, L. is an aromatic plant with a unique taste and aroma, recognized for its antioxidant properties. Rosemary extracts have been used in the treatment of various diseases due to their hepatoprotective, antiangiogenic effect, or as a curative treatment in Alzheimer's disease [73, 74]. On the other hand, it can be used in food preservation, preventing oxidation and microbial contamination, thus being a potential substitute for reducing synthetic antioxidants in food [75, 76]. EFSA (European Food Safety Authority) analyzed the safety of rosemary extracts [77]. It was concluded that it can be used in considerable amounts, ranging from 0.09 (elderly) to 0.81 (children) mg/kg per day of carnosol and carnosic acid. Currently, in the European Union, rosemary extracts are added to foods and beverages at levels up to 400 mg/kg (as the sum of carnosic acid and carnosol) [78].

R. officinalis is a rich source of phenolic compounds, and their properties are derived from its extracts and essential oils. The polyphenolic profile of this plant is characterized by the presence of carnosic acid, carnosol, rosmarinic acid, and hesperidin as major components. Rosemary essential oil contains mainly 1,8-cineole

(46.4%), camphor (11.4%), and α-pinene (11.0%) [78, 79]. Thus, rosemary oil, thanks to its phytochemical compounds (mainly caffeic acid, rosmarinic acid, and carnosic acid) has antibacterial, antifungal, and antioxidant properties. To support these bioactivities, there are studies that have demonstrated the antibacterial activity of rosemary oil against *E. coli*, *Bacillus cereus*, *S. aureus*, *Clostridium perfringens*, *Aeromonas hydrophila,* and *Salmonella choleraesuis* [78].

Animal studies have proven that rosemary used in smaller amounts in the feed mixture has beneficial effects on the gastrointestinal microbiota ecosystem. Another hypothesis is that the beneficial effects of essential oils result not only from their antimicrobial properties but also from their interference with digestive and absorption processes and with the immune system, improving the productive performance of animals and the state of health, in general [80]. In cows, rosemary leaves can be used to modulate the rumen microbiome and its function, being able to influence the abundance of rumen microbial populations responsible for protein and fiber degradation, and influencing methane and ammonia production [81]. In general, it can be concluded that rosemary extracts and essential oil can be used with confidence as feed additives, as a result of their multiple bioactivities with a favorable influence on production, product quality, and animal quality of life.

Thyme, a species of the genus *Thymus*, is an aromatic and medicinal plant, which includes two representative species *Thymus serpyllum* (wild thyme) and *T. vulgaris* (common thyme) [82]. The essential oil of *T. vulgaris* contains up to 30 monoterpenes, having a different chemical composition of the oils, depending on the area of origin of the plants. Thyme oil is of great commercial interest, being in the top 10 oils worldwide, used as a natural food preservative and aromatic additive to a wide variety of foods and beverages. It has considerable antioxidant, antibacterial, and antifungal effects, and is used as a flavoring in personal care products (soaps, cosmetics, perfumes, etc.) [83, 84].

Thyme essential oil has remarkable antibacterial effects associated with the presence of phenolic components, carvacrol, and thymol. Being rich in phenolic substances, thyme essential oil has the ability to modify both the permeability and the function of cell membrane proteins by penetrating the phospholipid layer of the bacterial cell wall, binding to the proteins, and blocking their normal activity. Due to the variety of molecules in thyme extracts, the antimicrobial activity cannot be attributed to a single mechanism, but to a number of diverse actions at different sites of the bacterial cell components, thus affecting the functions of the cell membrane, cytoplasm, enzymes, fatty acids, proteins, ions, and metabolites. Thus, this essential oil has been shown to have strong bacteriostatic and bactericidal effects against *C. jejuni*, *E. coli*, *S. enteritidis*, *L. monocytogenes,* and *S. aureus* [82].

The composition of thyme essential oil leads to antiseptic, antibacterial, antifungal, antioxidant properties and antimicrobial, anticoccidial, and anti-inflammatory actions in animals as well. Thyme essential oil has been shown to increase the production of digestive enzymes, which in turn improve the digestion of nutrients. This will result in increased weight gain, feed intake, and a better feed conversion ratio [85]. In numerous studies, thyme oil has proven its antibacterial effect, even for multidrug-resistant strains of *Salmonella*, *E. coli*, *Listeria,* or *Campylobacter* [86, 87]. The treatment with thymol oil determines the improvement of the general condition of the animals, through the bacterial balance established at the intestinal level. This fact leads to the obtaining of healthy animal products intended for human consumption.

Therefore, due to an increased demand to develop natural antimicrobial products capable of replacing classic antibiotics and not developing resistance, phytochemical extracts are gaining more and more ground. Thus, researchers are increasingly concerned with isolating and identifying new bioactive chemical compounds from plants to solve the problem of microbial resistance. Currently, approximately 50% of pharmaceutical and nutraceutical preparations are natural compounds and their derivatives [88]. Medicinal plants are an almost unlimited source of bioactive substances, and their capacity as antimicrobial agents can be exploited in different ways [29].

4. The use of plant additives in animals

Phytochemical substances are also called phytobiotic or phytogenic. These are natural bioactive compounds derived from plants and administered in animal feed to increase productivity. Natural alternatives to antibiotics should have the same beneficial effects on growth performance, ensure optimal production, and increase nutrient availability by improving the feed conversion rate based on the modulation of the gut microbiome and immunity [2]. The main bioactive compounds of phytochemicals are polyphenols, and their composition and concentration vary depending on the plant species, plant parts, geographical origin, harvesting season, and environmental factors [2, 3].

Recently, phytochemicals are increasingly used as natural growth promoters in the livestock industry. There are numerous studies that have tested a wide variety of essential oils or plant extracts from different herbs and spices in the diets of farm animals, ruminants, pigs, and poultry, proving a concrete improvement in health by developing innate immunity and reducing the effects negative effects of enteric pathogens, as well as a constant improvement of feed utilization efficiency and animal growth and production performance [89–92].

The mechanism of action of phytochemical substances is very diverse, depending on the concentration of active substances in the finished product used. Their beneficial effects are mainly attributed to their antimicrobial and antioxidant action. By including phytochemical substances in animal diets, the intestinal microbial population is modified and stabilized and the amount of potentially toxic microbial metabolites in the intestines is reduced. Also, due to their direct antimicrobial properties, including against various species of pathogenic bacteria, intestinal stress is reduced, as well as immune stress, thus improving animal performance [93]. Another important benefit of the use of phytoadditives in the current diet is the reduction of oxidative stress, and implicitly, the increase of antioxidant activity at the tissue level, which determines a significant improvement in health status [94]. Phytochemical substances show, including immunomodulatory action, through the rapid proliferation of immune cells, the development of antibody production, and the modulation of cytokines [3, 89, 93].

4.1 The use of phytoadditives in poultry

Until recently, in the poultry industry, enteric diseases, such as necrotic enteritis or coccidiosis, were traditionally controlled with classical antibiotics introduced into animal feed. As a result of the regulation of the use of natural growth promoters, the control of these diseases requires new prevention and treatment strategies with alternative natural sources without antibiotics. A growing number of scientific publications have emphasized the fact that the most important health-supporting action of phytoadditives is represented by their ability to improve the host's defense possibilities against microbial infections [3].

A wide variety of herbs, such as thyme, oregano, rosemary, marjoram, oregano, garlic, ginger, green tea, black cumin, coriander, or cinnamon, have been used in poultry as alternative solutions to stimulate growth. Various other essential oils, such as thymol, carvacrol, eugenol or coriander, garlic, ginger, star anise, cumin, basil, rosemary, turmeric, lemon, and sage, have been used either individually or in mixtures to improve the health and performance of animal husbandry [2]. Also, the use of a mixture based on thymol, cinnamaldehyde, and star anise essential oil improved body weight gain in broilers and improved feed utilization efficiency by improving feed conversion rate [89, 90].

Another method of maintaining health in poultry is represented by the ability of phytochemicals to increase the host's resistance to enteric diseases of various etiologies. An example of such phytoadditives is a mixture of phytonutrients containing carvacrol, cinnamaldehyde, and capsicum, which is the first commercial phytochemical product approved by the EU for use in animal feed. Research that used this product proved a development of innate immunity, and implicitly, an increase in resistance to the actions of enteric pathogens, resulting in a visible improvement in growth performance in broilers, including by improving the efficiency of feed use, nutrient conversion rate, and mortality reduction [2, 89, 92]. Moreover, the phytochemical substances in Hooker chives determined the amplification of the intestinal barrier function, by increasing the expression of proteins at the level of the intestinal mucosa in broiler chickens fed with lipopolysaccharides [95].

Regarding the ability of medicinal plants to activate the immune system, some extracts of dandelion, mustard, and safflower determined the stimulation of innate immunity and the inhibition of the growth of tumor cells in the tested poultry [3]. In another study, it was observed that the most important genetic effect induced by the use of cinnamaldehyde in poultry feed is correlated with the presence of the antigen and the developed humoral immunity, as well as the developed anti-inflammatory response in the case of enteric diseases [96].

The combination of several phytochemicals develops synergistic effects to counteract the negative consequences of enteric infections. The addition of a mixture of capsicum, lentinus, and curcuma to the broiler diet led to a better body mass gain, an increase in the production of serum antibody titers against profilin, as well as a reduction in the number of oocysts eliminated through feces in infected poultry with *E. acervulina*, compared to chicks, fed the control diet [97]. Detailed research on the effects of carvacrol, cinnamaldehyde, and capsicum extract highlighted a regulation of the expression of genes associated with the immunological, physiological, and metabolic status of the investigated chickens [98].

Many studies have demonstrated the beneficial consequences of phytochemicals in preventing diseases or improving the immune response, but few have analyzed the mechanisms underlying these effects. Some phytochemicals inhibit the innate immune response by targeting effects on pathogen pattern recognition receptors or their later developed signaling molecules [3]. In this context, future studies are needed to present the molecular and cellular mode of action of phytochemical substances for the control of diseases in industrial growth.

4.2 The use of phytoadditives in pigs

The weaning period is one of the most difficult and critical stages in the industrial breeding of pigs. The manifestation of its effects depends on several factors, including animal behavior, environmental factors, disease states, immune status, and nutritional balance. During this vulnerable period, the piglets are subjected to an accumulation of stress factors that result in health imbalances, with diarrheal manifestations, which can lead, in a short time, to the death of individuals [99]. In this context, numerous researchers have tried to highlight the beneficial effects of using phytochemical supplements in the feed of weaning pigs. Various studies in pigs have shown that phytochemicals improve intestinal health. The use of a mixture of phytochemical compounds containing carvacrol, cinnamon, and capsicum resulted in the identification of an increase in the amount of stomach contents, which suggests an increased gastric retention time, also obtaining an increased *Lactobacillus*: *Enterobacteria* ratio [3].

During the weaning period, diarrhea produced by *E. coli* is a frequent cause of death in pigs. This frequent pathology causes significant economic losses due to increased morbidity, decreased growth rate, drug treatment costs, and as the case may be, recorded mortality. *E. coli* enterotoxigenic variant is the most dominant and pathogenic type of *E. coli* that causes this type of diarrheal pathologies in piglets during weaning and after weaning [100]. Various phytochemical compounds, including capsicum, turmeric, or garlic extract, were tested in studies of infection with pathogenic *E. coli* in order to evaluate the beneficial effects in improving diarrhea and maintaining intestinal health in weaned pigs [101]. Studies have shown that supplementation with phytochemicals reduced the frequency of diarrhea in pigs, which underlines the fact that the inclusion of phytochemical extracts in pig diets increases the animals' disease resistance. Supplementation with phytochemicals also improved microflora balance and intestinal health, which indicated a reduced score of diarrheal diseases. Also, research on this topic indicates that the inclusion of low doses of phytochemicals in food reduces both systemic and local inflammation caused by *E. coli* infection. Other research on the most common viral infections encountered in pigs has shown that the inclusion of phytoadditives in the daily feed improves the immune response, reduces the viral load, and serum concentrations of inflammatory mediator factors, and decreases the duration of fever in infected individuals [102].

In conclusion, phytochemicals are the ideal compounds to replace antibiotics in order to obtain better health and growth performance in pigs. The potential positive effects of phytochemical extracts may differ due to a very varied chemical composition of the types of plant extracts. This situation requires the selection of suitable phytoadditives according to the purpose for which we want to use them and for the function we want them to fulfill in the body, as alternative sources to classic antibiotics, in the intensive breeding of pigs.

4.3 The use of phytoadditives in ruminants

In ruminants, host and ruminal microorganisms establish a symbiotic relationship through which the animal provides nutrients and fermentation processes suitable for the survival of the microbial population, and the microorganisms synthesize microbial proteins and degrade fibers as protein and energy sources for the host. Volatile fatty acids, resulting from the fermentation of carbohydrates, represent the key element in maintaining the microbial balance at the ruminal level. The possibilities of manipulating the proportions of volatile fatty acids through the use of phytochemical compounds ensure the ruminal health of cows and certify the increase in production for these species [103]. Protein degradation is important to ensuring the nitrogen requirement for the growth and development of the ruminal microbial population. When ammoniacal nitrogen is in excess, it is absorbed through the ruminal wall, converted into urea in the liver, and then excreted through urine. In general, in intensive production systems, as a result of nutritional imbalances, ammoniacal nitrogen in the rumen is produced in excess of the capacity of microorganisms to use it. This results in high production costs and an increase in the amount of nitrogen released into the environment. Therefore, the control of proteolysis, peptidolysis, and deamination are considered elements of interest regarding the modulation of ruminal fermentation [104].

Improving the efficiency of digestion processes in ruminants proves to be the best strategy for developing animal production performance. Therefore, the industry is looking for alternative feeding strategies and/or natural additives that allow to maintain or improve the production level without increasing the cost. Phytochemical substances from plants, including the diet, have the possibility to modify the nutritional value of feed by modulating the digestibility of nutrients in the digestive tract or by improving systemic metabolism. Those phytonutrients that have a strong antimicrobial activity and could cause imbalances in the ruminal microflora should be avoided. Research on alternative sources to antibiotics used as cattle feed supplements needs to be developed based on the use of phytochemical molecules and doses that induce only minor changes in microbial metabolism, but improve their growth rate, resulting in the improvement of the profile of fermentation [103, 104].

If studies on ruminal microbial vitality and action under the effect of phytoadditives have been intensively studied, there are less data on the effects of phytochemicals on productive performance in cows. Cinnamaldehyde supplementation and/or in combination with eugenol can improve milk production in cows, even if the increases are not significant [105]. On the other hand, the capsicum extract has the ability to modulate the immune function in animals by increasing the number of neutrophils and decreasing the lymphocytes when cattle receive their feed capsicum supplements with ruminal protection. In these cases, significant increases in milk production are also recorded, through the influence on carbohydrate metabolism and the redirection of glucose to the mammary gland [106]. This very interesting new application of phytochemical additives presents an opportunity to improve production, not only by reducing the use of classical antibiotics, but also by offering an alternative to the use of synthetic hormones.

These findings show the importance of the ability to establish clear objectives in the identification of alternative natural sources as growth promoters, through the identification of phytoadditives that can maintain the normal functioning of the rumen without affecting the decomposition of nutrients, the balance of the ruminal microbial population or the production of cows.

Finally, it can be emphasized that, although in human medicine, chemical substances derived from plants with strong medicinal properties are frequently used in various clinical studies for the treatment of a wide variety of diseases in humans and in veterinary medicine, research on the beneficial effects of phytochemicals on animal diseases are becoming increasingly widespread, many researchers being more and more interested in testing these substances [2, 3].

5. Conclusions

The antimicrobial activity of plant extracts represents a new hope for combating the danger of establishing the phenomenon of antimicrobial resistance. Through the phytochemical compounds that the plant products contain, they have the ability to fight against microbial agents, through the bactericidal or bacteriostatic action they exert, being also supported by the fact that they do not develop antimicrobial resistance. Phytochemical substances as alternative sources to antibiotics have been intensively studied and seem to be a promising solution due to the beneficial effects on animals and the possibility of eliminating the phenomenon of antibiotic resistance. It must be taken into account, however, that in some cases the effectiveness of phytochemical compounds has only been tested experimentally, outside the real conditions of raising animals, from intensive farms. Thus, it is considered that for the objective evaluation of plant extracts and to be able to take into account the recommendations to be used as phytoadditives, it would be necessary to select those researches carried out under farm conditions, repeated and tested by several authors and which certify close results. In this sense, the testing of phytoadditives should be supported by the management of intensive animal breeding farms in such a way that their practical applicability highlights concrete results. Also, the acceptance of the research results by the competent authorities and the development of a legal basis for use, according to a standardized method, would be imperatively necessary.

Plant extracts have proven great efficiency in supporting growth processes, intensifying productions, preventing illnesses, or treating various pathologies. But, in this continuous mediatization process of increasing the use of phytoadditives, the chemical characteristics of plant compounds and their mode of action, individual or synergistic, must be taken into account. In this sense, animal breeders are recommended to inform themselves very well or to request the advice of specialists before taking the decision to include some phytoadditives in animal diets, especially those with antimicrobial action. A lack of training can cause negative effects on animal health or production, which can also include an economic decline.

In this context, we recommend the use of phytochemicals as feed additives in animal feed, in order to replace antibiotics, eliminate antimicrobial resistance, intensify production, preserve animal welfare and protect animal and human health, after a rigorous analysis of the farm's needs and the expected effects.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

A Review of Application Strategies and Efficacy of Probiotics in Pet Food

Heather Acuff and Charles G. Aldrich

Abstract

In companion animal nutrition, probiotics (direct-fed microbials) are marketed as functional ingredients that add value to pet foods due to the impact they have on gastrointestinal and immune health of dogs and cats. The nature of the beneficial effect each probiotic strain exerts depends on its metabolic properties and perhaps most importantly, the arrival of a sufficient number of viable cells to the large bowel of the host. Pet food manufacturing processes are designed to improve food safety and prolong shelf-life, which is counterproductive to the survival of direct-fed microbials. Therefore, a prerequisite for the effective formulation of pet foods with probiotics is an understanding of the conditions each beneficial bacterial strain needs to survive. The aims of this chapter are: (1) To summarize the inherent characteristics of probiotic strains used in commercial pet foods, and (2) To review recently published literature on the applications of probiotics to pet foods and their associated challenges to viability.

Keywords: probiotics, viability, pet food, commercial processing, formulation

1. Introduction

Recent U.S. pet ownership statistics estimate that 70% of U.S. households own at least one pet, accounting for nearly 90.5 million homes [1]. Collectively in 2021, Americans invested $123.6 billion in their pets by purchasing pet foods, veterinary care, supplies, and non-medical pet care services, a clear indication that pets have become highly valued members of society. Over the past two centuries, the societal role of dogs has evolved from predominantly labor (i.e., guardianship, transportation, herding, and hunting), to a range of special operations (i.e., rescue, police, and military), therapeutic care (i.e., disease detection, assisting the sensory impaired, emotional support), and general companionship, deepening the reaches of the human-animal bond and a rising anthropomorphic view of companion animals [2]. Considering their increasing prominence in American lives, many pets today are viewed as members of the family and as such are being fed and nurtured with the goal of improving their wellness, longevity, and quality of life instead of solely production and performance.

A shift in feeding strategy for companion animals is perhaps most evident in the emerging market of functional foods and treats, which are foods considered to offer a positive health outcome that extends beyond providing essential nutrients [3]. Functional ingredients may include plant extracts, fibers with varying degrees of fermentability, joint supplements, non-essential nutrients, or microorganism and yeast-derived products, which can add value to pet foods by serving a preventative or therapeutic role [4]. Among these, direct-fed microbials (DFM) (commonly referred to as "probiotics") have been used for centuries to ferment staple human food products such as yogurt, cheese, wine, and bread and have only recently been embraced as health-promoting supplements [5]. The efficacy of probiotics in pets is a relatively new area of research, and innovations in the form of new application strategies, unique probiotic strain selection, and substantiating the potential health benefits is necessary to ensure the efficacy of products containing these beneficial microorganisms. The objectives of this chapter are to summarize the various sources and applications of probiotics to pet foods and their associated challenges to viability.

1.1 Historical highlights of probiotics

Probiotics have been present in food since early human civilization. It is presumed that our knowledge of bacteria in our food began when instances of spoilage and poisoning were encountered as early as 8000–10,000 years ago [6]. It wasn't until the mid-nineteenth century, however, that Louis Pasteur made the scientific community aware of acid-forming microorganisms and their role in the souring of milk and fermentation of wine [7]. This discovery prompted a succession of experiments aimed at identifying other microorganisms and uncovering their invisible but significant role in our food system. Nearly a half-century later in 1907, Nobel prize-winning scientist, Elie Metchnikoff, proposed that lactic acid bacteria in fermented milk were responsible for certain health benefits, particularly in delaying the onset of aging [8]. This came about from observing Bulgarian centenarians, who consumed the curdled milk ("yogurt") regularly. In one of his books, "The Prolongation of Life," Metchnikoff proposed that *Lactobacillus* might have a part in counteracting the putrefactive waste products of metabolism that contributed to disease and symptoms of aging, and thus the notion of consuming certain bacteria for promoting health was born. This intriguing theory inspired researchers over the next several decades to turn their focus to the health-promoting mechanisms behind the consumption of microorganisms.

Besides *Lactobacillus*, bacterial spore-formers were also discovered in the same time period. In 1876, Ferdinand Cohn recognized and named the bacterium *Bacillus subtilis* and shortly after Robert Koch described the life cycle of *Bacillus anthrax* [9]. *Bacillus coagulans* (originally named *Lactobacillus sporogenes*) was later described by the Iowa Agricultural Experiment station in curdled milk, and the organism was successfully isolated in 1932 [10, 11]. The unique sporulated condition of *Bacillus* microorganisms was credited with allowing them to survive in the environment as well as endure certain industrial processes such as the vacuum drying of evaporated milk. This provided early evidence that sporulated bacteria have the potential to survive an industrial food production process.

At the turn of the twenty-first century, the passing of the Dietary Supplement Health and Education Act of 1994 led to exponential growth in the sales of products marketed as probiotics for humans [12]. The global market of probiotic-fortified foods is expected to grow from $48 billion to $94 billion with a 7.9% compound annual growth rate between the years 2020–2027 [13]. This surge in interest in

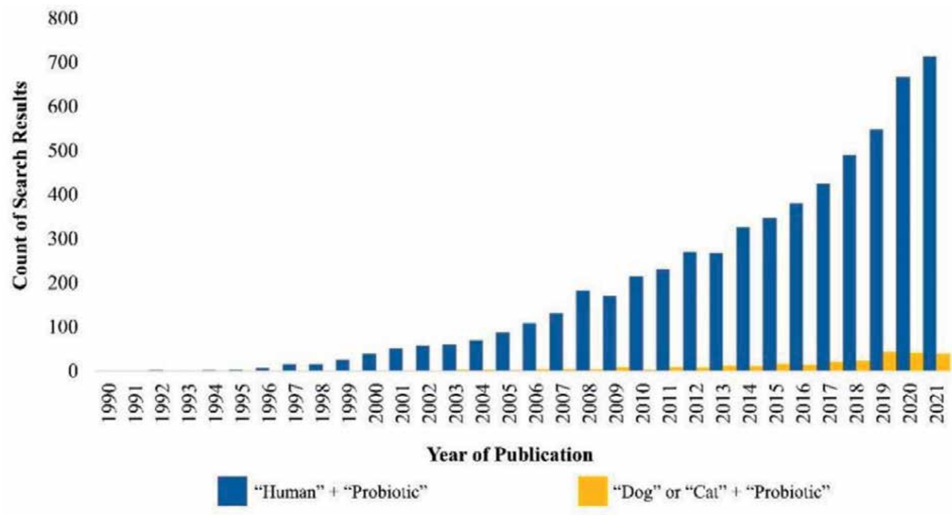

Figure 1.
Number of research publications returned by the PubMed database for search terms "human" or "dog" and "probiotic" between 1990 and 2021. Data presented for 2021 represents year-to-date publication counts available as of march 2021.

functional foods for humans inspired similar developments in the pet food industry, although far less research is available for the use of probiotics for dogs. For example, the PubMed open-access database returns >20,000 publications for "human" and "probiotic" between 1990 and 2021, whereas <250 publications are returned for "dog" and "probiotic" (**Figure 1**). Despite the small body of research available relative to that of humans, probiotics are still promoted for dogs in pet supplements, foods, and treats, and have garnered some support by veterinarians for use in clinical practice [14–16]. This rapidly growing market warrants a closer evaluation of novel probiotic strains, their viability through processing, as well as their ability to deliver similar health benefits as has been observed in humans.

1.2 Definitions and regulatory status

The term "probiotic" is derived from the Latin preposition "pro," which means "before, in front of" and the Greek word "biōtikós" meaning "of life" [17]. Over the last several decades, the definition of probiotics has been refined to incorporate various aspects of a probiotic's intended use and benefits (**Table 1**). The term "probiotic" is often used interchangeably with "direct-fed microbial" when referring to pet foods. However, the most current definition, and that which is used as the context for this chapter, is "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [24].

The criteria for receiving approval as an acceptable probiotic strain in animal feeds involves a framework for verifying the ingredient's compositional analysis, toxicological potential, and evaluation of animal exposure with a focus on potential adverse health effects [25]. The Food and Drug Administration's Center for Veterinary Medicine along with the Association of American Feed Control Officials (AAFCO) first issued a list of bacterial and yeast organisms for use in animal feeds in 1989 that has been revised over the years to include new organisms based on available research

Term	Definition	Year proposed	Reference
Direct-fed microbials	Live microorganisms that, when provided in adequate amounts in the diet, can improve gut microbial balance; the anaerobic bacteria that are able to produce lactic acid and stimulate the growth of other organisms	1965	[18]
Probiotics	Tissue extracts which stimulated microbial growth	1972	[19]
Probiotics	Organisms and substances which contribute to intestinal microbial balance	1974	[20]
Probiotics	A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance	1989	[21]
Direct-fed microbial products	Products that are purported to contain live (viable) microorganisms (bacteria and/or yeast)	1995	[22]
Probiotics	Live microorganisms which when administered in adequate amounts confer a health benefit on the host	2001	[23]
Probiotics	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host	2014	[24]

Table 1.
Published definitions of probiotics and direct-fed microbials.

mainly in swine and poultry. Today, there are 41 non-toxigenic bacteriological species that have been deemed safe for use in companion animals [26]. These microorganisms can be further classified based on physiological characteristics such as the structure of their cell wall, oxygen tolerance, and whether or not they are spore-forming (**Table 2**). Which traits these microorganisms share in common, and which make them unique, are important for the assessment of their potential use in specific food applications.

1.3 Strain selection criteria

In addition to meeting safety and regulatory guidelines, in general a probiotic candidate should have some degree of resistance to acid and bile salts, which are two principal chemical stressors that will be encountered in the gastrointestinal tract [27–29]. The canine digestive system has evolved with mechanisms to effectively inactivate pathogenic microorganisms and extract nutrients from a broad assortment of ingested materials. Comprehensive reviews of canine gastrointestinal tract physiology are available and serve as a useful reference for identifying the conditions that would exert the most stress on a potential probiotic microorganism (i.e., lowest gastric pH, and longest gastric and upper intestinal transit times [30]. For example, conditions mimicking gastric transit (1 h at pH 2.0), small intestinal transit (4 h at pH 6.80), and colonic transit (6–10 h at pH 5.6–6.9), with simultaneous exposure to other relevant biochemical components (i.e., digestive enzymes and bile salts) have been used in the development of *in vitro* canine gastrointestinal models [31, 32]. These conditions could also be applied for the screening of microorganisms intended for use in the diets of dogs.

In addition, any strains intended for application in commercially processed foods pet foods should exhibit high resiliency to process-related stresses, such as heat, prolonged shelf-life, and chemical composition of the food itself (i.e., matrix acidity, oxygen presence, water activity, or presence of microbial inhibitors [33]).

Taxonomic classification		Physiological characteristics		
Phyla and genus	**Species**	**Gram +/–**	**Spore-forming**	**Oxygen tolerance**
Firmicutes				
Bacillus	*amyloliquefaciens, coagulans, lentus, licheniformis, pumilus, subtilis*	+	yes	microaerophile and facultative anaerobe
Enterococcus	*cremoris, diacetylactis, faecium, intermedius, lactis, thermophilus*	+	no	facultative anaerobe
Lactobacillus	*acidophilus, animalis, brevis, bulgaricus, casei, cellobiosus, curvatus, delbrueckii, fermentum, helveticus, lactis, planatarum, reuteri*	+	no	microaerophile and facultative anaerobe
Leuconstoc	*mesenteroides*	+	no	facultative anaerobe
Pediococcus	*acidilactici, cervisiae, pentosaceus*	+	no	facultative anaerobe
Bacteroidetes				
Bacteriodes	*amylophilus, capillosus, ruminocola, suis*	–	no	obligate anaerobe
Actinobacteria				
Bifidobacterium	*adolescentis, animalis, bifidum, infantis, longum, thermophilum*	+	no	obligate anaerobe
Propionibacterium				
Propionibacterium	*freudenreichii, shermanii*	+	no	obligate anaerobe

Table 2.
Taxonomic classification and physiological characteristics of direct-fed microorganisms approved for use in dog and cat foods.

For pet owners, feeding probiotics as part of a food offers the convenience of daily administration to the pet while increasing perceived value of the product compared to conventional foods [34]. However, when probiotics are selected without consideration for these characteristics, the resilience of individual strains in commercial food applications is still open to question. In a study investigating the probiotic integrity of pet foods obtained from the marketplace, 53% of the sampled commercial products were found to be severely inadequate with respect to strain identity and colony-forming unit guarantees on the labels [35]. This highlights a need for validation of probiotic strains to ensure viability at the time of consumption by the animal.

When an organism can be guaranteed to be safely delivered to the gut, the metabolic activities of a bacteria are strain specific. Not all species of bacteria nor strains with a species favor the same metabolic pathways [36]. *Enterococcus*, *Lactobacillus* and *Bifidobacterium* are the most commonly used probiotics for animals, which produce lactic acid as a primary end product. Traditionally, lactic acid producing bacterial strains are Gram-positive anaerobes or facultative anaerobes, and non-spore-forming [37]. These strains also produce other substances such as hydrogen peroxide and bacteriocins which can influence the host microbiota [38]. The health benefits conferred to dogs have been summarized in several recent reviews, and include improvements to stool quality and mixed effects on apparent total tract digestibility, microbial fermentation end products, as well as immune system responses [39–41]. However, as vegetative cells intended for food applications, they are more susceptible to injury and death from the stresses associated with cooking and gastrointestinal transit. The survival of these microorganisms may be enhanced by the use of cell protection technologies, such as microencapsulation [42]. This is a growing area of research that is critical for the future of functional foods incorporating non-sporulating probiotics.

1.4 Inherent probiotic survival mechanisms

Many bacterial species have the ability to cope with rapidly changing and sometimes hostile conditions to protect themselves [43]. One of the most effective adaptations is forming spores in response to a nutrient-deficient environment, low water activity, unfavorable temperatures, or extremes in pH [44]. From the sporulated form, microorganisms regress to a state of dormancy characterized by low metabolic and respiratory activity [36–46]. Gram-positive bacteria, such as *Clostridia* and *Bacillus* species, can form thick protective barriers within the bacterial cell. The main layers of the spore include the core, peptidoglycan-rich germ cell wall and cortex, proteinaceous coats, and exosporium (**Figure 2**). Environmental sensing mechanisms allow the spore to germinate when favorable growth conditions are detected, such as the activation of nutrient and non-nutrient receptors located on the outer spore membrane [47, 48]. A metabolically dormant microorganism can be advantageous with regard to survival in prepared foods due to an increased tolerance to processing conditions and shelf-life during storage [49]. In addition, spores exhibit higher thermo-tolerance compared to vegetative cells and persist under conditions of low pH and in the presence of external proteases. Once the bacteria reach a suitable environment, the spores will initiate the germination process and be restored to a metabolically active state [50].

Bacillus spp. are a sporulating genus that has been evaluated in the diets of calves, broilers, and piglets over the past decade [51–53]. Key findings of these works include validating spore survival through the ruminant digestive tract, improvements to growth performance, and increases in apparent total tract digestibility. There is only one documented reports of *B. coagulans* in the diets of companion animals, despite this strain being included on the approved microorganisms list [54]. Even so, products containing *B. coagulans* are available nationally in stores for consumers to purchase. For example, *B. coagulans* GBI-30, 6086 is a lactic-acid producing, Gram-positive,

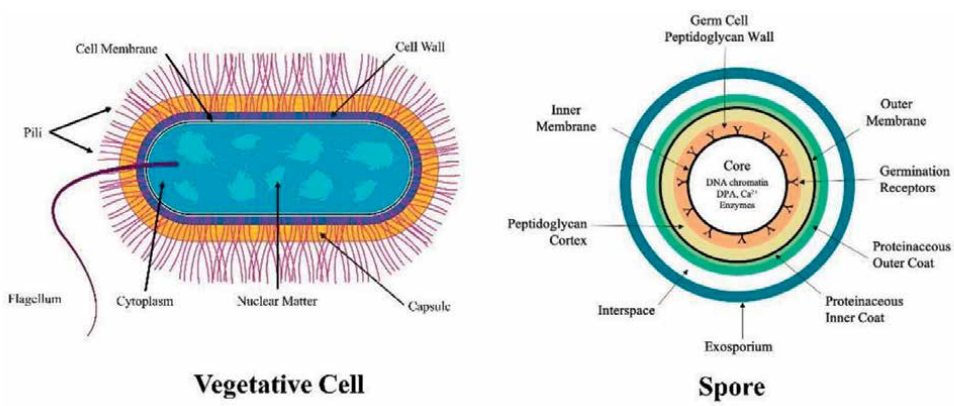

Figure 2.
Stylized illustration of vegetative cell and spore structural layers of probiotic bacteria.

spore-forming rod-shaped bacterium that is microaerophilic. This strain was developed by researchers at Ganeden Biotech (now a subsidiary of Kerry, Inc., Beloit, WI), under U.S. Patent No. 7,713,726. It was granted generally recognized as safe (GRAS) status in 2012 and became the first probiotic strain to receive a published monograph in the Food Chemical Codex (USP Monograph FCC 10). The isolate name GBI-30, 6086 signifies an optimal growth temperature of 30°C with an American Type Culture Collection designation number of PTA-6086. The spores of this strain are resistant to temperatures of up to 90°C, able to germinate in the body while resisting damage by gastric acids and bile salts as determined by both *in vitro* and *in vivo* evaluations [55, 56]. In addition, the safety of this strain with regard to toxigenic and genomic properties is well-established [57–60]. Thus, making this strain and others like it compelling candidates for incorporation into pet food products.

1.5 Enhancing probiotic survival potential

The careful selection of suitable probiotic strains and validation of survival through process conditions may still leave manufacturers unable to guarantee viability claims through the end of a product's shelf-life. Uncontrolled circumstances such as the handling and storage of the foods throughout distribution, retail merchandising, and in consumers' homes can contribute to adverse conditions and subsequent losses in viability over time. Thus, additional steps may be taken to lend further support to the survival of direct-fed microbials for the duration of a product's intended shelf-life. Microencapsulation is a technique that physically enrobes probiotic cells with an additional barrier against adverse external conditions. Spray-drying is one method of encapsulation for large-scale production. This process involves the dispersion of the cells into a liquid polymer solution, homogenization of the mixture, and evaporation of the solvent (commonly water) to form a matrix of dried microcapsules. Microencapsulation can also be accomplished by coextruding a bacterial culture emulsion with an outer gelling agent such as pectate, kappa-carrageenan, locust bean gum, gellan gum, or agar-agar [61]. The co-extruded material is then broken up into droplets that form capsules once dehydrated and cooled [62].

The encapsulation material should be approved for use in food products, nontoxic for the microorganism, and suitable for the food matrix. For example, the presence of singly charged ions such as phosphates, acetates, and citrates, may lead to the premature destruction of calcium alginate capsules through ionic competition. Furthermore, alginate is generally very sensitive to low pH values and heat, and loses its crosslinked structure and thus impair its functionality as a protectant very easily under these conditions [63]. Since many pet food matrices contain inorganic mineral salts and tend to be slightly acidic, this could lead to inferior performance of alginate encapsulations in certain matrices. It has been proposed that combining alginate with chitosan and poly-L-lysine to create multi-component microcapsules may enhance the stability of probiotics, while also reducing the destructive effects of substances that disrupt the structure of the encapsulation [64]. Egg whites, lecithin, whey protein, and carboxymethyl cellulose have also been proposed as compatible substances that may enhance alginate scaffolding for probiotic encapsulation in food applications [65–67].

Starches have also been shown to serve as successful encapsulating substrates [68, 69]. When considering starches as encapsulants, the starch amylose: amylopectin ratio has been reported to influence the effectiveness. For example, high-amylose corn starch granules led to greater resistance to heat and digestive enzymes compared native cereal starches [70]. Innovations in encapsulation technology include multi-component substrates, such as co-encapsulating prebiotics, probiotics, and other bioactive components to pet foods and treats [71]. Once in encapsulated form, the probiotic can be introduced into the food production process as discussed in the following sections.

2. Application of probiotics in commercially processed pet foods

After a desired strain and preparation is selected, probiotics have several hurdles to overcome before they can confer a benefit to the animal (**Figure 3**). For probiotics incorporated into food products, one of the most intense stressors is thermal processing. The vast majority of pet foods are cooked to some degree or commercially sterilized to extend shelf-life and reduce the risk of pathogenic microorganisms or their toxins from enduring in the finished, ready-to-feed product. The basic premise of thermal processing is to reduce or destroy microbial activity, which can be counterproductive to the inclusion of direct-fed microorganisms. Microbial eradication is enforced by federal regulations such as the Food Safety Modernization Act [72], the FDA's zero-tolerance policy for pet foods contaminated with Salmonella [73], and in 21 CFR Part 113 for thermal processing of low-acid canned foods packaged in hermetically sealed containers. As such, process controls are developed accordingly within food safety plans to ensure the target pathogenic species are effectively inactivated.

There are several mechanisms that have been proposed for the action of heat on vegetative cells, including damaging the outer cellular membrane and peptidoglycan wall, loss of cytoplasmic membrane integrity, and the denaturation of cellular organelles, RNA, DNA, and enzymes [74]. Depending on the organism and intensity of heat treatment, the action of heat may lead to one or more of these events, and the ultimate goal is to render pathogenic cells injured beyond repair. Spore-forming microorganisms are reported to exhibit greater wet-heat resistance compared to vegetative cells [75]. The mechanisms controlling heat resistance of spores have not been fully elucidated. However, known heat resistance factors include the accumulation of divalent cations such as Ca^{2+} and the dehydrated state of the spore core. Dipicolinic acid (DPA) also serves an important role by chelating the cations, which helps maintain a low moisture environment and high mineral density in the center of the core [76]. Microorganisms which possess genes encoding for DPA during the sporulation process tend to show increased heat resistance.

Figure 3.
Flowchart highlighting key considerations for the application of probiotic microorganisms into pet food products. Several variables are nested within each commercialization step, adding to the complexity of factors that influence probiotic survival and efficacy potential.

2.1 Extrusion cooking

Extrusion cooking is the most widely used technology in the commercial production of pet foods today, representing the largest category of pet food in terms of market share. Extruded pet foods are nutrient-dense, highly palatable, shelf-stable products which are produced in a continuous high throughput system. Extrusion is a high-temperature, short-time, high-shear process in which pre-conditioned raw materials are conveyed by a rotating screw through a barrel and forced through a small opening (a die) that results in vapor flash-off and expansion of the exiting product. Extruders are available as single- or double-screw configurations, and there are a variety of screw elements that can be combined to create a customizable screw profile in a given system. Throughout the conveying process, thermal energy (usually in the form of steam injected at the pre-conditioning step) and mechanical energy (generated by shear forces from the rotating screws contacting the material) cause the temperature inside the barrel to rise, which allows for the gelatinization of starch, cooking of the material, and serves as a key step in the destruction of spoilage and pathogenic microorganisms that may have been carried in with the raw materials [77]. It has been demonstrated that the ratio of specific thermal energy to specific mechanical energy applied to the food mass during extrusion influences the structural characteristics of pet food kibble [78, 79]. While thermal destruction of pathogens and surrogate microorganisms has been extensively studied, less is known about the effects of specific mechanical energy on microbes. It is possible that extrusion may influence microbial survival differently than other food processes.

Thermophilic organisms, such as *Bacillus* spp., are proposed as better suited for process validation studies since they would exhibit more thermotolerance and therefore be a reliable indicator for developing processes to achieve sterilization [80]. An experiment was carried out wherein different settings for the extruder barrel exit temperature, mash feed moisture content, and barrel retention time were combined to create 15 process combinations in order to compare the suitability of *Bacillus thermophilus* as a surrogate organism for Salmonella during single screw extrusion of animal feed. The results of the study indicated no survival of Salmonella when the feed was extruded at 24.5% moisture content, 3 s retention time, and 82°C or higher die temperature. On the other hand, *Bacillus stearothermophilus*, a spore-former, was detectable at all processing conditions in the range of moisture from 24.5–34.5%, retention times of 3–11 s, and extruder die temperatures of 77–100°C). This study demonstrates the potential for sporulated microorganisms to survive extrusion, while also allowing for destruction of pathogenic cells. Additional studies evaluating microorganisms of sporulating and non-sporulating taxa are summarized in **Table 3**.

2.2 Retort cooking

Retort cooking of most pet foods involves the heating of low-acid (pH >4.6) high-moisture (>0.85 a_w) products in hermetically sealed containers to a minimum of 121°C by injecting steam into a pressure vessel, with the goal of eliminating all vegetative pathogens and spoilage microorganisms as well as spores of *Clostridium botulinum,* rendering the final product commercially sterile. The retort is brought up to temperature during a 3–10 minute come-up period and held at 121°C for at least 2 min, depending on the food composition and packaging type. The hold time must be long

Microorganism	Food material	Process conditions	Viable cell loss	Reference
Bacillus cereus	commercial pet food diet	NR	1.08 log	[81]
Bacillus stearothermophilus	animal feed mash	Extruder: single screw RT: 3–11 s IBM: 24.5–34.5% Die Temp.: 110°C	1 log	[80]
Clostridium sporogenes	mechanically deboned turkey and white corn flour	Extruder: twin screw RT: 3.4 min IBM: 32% Die Temp.: 93.3°C	2 log	[82]
Clostridium sporogenes	mechanically deboned turkey and white corn flour	Extruder: twin screw RT: 3.4 min IBM: 32% Die Temp.: 115.6°C	4–5 log	[82]
Enterococcus faecium	dry dog food ration (corn flour, poultry by-product meal, corn gluten meal, rice meal, vitamins, and minerals)	Extruder: single screw RT: 71 s – 105 s IBM: 21.68% Die Temp.: 120–140°C	6 log	[83]
E. faecium	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT: NR IBM: 28.1% Die Temp.: 81.1°C	5 log	[84]
E. faecium	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT: 48–62.5 s IBM: 27.4–27.8% Temp 55.5–75°C	1.4–5.81 log	[85]
E. faecium	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT: 48–62.5 s IBM: 26.8–27.3% Temp: 80.3–100.5°C	2.3 to >5.87 log	[85]
Salmonella	oat flour	Extruder: single screw RT: 18–46 s IBM: 14–26% Die Temp.: 83–103°C	5 log	[86]
Salmonella typhiumurium	animal feed mash	Extruder: single screw RT: 7 s IBM: 28.5% Die Temp.: 83–103°C	8 log	[20]
Salmonella enterica	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT 48–62.5 s IBM 27.3–27.6% Temp 55.5–68°C	4–6.5 log	[85]
S. enterica	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT: 48–62.5 s IBM: 25.6–26.8% Die Temp.: 77–101°C	>6.86 log	[85]

Microorganism	Food material	Process conditions	Viable cell loss	Reference
Streptococcus thermophilus	whey protein isolate	Extruder: twin screw RT: 25 s IBM: 4–5% Die Temp.: 143°C	4.2 log	[87]
Streptococcus thermophilus	whey protein isolate	Extruder: twin screw RT: 35–40 s IBM: 4–5% Die Temp.: 133°C	4.9 log	[87]
B. cereus	commercial pet food diet	Coated on exterior of kibble after expansion-extrusion and drying; stored in commercial packaging at room temperature in a dry well-ventilated warehouse for 12 months	0.1–0.4 log	[81]

NR = not reported; RT = extruder residence time; IBM = in-barrel moisture content; and Die Temp. = maximum temperature measured at the die.

Table 3.
Summary of log reduction in microorganism viability under various extrusion processing conditions.

enough to achieve a 12-$\log_{10}$ reduction in the number of spores of this pathogen if it should happen to be present within the raw material matrix. The temperature inside the vessel is then cooled with injection of cold water until the pressure is reduced and the vessel can be safely opened. Steel or aluminum cans are the most common package used in pet food retort systems, however recent advancements in packaging technology have expanded into pouches, cups, and tubs made from a variety of starting materials (commonly polyethylene and its derivatives). Federal regulations have been established for manufacturers in 21 CFR Part 113 to mitigate the public health risk of botulism associated with past market recalls of foods processed using this method. Due to the intentionally severe conditions exerted on microorganisms present inside the food container during cooking, even the hardiest live microbials are not well-suited for retort applications. Opportunities for functionality do exist for the inclusion of pre-biotics and post-biotic ingredients, however.

2.3 Freeze-drying

Freeze-dried pet foods and treats have gained popularity in the past decade as the market demand for products with high bioavailability and less thermal processing has increased. Freeze-drying is considered a relatively gentle dehydration process due to the absence of heat and the slow rate of water removal using lyophilization, the phase transition of ice directly into vapor without passing through the liquid phase. This is achieved by first freezing the food preparation, applying a high vacuum to a sealed vessel to reduce the pressure, allowing the ice to sublimate from the product and collect on a condensing unit for removal from the system. Opposite to most pet food manufacturing technologies that aim to destroy viable microbes, freeze-drying is widely used as a preferred method for preservation of bacterial cultures. Cellular water can be removed to reversibly inactivate microorganisms to facilitate their storage. This makes freeze-dried pet food applications a good candidate for the application of direct-fed microbials.

Since the product is dehydrated without the use of heat, freeze-drying is not considered a cooking process. However, the ingredients used in freeze-dried pet food formulations can be pre-cooked or raw depending on the product's design. Many probiotic preparations that are used in pet foods are initially preserved by freeze-drying with the aid of a protective medium that helps prevent damage of cellular membranes and proteins as water is removed from the core of the cells. This prolongs the shelf-life of the probiotic cultures and allows for their downstream incorporation into many shelf-stable food applications. When blended into a food matrix, previously dehydrated probiotics have an advantage over vegetative bacteria when subjected to freeze-drying since their cellular water content is already low. The bulk of the water removal from the food matrix is from water surrounding the cells, rather than water within the bacterial core. For vegetative cells, the primary mechanism of cell injury is disruption of the cell membrane structure during intracellular ice formation [88]. A lower survival rate of Gram-negative bacteria relative to Gram-positive strains has also been reported, and this is thought to be due to the thinner peptidoglycan layer and the presence of lipopolysaccharides within the cell wall of Gram-negative species [89]. However, the damaging effects of freeze-drying on live cells is not significant enough to mitigate the risk of food-borne pathogens. Therefore, many freeze-dried pet foods and treats, particularly those containing raw ingredients, may undergo additional processing such as irradiation or high-pressure processing independent of the freeze-drying cycle for food safety. Adjunct processing for pathogen control can present additional challenges to probiotic viability but is not covered within the scope of this chapter.

2.4 Baking

Baking encompasses a wide range of products and processes including bread, snacks, cakes, tortillas, pastries, pies, pet treats, pet foods, and more. Baked products are traditionally composed of cereal flours, but meat-based formulations are also common in the pet food industry. Baking for food preservation is regarded as one of the oldest cooking methods documented in human civilization and was in fact the first process used to commercialize the first dog biscuits in 1860.

At a basic level, the baking process consists of combining ingredients to form a dough, forming the product into the desired shape, cooking the raw dough using dry heat in an oven, and cooling the baked product at ambient temperatures before packaging. The types of ovens in industrial-scale settings are gas-fired, oil-fired, and electric, fitted with a single or multi-pass conveyance system that transports the dough on a wire mesh belt. The transport of heat to the surface of the dough occurs through conduction, convection, and radiation, allowing for the evaporation of water from the surface of the product followed by a formation of crust layer. Standard baking times for bakery products range between 2 and 30 minutes, dependent on the oven design, starting moisture content, dough density, temperature, and desired finished product characteristics (color, size, appearance, and texture). Baking is generally a lower throughput process relative to extrusion and canning-retort, however it offers advantages such as the development of desirable colors and flavors that result from Maillard reaction product formation.

The primary stressor live microorganisms encounter during baking is heat. The duration and high temperature of typical baking are usually sufficient to inactivate

E. coli or salmonella pathogens, however formal scientific validation of the diversity of commercial baking processes for the inactivation of pathogens or direct-fed microbials has not been thoroughly studied. Across available data, a ≥ 5 log CFU/g reduction in *Salmonella enterica* serovars was demonstrated by 17 min of baking, and a 6.1 log CFU/g reduction by 21 min of baking at 190.6°C in an electric oven in muffins [90]. Higher temperatures were needed to achieve >6 log CFU/g in hamburger buns baked in a conventional oven for 13 min at 218.3°C [91]. This demonstrates variability in microorganism survival that may be dependent on the properties of the dough matrix and type of oven in addition to the microorganism's inherent thermal-resistance properties. To our knowledge, no such studies have been conducted on the inactivation of pathogens in baked pet foods and treats. However, from learnings gleaned from other thermal process technologies such as extrusion, it is reasonable to expect that dormant and microencapsulated probiotic preparations and those with higher thermal resistance attributes would be better suited for the baking environment.

To circumvent thermal stress, entrapment of probiotic cells in edible films or coatings on the surface of baked products is a promising approach. Using film-forming solutions based on sodium alginate, whey protein concentrates to suspend probiotics in a gel that can be applied as a topical coating to baked goods. Functional starch-based coatings have been successfully implemented using microencapsulated *Lactobacillus acidophilus* achieved 63% survival when the coating was comprised of 94% water, 5% starch, ad 1% microencapsulated probiotic applied to the loaf and baking at 180°C for 16 minutes [92]. The survival of *Lactobacillus plantarum* (strain CIDCA 83114) was reported to have improved retention during baking at 30°C for 40 minutes when applied as a corn-starch-based film (4 log reduction in viable cell counts) compared to a sodium alginate film (6-log reduction in viable cell counts) [93]. This suggests starch-based suspensions may be more effective than other films at protecting probiotic viability under baking conditions. However, validation of probiotic viability should be included as part of the commercialization process because of the wide range of direct microbial preparations, raw materials used in pet food and treat formulations, application strategies, and processing conditions.

3. Conclusion

Probiotics are one of a growing number of functional ingredients that contribute to the advancement of companion animal health and wellness, but delivering viable microorganisms in commercially processed food products presents many challenges to ensure the viability and efficacy they are marketed for. Pet food manufacturing processes are designed to improve food safety and prolong shelf-life, which is counterproductive to the survival of direct-fed microbials. Thus, making the selection of appropriate strains critical for their intended application. Among the most important characteristics to consider when selecting of probiotic strains used in commercial pet food applications are the strain physiological attributes (especially thermal resistance, oxygen tolerance, acid and bile resistance), stabilization method (such as sporulation, freeze-drying, or encapsulation), processing conditions (including time, temperature, pressure, moisture, water activity, pH), application method, and packaging and storage conditions. Verification of probiotic viability should be performed when working with novel probiotic strains, and when any modifications are made to processing conditions, product formulations, or packaging designs.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Chapter 4

Alternatives to the Use of Antibiotics in Animal Production

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Abstract

There is a growing demand for livestock products and by-products due to an increase in the human population globally. Farmers utilize feed additives and antibiotics to enhance growth and alleviate diseases to meet this increasing demand for meat and meat products. Although antibiotic use as growth promoters (AGPs) in the livestock industry has brought about a positive increase in production, the industry has also been negatively affected by the development of bacteria resistant to antibiotics and the presence of chemical residues in meat and excreta. Due to this, concerns have risen as this poses a health risk. Resistant bacteria can be transmitted to humans by consuming meat from antibiotic-fed animals or environmental spread from animal wastes. Therefore, action is required to curb this issue because it is estimated that the annual losses in GDP and death toll globally could increase because of the continuous use of antibiotics in livestock production. Hence, this review aims to examine natural alternatives that have the potential to replace antibiotics for food safety, health, and environmental reasons. These could bring a satisfactory impact on nutrient absorption for growth together with health-stimulating virtues.

Keywords: antibiotics, natural feed additives, livestock production

1. Introduction

The use of antibiotics in livestock production started as early as 1928 by Alexander Fleming, where their use was to fight against diseases in humans. An increase in the demand for meat and poultry in the latter years led to research studies being conducted [1]. It was then discovered that the continuous administration of antibiotics in small amounts in livestock diets was essential to alleviate diseases and improve growth. This led to antibiotics being used as growth promoters (AGPs) in livestock diets [2]. Antibiotics promote growth by inhibiting pathogenic bacteria growth, preventing the development of growth-suppressing metabolites, reducing the inflammation of the gut wall, and improving microorganisms in the gut. This enables optimal performance of animals through efficient utilization of nutrients. The positive influence of antibiotics led to their approval by the Food and Drug Administration of the United States of America to be used as growth promoters in animal diets [3]. Antibiotic use increased in the following years because of the growing global demand for meat and meat products [1].

However, antibiotic resistance in animals started when farmers were allowed to use antibiotics even without a veterinary prescription, leading to continuous overdose or abuse sub-therapeutically by zealous farmers. This resulted in certain bacterial death, and those that remain develop resistant genes [4, 5]. They develop several means of surviving the selection pressures brought about by antibiotics, such as antibiotic molecule deactivation by enzymes, efflux pumps, and the development of cell wall and ribosomal modification to protect cellular targets against antibiotics. When they contact these animals or consume meat from antibiotic-fed animals, these resistant bacteria are also transferred to human beings. According to Letchumanan et al. [6] and Low et al. [1], food safety remains under threat because of the high incidents of antibiotic resistance.

About 50–80% of the antibiotics produced are available for livestock production in developing countries, and these have the highest rates of resistance genes that can be passed on to human beings [7]. Therefore, action is required to address antibiotic resistance because it is estimated that the death toll could rise to 10 million by 2050 from 2.8 million in 2019, and losses on the annual GDP by 3.8% globally [1].

Similarly, an increase in cases of antimicrobial resistance associated with health risks resulted in the banning of antibiotics as growth promoters in livestock in 2006 by the European Union (EU) [4]. Although antibiotics for growth promotion were prohibited, they are still being used upon regulation on disease treatment. Regulations were put in place by the Food and Drug Administration of the United States of America to limit antibiotic use as growth promoters in 2014. However, not much has been done regarding this issue in some developed countries and many developing countries where there is still unregulated use of antibiotics as growth promoters.

The total removal of antibiotics in animal production is implausible, as it affects the livestock industry negatively. It is pivotal to search for naturally occurring, available, low cost and effective growth promoters as substitutes to AGPs in livestock diets, particularly in territories in which antibiotics were banned. Hence, researchers in recent times have been working increasingly on natural alternatives that could replace the use of antibiotics in livestock production for food safety, health, and environmental reasons [8]. Antibiotic alternatives are natural, organic ingredients that could be utilized as feed additives, resulting in promoting growth and the animal's health, primarily exerting their influence on the gastrointestinal tract [9]. Such natural antibiotic alternatives include prebiotics, probiotics, organic acids, photobiotic products (medicinal plants and products), exogenous enzymes, phage therapy, fossil shell flour, antimicrobial peptides, and bacteriocins [10–12]. They have attracted substantial recognition as additives in livestock production because of their satisfactory impacts on the absorption of nutrients and health-stimulating virtues [13].

2. Antibiotics alternatives

2.1 Phytogenic compounds

Phytogenic compounds are based on bioactive compounds derived from plants, and many such plant products can be broadly classified as essential oils, herbs, and spices [14]. Examples include lavender, green tea, cinnamon, garlic, pepper, oregano, rosemary, sage [15], and ginger [16]. Some of these plants contain secondary metabolites such as saponins, tannins, alkaloids, and flavonoids that could play the role of antibiotics in the body of animals.

Tannin-containing plants such as chestnut, *Acacia karroo*, and Grape pomace [17, 18] help alter rumen fermentation and mitigate disease-causing bacteria in the animal. Similarly, the presence of tannin in the diet reduces methane emissions from the rumen, thereby increasing the energy available for growth and production. Orlandi et al. [19] reported that 2–5% of tannins in livestock diets are highly beneficial to the development and health of the animal and for environmental safety against greenhouse gas. The mode of action of tannins includes protein binding, which affects the growth of bacteria in the rumen and fermentation. They reduce the amount of protein available for bacteria, thereby reducing nitrogen excretion and decreasing bloating and internal parasites [20]. Nawab et al. [18] noted that tannins could improve the production status, gut health, and immune status and reduce methane emission, which leads to improvement in the animal's overall performance. On the other hand, Saponins are involved in binding sterols, causing cell destruction and microorganism damage [15].

Other photogenic compounds that are often used as natural feed additives in animal diets are essential oils. These compounds are naturally extracted from plants and used in the cosmetic and fragrance industries and recently in livestock production [17]. They are extracted from leaves, stems, flowers, roots, seeds, and barks. Essential oils constitute compounds that are involved in the elimination of pathogenic bacteria in the rumen. Examples of essential oils used as feed additives in animal diets are rosemary oil, coriander oil, eucalyptus oil, garlic oil, cinnamon oil and clove bud oil [21]. Essential oils destroy gram-positive and gram-negative bacteria's cell membranes, making them ineffective in the animal's body. Since essential oils exhibit a lipophilic characteristic [22], they also have substances that weaken fungi, protozoa, and viruses through coagulating cytoplasmic contents [23]. According to Zhu et al. [22], supplementing broiler diets with essential oils and oregano or saponins improves growth performance and immunity by removing pathogens. Since essential oils constitute various components, bacteria have fewer chances to develop resistance than when fed antibiotics. In ruminants, essential oils improve immunity, decrease ammonia and methane production, and improve rumen fermentation, rumen microbes, and volatile fatty acid production. In monogastric animals, essential oils increase feed intake, growth performance, egg production, immunity, nutrient digestion, and utilization [21].

Herbs such as wormwood, also known as Tethwan, are natural herbs with a peculiar scent and various medicinal impacts. According to Beigh et al. [24], tethwan and oregano in livestock diets can enhance feed intake, the animal's ability to utilize nutrients efficiently, and rumen fermentation. However, there is a need to investigate further the mode of action, effect on microbial populations, and these compounds' ability to be utilized to provide a better understanding [3] since there is limited knowledge on how they function in the gastrointestinal tract of the animal.

3. Probiotics and prebiotics

Humans have been consuming them as natural constituents in diets or fermented foods for a long time. Probiotics are live microorganisms added to livestock diets to assist in enhancing microbial balance in the intestines by suppressing pathogenic bacteria [13]. The commonly used probiotics are *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Bacillus*, *Enterococcus faecalis* [25], and *Bifidobacterium* species [3]. Various *Bacillus* species are formulated in probiotic supplements as they are stable to heat and can withstand low pH conditions in the stomach. To improve variety and compliance, there is a need to introduce probiotics that are cheap commercially, have a long shelf life, are stable in the feed and can withstand the process of heat pelletization [25]. Honan et al. [17] deduced that probiotics could reduce methane emission production because of their effect on the rumen microbiome or metabolites. According to Abd El-Hack et al. [15], probiotics do not have any side effects on animals. They are specific to targeted strains of bacteria and resistant to acid and bile.

Tutida et al. [26] stated that the probiotics used in swine research studies vary and bring about variable effects, especially when administered under different conditions. Hence it is essential to consider the animal's age, feeding, and method of handling to aid in choosing the probiotic to add to the diet. Probiotics improve the overall health of the animals by colonizing the intestines, removing pathogenic microorganisms, releasing metabolites, and boosting the immune system [14]. However, the limitation of probiotic use is the risk of spreading and transferring genes resistant to antibiotics, as probiotics are directly involved with disease-causing bacteria in the gut.

Prebiotics are indigestible carbohydrates (oligosaccharides, fructans, pectin) that aid in the growth of beneficial bacteria in the intestines [13]. Thereby improving the overall performance of the animal [14, 26]. They are involved in eliminating pathogens such as *E. coli* and Salmonella and preventing cancer in the colon. Shehata et al. [25], observed that prebiotics could improve poultry's immune system, leading to faster disease clearance, decreased pathogenic bacteria such as *streptococcus* and *staphylococcus*, and improved intestinal morphology. Specific prebiotics have been known to enhance butyrate production in the rumen by providing a favorable environment for the growth of bacteria responsible for butyrate production [3].

When probiotics are used together with prebiotics, they are regarded as synbiotics. This combination promotes the growth and function of beneficial bacteria in the gastrointestinal tract [27]. Hence, it is beneficial to include synbiotics in livestock diets as the combined effect produces better output than including either. Since the aim is to improve livestock production by replacing antibiotics with these substances, combining them would benefit the animals and reduce their use.

4. Phage therapy

Bacteriophages are bacteria-infecting viruses with high specificity to target organisms [1]. They were first discovered during the early 1900s, and their use increased in the following years. However, increased use and benefits of antibiotics led to a decline in phage therapy use. Recent cases of antibiotic resistance and chemical residues in livestock brought about by antibiotics as growth promoters have caused researchers to gain interest in phage therapy. Phage therapy could be useful as an alternative to antibiotics to curb antibiotic resistance in livestock [28]. This is because phages are specific and can multiply when they detect an infection. Phages also can evolve and are cheaper, unlike antibiotics [1, 7]. Bacteriophages aid in growth promotion and coccidiosis prevention by eliminating pathogens in animal production.

Łusiak-Szelachowska et al. [29] pointed out that a combination of antibiotics and phage therapy could significantly reduce pathogenic microorganisms such as *Staphylococcus aureus* than utilizing one in livestock diets. The phage-antibiotic combination could result in increased production of phages and cell elongation by antibiotics. This could reduce the use of antibiotics and their concentration, thereby decreasing the rate of antibiotic resistance.

5. Organic acids

Organic acids are carboxylic acids with the general structure R-COOH [30]. For a couple of years, they have been used in the poultry industry as a substitute for antibiotics [31]. Their ability to function efficiently lies in the targeted bacteria or fungi, chemical composition, molecular weight, and form [4]. Organic acids interfere with bacterial growth and cause death by entering the bacteria's cell membrane, leading to a reduced pH in the alkaline environment of the bacteria, thereby altering their mode of function [5]. Organic acids enter gram-negative bacteria cell walls and release H^+ ions which reduce the pH and interfere with replication and protein synthesis in the cytoplasm of the pathogen [30]. Kiarie et al. [32], suggested that organic acids are useful as feed additives for weaned piglets. Organic acids such as benzoic acid reduce the presence of pathogens such as *E. coli*, promote digestion of nutrients, and improve gut health in swine. Piglets fed with a diet containing benzoic acids had been reported to have better overall performance in the first 3 weeks after weaning compared with those fed diets fortified with antibiotics as growth promoters. **Table 1** shows the effects of various organic acids, their blends, and recommended inclusion levels as discovered in research studies.

Supplementing diets with short-chain fatty acids and medium-chain fatty acids improves disease protection, performance, and digestion rate and prevent the growth of pathogenic bacteria respectively in poultry and pigs [5]. This suggests that organic acids have the potential to be used as alternatives in livestock production [3]. However, there is inconsistency in research studies on the potential and effects of combinations of organic acids in animal production. These inconsistencies could be associated with variations in the composition and incorporation levels, feed type, environment, and breed [30]. Hence more research is essential.

6. Enzymes

Enzymes are proteins in nature, and they are regarded as biological catalysts that aid in speeding up the rate of chemical reactions [15]. They improve gut

Organic acid	Recommended inclusion rate (%)	Effect/s	Reference
Citric acid	0.5	Enhanced feed intake, growth, carcass yield	[4]
Combination of caproic, caprylic, fumaric, citric, and malic acids	0.2	Enhanced average daily feed intake, and growth rate, eliminated pathogenic microorganisms in swine.	[26]
Synergistic blend	—	Improved digestibility and absorption in poultry.	[30]

Table 1.
Organic acids, recommended inclusion rates, and effects.

health in broilers and result in improved performance [22]. The nutritional value of the feed improves when diets are supplemented with enzymes. This results in improved feed efficiency, utilization, and decreased nutrients excreted. They are grouped into different classes based on the source and the substrates they act upon [15]. Adding exogenous enzymes to poultry diets enhances digestibility and utilization, reducing the quantity of nutrients excreted. Including exogenous enzymes in livestock diets also aid in lowering feed cost by providing a more significant return on investment [33].

Inclusion of the enzyme phytase in animal diets aids in the digestion of phytate to inositol ad inorganic phosphate. This is usually done because the phosphorus from cereal grains cannot be digested by poultry without phytase addition. The addition of phytase in poultry diets is economical because it efficiently utilizes phosphorus, which is regarded as the most expensive mineral in poultry production [15]. It is essential to include fiber and starch digesting enzymes in poultry diets as they assist in digesting non-starch polysaccharides. Xylanase and β-glucanase addition to poultry diets improve feed conversion ratio, digestibility, growth performance, and nutrient utilization and reduce wet litter [34]. The inclusion of enzymes in livestock diets is of great benefit not only to the animal through improved health, nutrient utilization, and growth but to the farmer also through reduced cost and increased returns.

7. Fossil shell flour

It is made from fossilized remains of diatoms, which are minute single-celled organisms found in seas, lakes, and soils [35, 36]. Fossil shell flour, also known as Diatomaceous Earth (DE), is regarded as a substance with multiple purposes and has the potential to be utilized as a substitute for antibiotics in livestock production. Anand et al. [37] stated that fossil shell flour is abundantly available and cheap than chemical-based feed additives. It is an anti-caking agent; it averts the formation of clumps in animal feed. This results in increased surface area of the feed that comes in contact with microbes and enzymes during digestion, leading to increased nutrient availability and utilization [38]. Fossil shell flour improves the animal's well-being as it has over 14 minerals, including calcium, phosphorus, potassium, magnesium, copper, zinc, and iron silica. These are usually not available abundantly in most feed crops [39]. It also aids in reducing parasite load due to its sharp edges (that are seen with a scientific microscope), which can harm bacterial cell membrane surfaces, thereby causing dehydration and eventually leading to death [35].

Table 2 shows different inclusion levels of Fossil shell flour among other species and its effects discovered by researchers. Wikoff et al. [43] found that 2% inclusion of red lack diatomaceous earth (a naturally occurring blend of diatomaceous earth and calcium bentonite) in livestock diets does not pose a health risk to human beings. This signifies that fossil shell flour has the potential to be utilized in livestock diets as a substitute for antibiotics. Further studies need to be conducted to validate the safety of Fossil shell flour and the maximum inclusion rate based on each livestock species.

8. Antimicrobial peptides (AMPs)

These are structurally heterogeneous cationic, amphiphilic peptides expressed by most multicellular organisms as part of their innate immune system [2]. Most

Recommended inclusion rate	Species	Effect/s	Reference
2000 mg/kg	Broilers	Improved lymphoid organs, reduced aflatoxin availability	[40]
2%	Layers	Reduction in parasitic load increased body weight gain and egg production.	[38]
Up to 2%	Layers	Improved egg quality	[36]
2%	West African Dwarf ewes	Weight retention during lactation, improved feed intake, twin survival.	[41]
4%	Dohne Merino weathers	Improved weight gain, nutrient digestibility, overall performance	[35]
40 g FSF/kg	Dohne Merino rams	Reduced heat stress and improved growth performance	[42]

Table 2.
Research studies of FSF effects and recommended inclusion rates.

of the antimicrobial peptides are derived from the cleavage of proteins during the replacement of proteins by protease enzymes [44]. They assist in fighting against disease-causing microorganisms in every animal, and approximately 5000 antimicrobial peptides have been discovered to date. Targets for antimicrobial peptides differ because of their nature; they can target gram-negative, gram-positive fungi, or viruses. This leads to different modes of action against pathogenic organisms. Some of them destroy the plasma membrane of the cell, which alters the proper functioning of the bacteria; others affect DNA, protein, and cell wall formation.

It is unlikely for bacteria to develop resistance against antimicrobial peptides because they break down the cell membrane of the bacteria's cell through non-specific disturbance of lipid bilayers. Antibiotics are regarded as bacteriostatic, but antimicrobial peptides are considered bactericidal and advantageous over antibiotics [45]. The typical families of antimicrobial peptides in livestock are cathelicidins and defensins. They are proteins involved with the innate immune system, proteolytically active, and in the animal's immune response to prevent and eliminate infections [44]. However, there is little knowledge on clinically antimicrobial peptides, mode of action, and availability. Hence, more research studies need to be conducted to understand AMPs in animal production better.

9. Bacteriocin

Another group of antimicrobial peptides (AMPs) is Bacteriocins. They are regarded as proteins or peptides produced by bacteria that exhibit repressive actions against numerous bacteria. They are produced in the late log growth phase and at the beginning of the stationary phase, unlike antibiotics, which are a product of secondary metabolism [46]. Schulze et al. [2] reported that bacteriocins are small bactericidal or bacteriostatic peptides synthesized by bacteria that play a regulatory role in bacterial ecosystems. They emit strains of bacteria that aid in preventing the growth of pathogens.

Bacteriocins are effective antibiotics and preservatives in the food and pharmaceutical industries. According to Murugaiyan et al. [28], bacteriocins are stable to heat and are less toxic. Hence they have the potential to replace or be used as a substitute for antibiotics. Bacteriocins also reshape the microbiota by killing the targeted pathogenic bacteria without harming the surrounding microorganisms, making them advantageous over antibiotics [47]. However, inconsistencies in research are the barrier to providing proper and complete knowledge, function, and potential of bacteriocins as a substitute for antibiotics in livestock production.

10. Summary

Organic acids, phytogenic compounds, antimicrobial peptides, phage therapy, bacteriocins, fossil shell flour, enzymes, probiotics, and prebiotics can bring success, profits, and the possibility of replacing antibiotics in livestock. As they can enhance growth, alleviate diseases, and improve production. However, it should be noted that none of these antibiotic alternatives is more efficient at a large-scale farming level. They can compensate for the total exclusion of antibiotics in livestock diets to a certain extent. Hence the researcher recommends that blending these alternatives can be a possibility to improve production and ensure more returns to farmers in place of antibiotics. Utilizing these natural alternatives in place of antibiotics is beneficial for food safety, health, and environmental reasons. They could bring a satisfactory impact on nutrient absorption for growth together with health-stimulating virtues.

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Chapter 5

Potential Substitutes of Antibiotics for Swine and Poultry Production

Ho Trung Thong, Le Nu Anh Thu and Ho Viet Duc

Abstract

Early of the last century, it was detected that antibiotics added to the animal feeds at low doses and for a long time can improve technical performances such as average daily gain and gain-to-feed ratio. Since then, the antibiotics have been used worldwide as feed additives for many decades. At the end of the twentieth century, the consequences of the uses of antibiotics in animal feeds as growth promoters were informed. Since then, many research studies have been done to find other solutions to replace partly or fully to antibiotic as growth promoters (AGPs). Many achievements in finding alternatives to AGPs in which probiotics and direct-fed microorganism, prebiotics, organic acids and their salts, feed enzymes, bacteriophages, herbs, spices, and other plant extractives (phytogenics), mineral and essential oils are included.

Keywords: antimicrobial growth promoters (AGPs), bacteriophages, probiotics, prebiotics, antimicrobial peptides (AMPs), phytogenics, hyperimmune antibodies

1. Introduction

The antimicrobial growth promoters (AGPs) or antibiotics have been used in animals at treatment/sub-therapeutic concentrations for enhancing the productivity and preventing diseases. The beneficial effects of using antibiotics were first advocated by Moore *et al.* when they found that chicken fed streptomycin with adequate amounts of folic acid exhibited increased growth responses [1]. Later, in 1951, the US Food and Drug Administration (FDA) approved the use of AGPs in animal feed without veterinary prescription [2]. Subsequently, the use of AGPs has become globally practiced, significant rising by 10–20-fold since 1950s.

Despite the positive effects of AGPs being well documented, their use was also controversially argued for a long time due to the risks of antimicrobial resistance, posing a potential threat to human health [3]. For instances, the resistance to antibiotics has been increasingly observed since the first cases of streptomycin resistance in food animals were recognized in 1951. In fact, there is irrefutable evidence that foods from many animal sources and all food processing stages contain a large number of resistant bacteria [4]. Consequently, there are approximately 23,000 and 25,000 deaths annually occurred due to the antibiotics resistance in the USA and Europe, respectively [5, 6]. Antimicrobial resistance had led to the failure of treatment in 195,763 cases of pneumococcal disease, which contributed to 2925 child deaths annually in Ethiopia [7].

Since the big concern of antimicrobial resistance to global public health, the European Union issued a ban all AGPs on precautionary grounds in 2006 [8, 9]. In the World Health Day 2011, the subject "combating drug resistance: no action today, no cure tomorrow" was discussed to reinforce all countries in the whole world to take proactive actions against antimicrobial resistance. Furthermore, The World Health Assembly 2015 approved global action plan to tackle the issue of bacterial resistance. In addition, the 2016 United Nations High-Level Meeting on antimicrobial resistance and the G20 Summit in Hangzhou, China, launched strong commitments to control the crisis of antimicrobial resistance [10].

Both political and consumer pressures are prompting a reduction in the use of AGPs in animal production; therefore, the identification of alternatives might be reasonable approach that may help reduce the risks and prevent the spread of drug-resistant bacteria and may promote the animal breeding industry. A great deal of studies have focused on the development of alternatives to AGPs including probiotics, prebiotics, synbiotic, enzymes, phytogenics, antimicrobial peptides, bacteriophage, and antibody therapy [3, 11–20]. In addition, in recent years, the CRISRP-Cas9 gene editing tool showed effective impacts on preventing infectious diseases that is a promising approach to alternative to AGPs in the future [21]. This review, therefore, focuses on such alternatives along with a description of their efficacy in swine and poultry production.

2. Modes of action of AGPs

It is believed that the successful development of antibiotic alternatives relies on understanding the mechanism of action of AGPs. Current evidence shows that at least two major modes have been proposed to explain the function of AGPs: (i) bacteria-centric and (ii) host-centric. Further, other environmental factors including stress, diet, and nutrition will influence both the host and the microbiota, and interactions among these factors with AGPs are also important to their function [22].

2.1 Bacteria-centric mode of action

The intestinal mucosa is central both to nutrient absorption and to maintaining the immunological homeostasis, and the complex microbial communities that harbors gastrointestinal tract (GIT) are involved in the host immunological and metabolic processes [23]. AGPs are utilized as feed additives, and thus, the GIT is considered as a primary site of AGPs action. The initial study on germ-free mice with AGP administration did not exhibit the increased growth suggested that modulation of the intestinal microbiota is central to the action mechanism of AGPs [24]. The growth-promotion phenotype was shown to be transferrable to germ-free hosts by low-dose antibiotic-selected microbiota, indicating that the altered microbiota and not the antibiotics played a causal role [25]. It was also shown from the studies in mice that exposure to low-dose antibiotics early in life induces long-term host metabolic effects by accelerating normal age-related microbiota development and altering ideal expression of the genes involved in immunity [26]. Bacteria-centric hypotheses propose that AGP-induced changes to bacterial communities lead to enhanced growth by modulating the microbiota to create a more efficient system. This may include altering competition for nutrients, preventing pathogen colonization, and/or selecting for bacteria that are able to extract more energy from the diet [22].

2.2 Host-centric modes of action

The intestine not only plays vital roles in nutrient absorption but is also a major immunologically responsive organ. The "host-centric hypothesis" is supported by evidence that several antimicrobial agents have immune-modulating properties at therapeutic concentrations, which include downregulation of prolonged inflammation, increased mucous clearance, and modified phagocyte activity [22]. The direct effects of AGPs on the host physiology were indicated by Brown et al. [27]. They found that Altered Schaedler Flora (ASF) mice treated with chlortetracycline or tyrosine phosphate had lower expression of *βd1* and *Il17a* in the intestine and had a strong induction of *Il17a* and *Il10*. In addition, by treating with AGPs, mice exhibited a lower hepatic expression of acute-phase proteins (*Saa1*, *Hp*, and *Cp*) in the liver tissue and *Citrobacter rodentium*-induced reductions in the expression of genes involved in lipogenesis (*Hmgcl* and *Fabp1*) [27]. Although the effects observed in mice cannot be directly extrapolated to farm animals, they might provide an insight into a possible mechanism of action and highlight important considerations in the development of alternatives to AGPs.

3. Alternative approaches to AGPs

3.1 Using available alternative compounds in the absence of AGPs

An ideal alternative approach should have the same beneficial effects of AGPs, enhancing the growth and preventing the diseases. Considering the proposal AGP mechanisms of action, the practical alternative compounds should possess the properties of modulating the gut microbiome and immune responses. Over the past decades, scientists have investigated and evaluated a variety of alternatives for antibiotics to control health issues related to animal production including probiotics, prebiotics, synbiotics, organic acids, enzymes, phytogenics, and trace minerals. The recently discovered novel alternatives such as hyperimmune egg yolk IgY, antimicrobial peptides (AMPs), and bacteriophages therapy have been developed.

3.1.1 Probiotics

FAO and WHO [28] defined probiotics as live microorganisms (yeast, fungi, and bacteria), which, when supplemented in adequate amounts, affect the host intestinal microbial ecosystem and may help prevent the growth of pathogens resulting in improved health and prolonged life. A multitude of studies on useful microorganisms for probiotics were performed and led to the successful development of commercial probiotic products as food supplements for humans or feed additives for farm animals [29]. Commercial strains of probiotics are commonly isolated from the intestinal microflora and selected based on the criteria including resistance to stomach acids and bile salts, ability to colonize the intestine or antagonize potentially pathogenic microorganisms [30]. The probiotics genera are mainly *Bacillus, Lactobacillus, Bifidobacterium, Enterococcus, Pediococcus*, and *Streptococcus* [31]. Further, other microbes such as *Saccharomyces*, *Aspergillus oryzae,* and many more along with their products are also classified as probiotics.

The use of probiotics in poultry production had considerably positive effects on productivity and health. A numerous of scientific studies have quantified the efficacy of probiotics for growth promotion in both broilers and layers [32–37]. For examples, Huang *et al.* reported that *Lactobacillus* strains, *Lactobacillus casei* (low dose) and *Lactobacillus acidophilus* (high dose), and *S. acidophilum* (high dose), a strain of fungus, were able to promote the growth of broiler chickens [35]. Fesseha *et al.* recently revealed that *Lactobacillus paracaseis sparacasei* and *Lactobacillus rhamnosus* were beneficial for the growth performance by improving body weight gain (BWG), feed conversion ratio (FCR), feed intake (FI), and positively affects the growth of the chicken [36]. In addition to the improved growth performance, the use of probiotics was also shown to enhance the general immune function of broilers, modulate the intestinal microbiota, and increase the number of beneficial bacteria [38–42]. Park *et al.* indicated that the dietary *B. subtitis* supplementation enhanced growth, intestinal immunity, and epithelial barrier integrity when chicken were infected with *E. maxima* [42].

The use of probiotics for health and swine production has been widely reported in the literature. As early as in 1970s, some studies showed that the *Lactobacillus* supplements improved average daily gain (ADG) and FCR in starter pigs [43, 44]. Huang *et al.* demonstrated that dietary *Lactobacilli* supplementation improved ADG and average daily feed intake (ADFI) of the weaning pigs [45]. Le Bon *et al.* recently reported that using probiotics had positive effects on FCR of weaned pigs, the *E. coli* counts in the gut were reduced dramatically when compared with the non-treated pigs [46]. More studies of probiotic effects on the growth performance of pigs, including suckling, weanling, growing, and finishing pigs, have been reviewed in detail [47]. In addition to the growth performance, studies on the effects of probiotics on the reproductive performance of swine are also reported. Alexopoulos *et al.* reported that the sows fed BioPlus 2B (containing *Bacillus licheniformis* and *B. subtilis*) at a dose of 400 g/ton during the interval from 2 weeks prior to the expected farrowing date up to the weaning day improved the subsequent fertility, reduced piglet diarrhea, reduced pre-weaning mortality, and increased piglet body weight at weaning [48]. Ahasan *et al.* summarized the results of previous studies and showed that some probiotic species including *Bacillus and Streptococcus* improved the litter size and vitality, colostrum quality, milk quality, and quantity [29]. Moreover, the supplementation of probiotics was also shown to enhance the immune responses in swine. By using *in vitro* model for studying the interaction between microorganisms and the host, Liu *et al.* found that the *L. acidophilus* or *L. rhamnosus* GG treatment of the cells did not reduce the replication of porcine rotavirus, but the *L. rhamnosus* GG alone treatment post-rotavirus infection reduced the mucin secretion response induced by the virus. The *L. acidophilus* treatment prior to the virus infection increased the interleukin 6 (IL-6) response to the infection, whereas the *L. rhamnosus* GG treatment post-rotavirus infection downregulated the IL-6 response [49]. This beneficial effect in turn can lead pigs with better capacity of nutrient digestion and absorption, and better nutrient utilization and production performance [31]. Various studies demonstrated that supplement of probiotics such as *Lactobacillus fermentum*; *Lactobacillus reuteri*, and *L. plantarum* complex can improve the digestibility of dry matter, organic matter, energy, crude protein, crude fiber, and phosphorus compared with those with non-probiotic-treated pigs [50–52].

Generally, the sub-therapeutic use of antibiotics to improve growth and efficiency of farm animal production has been restricted or banned in more than 30 countries, but the application of AGPs in feed to prevent diseases and improve production performance of pigs as well as poultry is still a common practice in other parts of the world. Thus, the replacement of AGP with probiotics, to address the issue of antibiotic resistance, is very critical for public health and the global poultry and swine production.

3.1.2 Prebiotics and synbiotics

Among feed additives that have been studied as alternatives to AGPs, prebiotics have been exploited and applied broadly into swine and broiler diets in the recent decades. Gibson and Roberfroid defined prebiotics as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health" [53]. The dominant prebiotics are mannan oligosaccharides (MOS), fructo-oligosaccharides (FOS), raffinose, resistant starch, and resistant dextrins.

In broiler production, Rehman *et al.* reported that the dietary supplementation of MOS significantly improved BWG and FCR in both starter and finisher phases, and antibody titer for infectious bursal disease was improved by the interaction effect between probiotics and prebiotics, when compared with the control group [33]. Similarly, the effect of MOS from one commercial product on growth rate, gut health, and control pathogen colonization of broilers under *Clostridium perfringens* (*C. perfringens*) challenge was indicated. The results showed that FCR and BWG in broiler group treated with MOS were significantly better than the control group, and MOS level of 0.05% was enough to achieve a response competitive with that of the antibiotic. Other studies also indicated that supplementation of MOS from 0.04 to 0.08% could alter cecal microbial community composition by increasing the genus Bacteroides and decreasing the counts of coliforms and *C. perfringens* [13, 54, 55]. In addition to its effects on cecal microbiota, MOS also improved microbial community in other sections of the intestine, including the jejunum, the ileum, the jejunal mucosa, and the ileal mucosa [56, 57]. Similar to MOS, FOS, which is derived from plants, has also been reported to enhance performance and modulate the gut microbiota in broiler chickens [58–60].

In the swine production, dietary supplement of prebiotics such as MOS (0.04–0.08%) increased the growth performance of sows and piglets and modulated the composition of the swine gut microbiome [17, 61]. Similarity, Zivkovic *et al.* indicated that introduction of prebiotics (MOS and FOS) in the diets had positive effects on performances of sows and suckling piglets such as greater FI in lactating sows by 13.75%, more born piglets by 14.7% and heavier by 3.6% at birth, greater body weight of litter by 3.1% at weaning, better FI of pre-starter by 6.7% per litter during lactation [62].

There are also abundant studies indicating the beneficial effects of combination of probiotics and prebiotics termed as synbiotics. The use of synbiotics was based on the concept that a mixture of probiotics and prebiotics affects the host by improving the survival and implantation of probiotic organisms and by selectively promoting the growth or metabolism of beneficial bacteria in the intestinal tract [63]. Supplementation of diets with a synbiotic product was shown to significantly improve body weight, average daily gain, feed efficiency, and carcass yield percentage compared with controls or probiotic-fed broilers [64–67]. There is a great potential for synbiotics to be used as antibiotic alternatives for improving performance and reducing pathogenic load in the intestines of poultry. However, when combined utilization of various probiotics and prebiotics to be used as synbiotics, it is critical to carefully consider and research trials should be conducted to investigate their synergistic effect compared with the use of either product alone.

3.1.3 Organic acids

Dietary supplement of organic acids has been considered as potential alternatives to AGPs, owing to their antibacterial nature [3]. For examples, Dibner and Buttin showed that organic acids can stimulate pancreatic juice secretion and increase villi height [68]. Fascina *et al.* reported organic acids enhanced immune responses and intestinal quality of broilers [69]. Ao *et al.* claimed that organic acids can reduce gut pH, thereby suppressing pathogenic bacteria growth, which in turn enhances gut health and nutrient intake [70]. Therefore, organic acids have been widely used and shown to have significant benefits in swine and poultry production over the years.

Most of organic acids used as feed additives can be described as volatile short-chain fatty acids (e.g., fumaric, acetic, propionic, lactic, butyric acids), medium-chain fatty acids (MCFA), and long-chain fatty acids (LCFA) [71]. Adil *et al.* indicated that birds fed diets supplemented with different organic acids including butyric, fumaric, and lactic acid showed significantly higher BWG and FCR [72]. Maximum improvement was achieved in the group fed 3% fumaric acid in the diet. Addition of organic acids in broiler diets also increased the villus height in all the segments of small intestines [72]. Ma *et al.* recently reported the effect of administered levels of organic acids on intestinal health, enzyme activity, and antioxidative characteristics in broilers. The results showed an increased concentration of IgA, D-lactate, and IL-10 in the serum of broilers diets with 6000 mg/kg mixture of organic acids. Dietary of 3000 mg/kg mixed organic acid decreased the pH value of duodenum and enhanced the amylase activity of the pancreas, the tight junction protein (mainly Claudin-1, Claudin-2, and ZO-1) in the duodenum of broilers. Also, the modulated structure microbiota and the reduced abundance of *E. coli* were observed in birds fed with both high and low level of organic acids [73].

In swine, use of organic acids has demonstrated efficacy as AGPs and has positive impacts on disease prevention [74, 75]. For examples, Upadhaya *et al.* reported that the supplementation of organic acids mixture (10% malic, 13% citric, and 17% fumaric acids) to the diet of growing and finishing pigs improved the growth, digestibility of dry matter, N, and energy, decreased *E. coli* counts, and increased *Lactobacillus* counts [76, 77]. Likewise, Ahmed *et al.* indicated that weaned piglets fed the diet with 0.4% organic acid mixture (4.1% propionic, 9.5% phosphoric, 10.2% lactic, and 17.2% formic acids) and diet with 0.5% pure citric acid increased *Bacilli* and *Lactobacilli* counts and reduced *E. coli* and *Samonella* counts [78]. Li *et al.* also reported that weaned pig fed diet with 0.2% organic acid mixture (butyrate, MCFA, phenolics) and diet with 0.3% short-chain fatty acid plus MCFA improved the gut health and showed a similar growth-promoting effect as antibiotics [79]. Hong *et al.* also reported that dietary with a blend of MCFA (caprylic and caproic acids) improved the performance and nutrient digestibility in weaned pigs [80].

3.1.4 Exogenous enzymes

The use of in-feed enzymes for replacement of AGPs in swine and poultry production has been proposed for the past decades. The potentials of using enzymes are to improve the digestive and absorptive function of the gut, which can allow the host to absorb nutrients to a greater extent [81, 82]. Further, the use of exogenous enzymes may improve the integrity of intestinal mucin, increase gastric residency of feed, and reduce inflammatory responses and other beneficial effects on immune function and resilience [83]. Generally, the enzyme systems available for animal feed are derived from microbes (fungi and bacteria) through the fermentation or genetic engineering. The main classes of enzymes include phytase, carbohydrase (xylanase, cellulase, α-galactosidase, β-mannanase, α-amylase, and pectinase), and proteases [3].

The beneficial effect of various enzymes in improving the growth and feed efficiency in poultry is well documented. For examples, a meta-analysis conducted by Swann and Romero investigated the effects of a mixture of xylanase, amylase, and protease on nutrient digestibility of broiler chickens. Their results showed that the enzyme combination increased the apparent digestibility of undigested crude protein, starch, and fat by 22.7, 88.9, and 33.4%, respectively [84]. McCormick *et al.* reported that the supplementation with 500 or 1500 FTU/kg of phytase in broiler diets significantly improved the growth performance, tibia ash, and apparent ileal digestibility and retention of phosphorus [85]. Guo *et al.* indicated that 5500 U/kg xylanase supplementation of wheat-based diets improved FCR in birds irrespective of *C. perfringens* infection and elevated apparent ileal digestibility of crude protein and mRNA expression of nutrient transporters in infected birds [86]. Nuseirat *et al.* recently showed that birds fed with the combination of xylanase (10XU/g feed) with probiotic *Bacillus* spp. ($1x10^5$ CFU/g feed) showed the improvement of live performance, reducing environment microbial load, as well as improving energy utilization [87].

The supplementation of swine diets with exogenous enzymes to enhance performance is not a new concept, and research articles in this field date back to the 1950s. However, the response of pigs to supplementation with traditionally fermented enzymes is less consistent than has been observed with poultry. It is possible that exposure to the low pH in the stomach of the pig is either partially or totally denaturing the enzyme accounting for the lower magnitude of responses obtained when carbohydrases are fed to pigs compared with poultry [20]. Recently, several carbohydrases have been developed by genetic engineering, which have considerable potential for animal feed application. Diets supplemented with recombinant β-mannanase increased weight gain by 16.4% and feed efficiency by 17.7% in growing pigs, while pigs fed the diet with traditionally fermented β-mannanase improved BWG and FCR by 3.4 and 4.9%, respectively [88, 89].

3.1.5 Phytogenics

Phytogenic compounds have been widely recognized as potential alternatives to AGPs. Phytogenics, also referred as phytobiotics or botanicals, are plant-derived natural bioactive compounds used to enhance animal productivity and health [90]. A wide variety of plants and their extracts including herbs and spices (e.g., garlic, cumin, pepper, mint, cinnamon, turmeric, clove, alfalfa, thyme, sumac, aloe vera, mulberry leaf); essential oils (plant-extracted oils of the families *Alliaceae* (onion), *Apiaceae* (celery), *Asteraceae* (aster), *Lamiaceae* (oregano, thyme, lavender, peppermint, sage oils), *Lauraceae* (cinnamon oil), *Liliaceae* (garlic oil), *Myrtaceae* (tea tree oil), *Poaceae* (grass) and *Rutaceae*); and oleoresins (volatile and nonvolatile components responsible for the characteristic flavor and aroma) are often classified as the common phytogenic compounds [3, 15, 91]. In addition to these phytobiotics, essential oil nanoemulsion (NE) that is known as an isotropic mixture, a combination of oil and surfactant, which spontaneously forms fine emulsions of oil in water, is also considered as a type of potential phytogenics [92].

In recent years, the phytogenics used individually or as blends in feed have been investigated and shown positive effects on feed efficiency, antimicrobial, and immune stimulating in poultry and swine production. For examples, the supplementation with various levels and forms of Aloe vera [93–96], garlic [97–99]; pepper [100–103]; turmeric [104–107] in feed improved the productivity of both broilers and laying hens, enhanced the immune responses, increased *Lactobacillus* counts, and reduced *E. coli* counts. Also, Guo *et al.* showed a significant increase in BWG and improvement in feed efficiency when broilers were given diets supplemented with a mixture of 14 herbs [108]. A similar study showed that a mixture of essential oils (EO) derived from caraway, basil, lemon, laurel, sage, oregano, thyme, and tea enhanced the growth of broilers [109]. Likewise, Eucalyptus and peppermint EO showed higher hemagglutiin-inhibition antibody titers against both avian influenza and Newcastle vaccines as compared with control [110]. However, the volatile bioactive components in the EO make it possess the antimicrobial activity, and also become a limiting factor in EO application. Nanoemulsions carrier systems can be a solution to tackle that problem. Nanoemulsion is increasingly being utilized for improving the bioavailability of certain types of volatile components, where most of them are lipophilic substances. Noori *et al.* found that nanoemulsion-based edible coating containing ginger EOs can help increase the life of breast fillets [111]. Similarly, Keykhosravy *et al.* also reported that edible chitosan-loaded nanoemulsions containing two essential oils (Zataria Multiflora Boiss and Bunium persicum Boiss) could play an effective role in the preservation of the microbial qualities of turkey meat [112].

A multitude of studies about effects of phytogenics on swine growth and health have been also investigated. For instances, a recent study reported that pigs fed diets with a phytogenic mixture (including garlic oil, cinnamic, aldehyde, thymol, carvacrol and eugenol) and/or encapsulated sodium butyrate increased ADG and FCR [113]. An earlier study also showed that piglets fed with herbal extracts (sage, lemon balm, nettle, and purple coneflower) grew faster than control animals and showed significantly higher final average body weights. The herbal extracts improved the structure of the ileal epithelium by considerably increasing the villus height. Better digestibility of nutrients could be due to higher villi in these animals [114]. Supplementing EO has been reported to improve the immune status of piglets after weaning, as indicated by an increase in lymphocyte proliferation rate, phagocytosis rate, as well as in IgG, IgA, IgM, C3, and C4 serum levels [115–117].

In addition, there is also limited information concerning the interaction between EO and feed ingredients. Jamroz *et al.* investigated the influence of diet type (corn vs. wheat and barley) on the ability of plant extracts (100 mg/kg containing 5% carvacrol, 3% cinnamaldehyde, and 2% of capsicum oleoresin) to modify morphological and histochemical characteristics of the stomach and jejunal walls in chickens [118]. Their results demonstrated significantly more jejunal wall villi in birds fed the maize diet with plant extracts. The incorporation of carvacrol, cinnamaldehyde, and capsicum oleoresin promoted positive and negative changes in digestive function, intestinal epithelium, microbial ecology, and fermentation in weaned pigs depending on the amount of protein included in the diet [119]. In general, phytogenics enhance the production of digestive secretions and nutrient absorption, reduce pathogenic stress in the gut, exert antioxidant properties, and reinforce the animal's immune status, which help to explain the enhanced performance observed in swine and poultry [120].

3.1.6 Antibody therapy

Antibodies can cause agglutination of bacteria and viruses, thus lessening the number of infectious units, restrict mobility of the pathogen, and inhibit microbial metabolism and growth when antibodies bind to bacterial transporter proteins [121]. In poultry, maternal antibodies are transmitted to the offspring *via* the yolk of the eggs [122]. As a consequence, egg yolk was one of the sources of antibodies that has gained much interest as an inexpensive nonantibiotic alternative for prophylaxis and therapy of infectious diseases in an agricultural setting [123]. Several studies have successfully tested the ability of egg yolk immunoglobulins to control infectious diseases in chickens [3]. For examples, Lee *et al.* [124, 125], Xu *et al.* [126]; Juarez-Estrada *et al.* [123] demonstrated that the oral immunotherapy using egg yolk IgY against *Eimeria* sp. represents an effective and natural resource against severe *E. tenella, E. acervulina, E. maxima* infection favoring the gradual withdrawal of the anticoccidial drugs and antibiotics. Rahimi *et al.* investigated the effect of supplementation of *Salmonella enteritidis*-specific IgY on 3-day-old infected chicks and found lower fecal shedding and *S. enteritidis* concentration in the cecal content. They also observed a lower isolation of *S. enteritidis* from the liver, spleen, and ileum of birds [127]. Chalgoumi *et al.* also reported that antibodies simultaneously directed against *S. enteritidis* and *Salmonella typhimurium* can be efficiently produced in the same egg yolk of hens immunized with *S. enteritidis*— bacterial outer membrane proteins and *S. typhimurium*—bacterial outer membrane proteins in a half-dose mixture. This antibody mixture can be used as an additive in broiler chicken diets to fight both *S. typhimurium* and *S. enteritidis*, which are the predominant cause of salmonellosis in human often associated with poultry meat consumption [128]. In addition, *Campylobacter jejuni* is one of the most important causes of foodborne gastroenteritis. Chickens are considered a reservoir host of *C. jejuni*, and epidemiological studies have shown that contaminated chicken meat is a primary source of human infection. AI-Adwani *et al.* investigated the effect of IgY against the five *C. jejuni* colonization-associated proteins or CAPs (CadF, FlaA, MOMP, FlpA, and CmeC). They showed that α-CadF, α-MOMP, and α-CmeC IgY significantly reduced adherence of *C. jejuni* to the chicken hepatocellular carcinoma cells, suggesting that these α-*C. jejuni* CAP-specific IgY may be useful as a passive immunotherapeutic to reduce *C. jejuni* colonization in chickens [129].

Furthermore, it has been well documented that oral administration of IgY acts as potential AGPs for controlling diarrhea and exerting growth-promoting activity in pigs [130]. Diarrhea due to enterotoxigenic *Escherichia coli* (ETEC) is by far the most common enteric colibacillosis encountered in neonatal and post-weaned pigs. A research group of Jin et al. and Marquardt et al. investigated the effects of egg-yolk antibodies against ETEC K88 in *in vitro* piglet intestinal mucus and in neonatal and early-weaned piglets. The *in vitro* studies showed that anti-K88 antibodies from chicken egg-yolk when added to ETEC K88 prevented their binding to receptors in the mucus isolated from the intestine of piglets. Further, they also indicated that the neonatal and early-weaned piglets that received the egg-yolk antibodies were protected against ETEC infection [131, 132]. Porcine epidemic diarrhea virus (PEDV) is another important enteric viral pathogen that is responsible for neonatal piglet diarrhea. The studies of Weiping *et al.* and Cui *et al.* revealed that the survival rate was increased significantly in pigs treated with IgY compared with a control group suggesting that IgY can be an alternative method for conferring protection in piglets against PEDV [133, 134].

3.1.7 Antimicrobial peptides

The antimicrobial peptides (AMPs) are a class of short peptides widely found in nature, and they are an important part of the innate immune system of different organisms. They have inhibitory effects against bacteria, fungi, parasites, and viruses [135]. Since the emergence of antibiotic-resistant microorganisms, the AMPs have rapidly captured attention as potential drug candidates for replacement of antibiotics [135]. A recent review showed that there are more than 700 AMPs known to exist. And they are generally classified based on source, activity, structural characteristics, and amino acid-rich species [20]. Among AMPs, interest in bacteriocines has been widely increased due to their antibacterial properties [136], and a few hundred bacteriocines were currently described [137]. Numerous evidences showed the potentials of using bacteriocines to improve the animal growth performance and inhibit the pathogens growth. Ogunbanwo *et al.* investigated the potential therapeutic efficacy of bacteriocin and bacteriocin-producing *Lactobacillus plantarum* strain in an experimental *E. coli* infection of broiler chickens. They found that the significant reduction of clinical signs of colibacillosis, improvement in the growth rate of the studied birds, lower percentage of re-isolation of *E. coli,* and reduction of abnormally high globulin were exhibited in chickens infected with *E. coli* and are treated with bacteriocin or bacteriocin-producing *L. plantarum* [138]. Grilli *et al.* reported that pediocin A, a bacteriocin produced by *Pediococcus pentosaceus*, was highly active against *C. perfringens* in an *in vitro* assay [139]. They also showed that a partially purified fraction of pediocin A, alone or in association with the producer strain, significantly improved the growth performance of broiler chickens challenged with *C. perfringens* [139]. Wang *et al.* demonstrated the efficacy of bacteriocin (albusin B) as a potential alternative for feed antibiotics. In this study, the albusin B, which is produced by *Ruminococcus albus* 7, has been reported to improve broilers body weight gain, elevate mRNA expression of sGLT1, GLUT2, and PEPT1 in the jejunum, decrease *Samonella* load, and increase the fecal *Lactobacillus* counts [140]. Futhermore, nisin, one of the most commonly used bacteriocins for food preservation, has been also reported to enhance the growth performance and modulate GIT ecology of broilers [141].

Post-weaning diarrhea is responsible for major economic losses in the swine industry. ETEC is the major cause of this enteric disease in pigs, being responsible for approximately 50% of piglet mortality. Colicins, a class of bacteriocines, have been shown to be effective against ETEC strains and could be a potential alternative to antibiotics in swine production [142]. Culter *et al.* reported that dietary inclusion of colicin E1 was shown to decrease the incidence and severity of post-weaning diarrhea caused by F18-positive ETEC [142]. In addition to ETEC, *Streptococcus suis* is a major swine pathogen that has been associated with severe infections such as meningitis, arthritis, endocarditis, pneumonia, and septicemia, and major responsible for economic losses in the swine industry [143]. The nisin-producing strain *L. lactis* ATCC 11404 proved to be capable of inhibiting the growth of *S. suis*. And, all the *S. suis* isolates tested were susceptible to purified nisin, with the minimum inhibitory concentration ranging from 1.25 to 5 μg/mL [143]. Furthermore, Hu *et al.* have demonstrated that the *Lactobacillus gasseri* LA39 and *Lactobacillus frumenti* as potential microbes associated with diarrhea resistance in early-weaned piglets, and thus, microbiota-derived bacteriocin gassericin A targets host intestinal epithelium may also help prevent diarrhea suggesting that secretory gassericin A may also serve as a specific biomarker for diarrhea resistance in early-weaned piglets [144]. In general, bacteriocins not only represent alternatives to AGPs but are also considered as a promising therapy for preventing and controlling animal diseases.

3.1.8 Bacteriophage therapy

Bacteriophages are bacteria-specific viruses that have been utilized as therapy to against pathogens and are thus considered as alternatives to antibiotics in the age of multi-drug resistance. The bacteriophage is probably the most abundant biological entity on the earth with estimation of 10^{31-32} phages [145]. Bacteriophages can be generally classified based on morphology, nucleic acid, phage life cycle, and bacterial target and site. Regarding the morphology, the tailed phages constitute the order Caudovirales, which is divided into families: *Siphoviridae* (61% of tailed phages), *Myoviridae* (25%), and *Podoviridae* (14%) families [146]. Further, phages are categorized into two types based on their life cycle, namely virulent and temperate. But temperate phages cannot be utilized as antimicrobial agents for therapeutic purposes because they may transfer genetic material from one bacterial cell to another. In contrast, virulent phages rapidly exterminate the bacteria, enabling them to be used as efficient antibacterial agents [147]. There has been an explosion of research and interest in the usage of bacteriophages in the poultry industry. Most of the phage-based products are targeted against the main foodborne pathogens, such as *C. jejuni*, *Salmonella* spp. (*S. Enteritidis*; *S. Typhimurium*), *E. coli, Listeria monocytogenes, Staphylococcus aureus,* and *C. perfringens*. A recent study demonstrated that a phage cocktail containing virulent Campylobacter phages was used by oral route to treat broiler chickens challenged with *C. jejuni* [148]. Bacteriophage predation of *C. jejuni* did not affect the microbiota but selectively reduced the numerous of *C. jejuni*. They have concluded that bacteriophage control to reduce *C. jejuni* levels in chickens could reduce human exposure and disease acquired through the consumption of contaminated poultry products [148]. The previous similar studies have been performed to investigate the effects of phages against *Salmonella* spp. infection in chicken. Bardina *et al.* and Hong *et al.* reported that a cocktail phage containing virulent *Salmonella* spp. significantly reduced the *Salmonella* cell numbers in chicken challenged with *Samonella* by oral administration [149, 150]. Consequently, in 2019, the first results were reported from the use of Salmonella phages at a commercial scale in the poultry production system [151]. In addition, *E. coli*-associated infections are widely distributed among poultry of all ages and categories. Huff *et al.* have demonstrated that aerosol spray of bacteriophages administered to 7-day-old chickens prior to the triple challenge with *E. coli* can prevent airsacculitis caused by *E. coli* [152]. Eid *et al.* indicated that phage therapy found to be an attractive option to prevent and control multidrug-resistant colibacillosis in broilers [153].

Furthermore, although phage therapy has been used successfully in swine since the early 1920s, it has only recently started to attract the attention of the research community as a tool for use against bacterial diseases in swine [147]. For instances, Kim *et al.*; Yan *et al.*; and Gebru *et al.* reported that dietary supplementation with anti-*Salmonella* phage improved dry matter, nitrogen, and energy digestibility for growing pigs, and thus improved the performance of pigs [154–156]. Albino *et al.* isolated a *Salmonella* phage belonging to the *Podoviridae* family, which significantly reduced *Salmonella* counts in an *in vitro* experiment [157]. Morita *et al.* investigated the characterization of a virulent bacteriophage, named PP01, specific for *E. coli* O157:H7 isolated from swine stool sample. The phage concentration in stool estimated was 4.2×10^7 PFU/g of the *E. coli* O157:H7. The results indicated that phage PP01 might suppress its host *E. coli* O157:H7 in the GIT ecosystem [158].

3.1.9 Metal and clay minerals

The use of trace minerals as alternatives to antibiotics in animal production has been gaining increasing attention in the recent years. Copper (Cu), a crucial trace element involved in various physiology and biochemical processes, includes hemoglobin synthesis, wound healing, bone development, and more importantly serves as a cofactor for many metabolic enzymes [20, 159]. However, animals can only absorb a small fraction of Cu and the most is discharged into the environment. Hence, the use of Cu as a growth promoter is not only important to health but also to environmental issue. In recent years, many studies have reported Cu nanoparticles (Cu-NP) as a promising alternative to AGPs. The main purpose of using Cu-NP as feed additives in poultry and swine production is to improve the growth performance, and reduce the pathogen growth and excretion of Cu into the environment [159]. Zheng *et al.* indicated that broilers were fed with supplementation of 2 g/kg Cu-NP in diet and exhibited the regulation of the intestinal microflora, the growth of beneficial bacteria, and inhibition of harmful ones, enhanced nitrogen metabolism, and reduced ammonia emission [160]. Similarly, the addition of Cu-NP in pig diets also improved the digestibility of crude fat and energy, enhanced IgG γ-globulin and total globulin protein levels, and increased superoxide dismutase activity [161].

In addition to Cu, zinc is another essential trace mineral that plays an important role in cell proliferation, immune response, reproduction, gene regulation, and defense against damage, and also has been commonly used as a growth promoter in animal production [3]. Nguyen *et al.* reported that chickens fed with different levels of nanoscale metal components including iron, Cu, zinc oxide, and selenium exhibited improved growth (hen's body weight at 38 aged weeks and egg weight ranged from 2.53 to 2.60 kg/hen and 50.86–51.55 g/egg, respectively), the more efficient absorption of feed minerals, consequently decreasing the risk of environmental pollution [162]. Thema *et al.* evaluated different combinations of a probiotic (*B. licheniformis*), an organic acid mixture (benzoic and fumaric acids), a protease enzyme, and chelated minerals (Cu, Zn, and Mn) as alternatives to zinc-bacitracin antibiotic. They concluded that the diets could replace zinc-bacitracin antibiotic in broiler diets as they promoted similar growth performance and carcass characteristics [163]. Furthermore, the use of zinc in the pig diets also has positive effect on the growth performance. Kociova *et al.* investigated the effect of two formulations of zinc phosphate-based nanoparticles (ZnA and ZnC NPs) on pig growth performance, intestinal microbiota, antioxidant status, and intestinal and liver morphology. They found that all piglet groups fed with ZnA exhibited significantly higher piglet weight gain. The substantial occurrence of *E. coli* virulence factors was found on day 5, mainly in fimbrillary antigen and thermostable toxins, except for piglets fed by ZnC. The antioxidant status was affected only by ZnA group of piglets. The positive changes in the liver and the intestinal morphology of piglets with NPs were also observed [164].

Moreover, clays are crystalline, hydrated aluminosilicate molecules composed of alkali and alkaline earth cations along with small amounts of various other elements. Clay minerals are also used in particular in animal nutrition due to their absorption and decontamination properties significantly contributing to the health of the animals [165, 166]. For instances, AI-Beitawi *et al.* [167] investigated the effect of three levels of nanoclay minerals (1, 1.5, and 2%) on growth performance, internal organs, and blood biochemistry of broiler chickens compared with vaccines and antibiotics. The results showed that 2% nanoclay minerals fed at the two intervals significantly improved the growth performance of broiler chickens. Blood biochemistry, high-density lipoprotein, which are known to be beneficial for humans, significantly increased by feeding 1.5% nanoclay minerals at the two ages compared with control groups and other treatments. A recent report on pig showed that supplementation of the diet with 3 g/kg of an aluminosilicate mineral product comprising 72.6% SiO_2, 8.18% Al_2O_3, 9.42% Fe_2O_3, 5.25% K_2O, and 1.41% Na_2O could increase weanling pig performance [168].

3.2 CRISPR-Cas9 approach: a gene editing tool to against antibiotic-resistant bacteria

The CRISRP-Cas9 is a promising gene editing tool for controlling the prevalence of antibiotic resistance genes in bacterial populations and eliminating pathogens with high precision [169]. Thus, in recent years, various studies investigated the potentials of CRISPR-Cas9 system as an alternative therapeutic to antibiotics [21, 170].

As above described, the bacteriophages, a tool for use against bacterial diseases, has been widely utilized in the veterinary medicine. However, due to the structural diversity of phages, traditional nanoparticle delivery strategies are not practical. Drug therapy delivery often includes using different nanoparticles to absorb treatment cargoes. But these methods are ineffective for large, non-symmetrical phage cargoes, and pore sizes are too small to absorb phages. A potential alternative approach has been recently investigated is development of phages encoded with CRISPR-Cas9 offering species specific delivery of novel antibacterials. Citorik *et al.* and Bikard *et al.* use phage encoded with CRISRP-Cas9 to target antibiotic resistance invirulent strains of *E. coli* and *S. aureus*, respectively [171, 172]. They observed that the addition of phage encoded with CRISPR-Cas9 resulted in rapid killing of specific bacteria. In brief, although CRISRP-Cas system is far from commercial use, this alternative approach is currently being explored and considered as revolutionary tool in the fight against antimicrobial resistance.

4. Conclusion

Alternatives to AGPs are essential tools for minimizing the antimicrobial resistance crisis, reducing antibiotic use, and increasing animal productivity. The promising alternative approaches could be probiotics, prebiotics, organic acids, enzymes, phytogenics, AMPs, hyperimmune antibody, bacteriophages therapy, and CRISRP-Cas9 system. And, it is also believed that there is no single alternative that can replace the current use of antibiotics. It, therefore, is anticipated that the controlled combination of alternatives and/or with advanced tools may help address the issue of antibiotic resistance significantly such as CRISRP-cas9 encoded phages, synbiotics, essential oils, mineral, recombination enzymes.

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Chapter 6

Swine Production: Probiotics as an Alternative to the Use of Antibiotics

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Abstract

Animal food production is one of the most powerful European economic sectors; however, this sector is facing new challenge due to the development of bacteria with resistant genes, and consequently, restriction on the administration of antibiotics. Limitation, at the moment, is focused on those antibiotics employed in human medicines. Therefore, it is necessary to improve as much as possible animals' health and reduce diseases. Among others, alternatives include adequate animal handling, hygienic facilities, quality food, or vaccines. Probiotics also arise as a good alternative due to their already known properties as intestinal microbiota modulators, improving the immune functions and reducing the risk and the development of illness. Significant data can found scientific literature that demonstrates probiotics benefits when they are administrated to the animals through diet. However, to be able to apply all these findings in a specific animal species, at a particular production animal life stage and at a industrialize scale, it is necessary to compile and organize reported information. This chapter presents the most recent and relevant finding on the use of probiotics in swine production.

Keywords: probiotics, swine, animal production, antibiotics, antimicrobial-resistant bacteria, pork

1. Introduction

The abuse on the use of antibiotics in veterinary and human medicine has triggered the exponential development of resistant genes. These genes gave to the microorganism the ability to resist antimicrobial treatments, especially antibiotics; therefore, infections become more a more difficult, since usual therapies are not effective anymore. Special attention is required for the development of superbugs, resistant bacteria to most antibiotics, such is the case of *Salmonella*, *Campylobacter*, and *Escherichia coli*, resistant to a wide range of commonly used antibiotic [1]. It is estimated that in 2050, there will be more deaths from multidrug-resistant bacteria than from cancer worldwide [2]. According to the Spanish Ministry of Health, Social Services and Equality (2014) [3] antibiotics with critical importance for human health are macrolides (7.5%), polymyxins (6.6%), fluoroquinolones (1.9%), and cephalosporins of third and fouth generation (0.2%).

Implications of resistant bacteria in food safety are no longer in doubt, and different studies and investigations have shown that food contain genes of resistant bacteria, and these genes can be transmitted from animal to human through the food chain [4–7]. Therefore, food safety authority must control transmission of pathogen, residue of veterinary medicines, resistant bacteria, and resistant genes. Maciel *et al*. [8] isolated resistant *Salmonella* spp. from swine and poultry products, and theirs results indicated that 55% of the isolated were resistant to three or more classes of antibiotics (amoxicillin, ampicillin, ceftiofur, enrofloxacin, florfenicol, gentamycin, tetracycline, and sulfa-trimethoprim). This is not only a human health problem, which causes more than 33000 deaths in Europe each year [9] compared to those caused by the combination of influenza, tuberculosis, and HIV/AIDS, but also a veterinary problem and, consequently, a food safety problem. Forslund and collaborators [10] observed in human stool samples higher resistance genes to antibiotics employed in human and veterinary medicine than to those antibiotics only employed in human medicine.

Aiming to control and reduce the development of resistant genes, international organizations and public bodies, organisms including WHO, have establish different strategies and protocols to reduce the use of antimicrobials in human and veterinary medicine with high restriction for the food animal production. As a result, the European Regulation 2019/6 was published in 2019 [11], this regulation established limitation on the use of antibiotics in veterinary medicine, avoiding their application for routine prophylactic and metaphylactic, with special restriction to those antimicrobials that are of critical importance for preventing or treating life-threatening infections in humans. The objective of this regulation is to warrant food safety and human health by protecting consumers from the consumption of resistant genes or residues of antibiotic through food of animal origin.

Therefore, the European animal food-producing sector must face the great challenge of reducing, and avoiding as much as possible, the use of antibiotics, without spreading pathogens. Possible alternatives include biosecurity plans, hygiene measures, infectious disease prevention protocols, correct housing design, production integration systems, correct animal management to reduce stress, the use of quality food and water, vaccination programs and the use of bioactive substances such as probiotics, prebiotics, antioxidants, and vitamins, among others [3, 12].

This chapter aims to collect the most employed probiotic species in swine production as well as to report the results to evaluate if, effectively, the use of specific probiotics, administered alone or as a mixture of species or strains may an interesting alternative to antibiotics to prevent diseases. Other aspects as the average daily feed intake or the daily weight gain have been evaluated.

2. Pig production

The livestock sector is the main produce of animal-based protein for human consumption and is the pork sector which accounts for more than one-fourth of the total protein consumed worldwide [13]. In the last decade, this sector has increase in number of pigs, number of pig farms, and meat production. According to the FAO statistical yearbook published in 2021 [14], in 2019 a total of 337 million tonnes of meat were produced, 44% more than in 2000. After China, the UE is the second leader pig meat producer, being Spain the fourth producer, after China, EEUU, and

Germany. Only in Europe, 150 million of pigs are reared representing the largest livestock category before bovines. Pork meat demand will certainly increase due to continuous human population growth, and this will increase farms size and numbers, leading to more animals' densities in the farms. The major problem of swine production is infection disease as they are transmitted easily between animals, and they decrease productivity by reducing animal growth and in many cases causing animal death. Pig diseases need to be controlled during meat production, not only to obtain major production benefits but also to warranty food safety.

2.1 Production cycle and diseases

The prevention of infection is of great importance in food-producing animals to guarantee and maintain animals' health to achieve good production rates and quality food. To control and reduce disease in swine production, it is important to understand the production cycle and the most prevalent pathogens in each phase.

2.1.1 Gestation and lactation phase

This phase lasts for 114 days (gestation) and 21–28 days more for lactation. At the fourth week of gestation, sows are separated in groups of approximately 15–20 individuals. During gestion and lactation, sows are subject to various stress factors such as farrowing, lactation, housing conditions, management, as well as feeding [15]. Parvovirus is the most frequent disease at this stage, and sow vaccination is required. Additionally, numbers of newborn piglets can decrease by increasing mummified fetuses and neonatal deaths [16] by virus attacks such as porcine teschovirus (TVP), circovirus type-28 (PCV2), rotavirus, reproductive syndrome virus, and porcine respiratory virus (PRRS). In fact, mortality rate of piglet, at this stage, is one of the main problems for profitability in swine production [17].

Intestinal microbiota of both mother and piglet plays a relevant role in animals' health. At this stage, piglets are sensitive to diseases since they have an undeveloped digestive and immune systems, and they can easily die if infections are not treated quickly. Key aspects for piglet microbiota development include, among other, the type of parturition, use of antibiotics, and lactation period. Sow milk provides to the piglets all the necessary nutrients and antibodies to grow and face infections [18]. Piglet mortally, at this stage, is related to diarrhea caused by *E. coli*, *Salmonella* spp., *Campylobacter* spp., rotavirus, coronavirus, and protozoa of the genus *Cryptosporidium*. Other microorganisms and diseases that can also affect piglets' health are *Actinobacillus suis*, which causes respiratory processes, porcine circovirus type 1 and 2, responsible of swine dermatitis and nephropathy, *Streptococcus suis*, causing respiratory disease, African swine fever and Classical swine fever, causing respiratory and dermatological symptoms and sudden deaths, foot-and-mouth disease virus responsible for dermatological, respiratory, and lameness symptoms, and respiratory syndrome virus (PRRS) that can cause neonatal deaths [16].

2.1.2 Piglet weaning phase

Piglets are separated for theirs mothers at 3–4 weeks of age (21–28 days), although it will naturally occur at 17 weeks (119 days), and this transitional phase or weaning lasts until 60–70 days of life. Groups of animals, of 20 to 25 individuals from different litters, are now formed. Separation of piglet from their mothers is a sudden, quick, and stressful for the animals, because a lot of changes takes place; animal diet and social and environmental living conditions are modified. These modifications have an impact on the health status of the piglets and can cause decreases in its performance and even on his death. The critical moment are the first 5 days of the transition, which are very important, and the environmental, handling, and feeding conditions must be checked and verified very well in order to reduce the incidence of post-weaning and post-weaning diarrhea and improve the pig growth [16, 18, 19].

At this stage, the piglet intestinal microbiota undergoes changes, losing diversity, since in the previous stage it was largely modulated by lactation. Decrease has been observed in the bacteria of the *Lactobacillus* and *Clostridium* genera and *Escherichia coli* species, and their increase has been associated with the appearance of enteric infections that can lead to diarrhea [18, 19]. Among other, most common pathogens in this period are *A. suis*, *Pasteurella multocida*, *S. suis*, and *Bordetella Bronchiseptica* causing respiratory processes, porcine circovirus type 1 and 2, porcine herpesvirus type I responsible for Aujeszky's disease, *Staphylococcus hyicus* responsible for epidermitis exudative, porcine epidemic diarrhea virus, foot-and-mouth disease virus, and PRRS virus [16].

2.1.3 Fattering phase

This phase begins 60–70 days after birth, when the animal weighs around 30 kg. It lasts for 80–100 days, until the animals reach the optimum weight for slaughter, which is approximately 100 kg [16, 20]. Diseases most frequently reported in this phase are those caused by PRRS virus that causes respiratory symptoms and weight loss in pigs, swine circovirosis caused by PCV2, swine flu or influenza, caused by

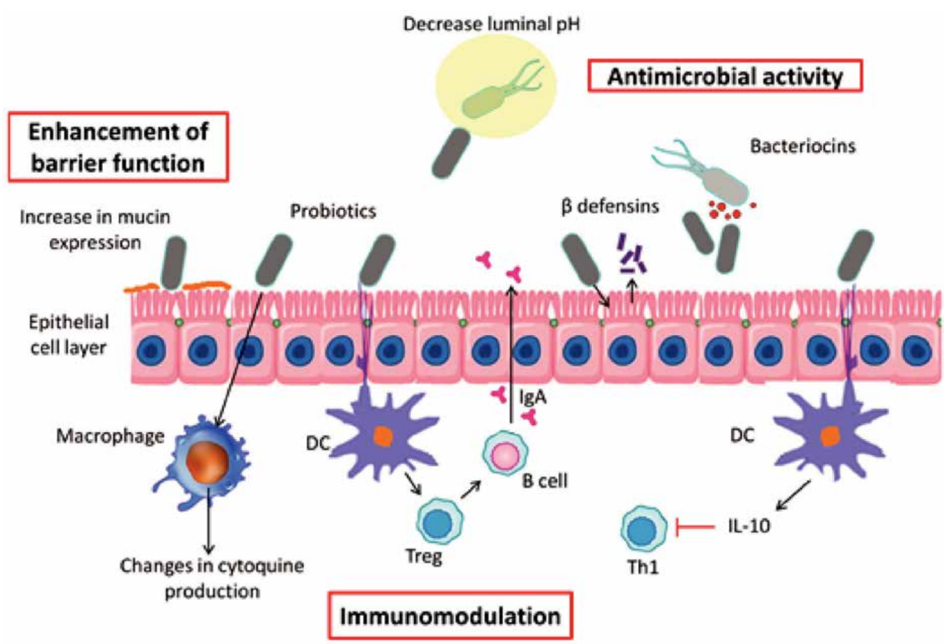

Figure 1.
A schematic diagram about probiotic mechanisms within the intestine. Reproduced from Cerdó et al. [26] (CC BY license).

influenza virus type A, and swine enzootic pneumonia caused by the bacterium *Mycoplasma Hyopneumoniae*. Other agents that also cause relevant diseases during this phase are coronavirus, that gives rise to transmissible gastroenteritis (TGE), *Salmonella* spp., mainly associated with diarrhea, *Lawsonia intracelluralis*, that causes ileitis [21, 22], *Brachyspira hyodysenteriae*, a spirochete that causes swine dysentery in fattening pigs [23] and, *Actinobacillus pleuroneumoniae*, that causes porcine pleuropneumonia and pastry/streptococcal pneumonia [16].

3. Probiotics: an alternative to reduce antibiotics

The use of bioactive substances is a possible and good alternative to reduce the use of antibiotics. Probiotics, in particular, have attracted attention from the scientific community and producers due to their already recognized efficacy in humans.

According to the Food and Agriculture Organization of the United Nations (FAO)/WHO, a probiotic is defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [24]. Numerous studies have been shown the beneficial effects of probiotics for human and animal, including the swine sector. Positive effects include modulation of the intestinal microbiota, regulation of the immune system, as well as improvement of growth, efficiency of feed conversion, and reproductive improvement of pregnant sows [25].

Probiotics can act against pathogenic microorganisms through various mechanisms including anti-adhesive effect, production of antimicrobial substances, strengthening of the epithelial barrier of the intestine, and modulation of the immune system (**Figure 1**) [19]. The anti-adhesive effect is characterized by the ability of probiotic bacteria to adhere to intestinal epithelial cells, taking place a competition between probiotic and the pathogen for the same receptor. In addition to competitive exclusion, other modes of probiotic anti-adhesion described are degradation of carbohydrate receptors through the secretion of proteins, establishment of a biofilm, production of analogs receptor, and induction of biosurfactants [27]. Probiotics can also produce antimicrobial substances such as antimicrobial peptides, also called bacteriocins, which are produced, among others, by lactic acid bacteria, and they can also generate deconjugated bile acids with superior antimicrobial activity to bile salts synthesized by the body itself [28]. Another mode of action of probiotics is through the strengthening of the intestine epithelial barrier [28]. Probiotics are also capable to modulate the immune system as it can be observed in **Figure 1**.

4. Probiotics applied in swine production

Probiotics tested in swine include a variety of bacteria, and they were tested in the three main stages of production: gestation and lactation, piglet weaning, and growing and finishing phase. The most employed species include *Bacillus subtilis*, *Clostridium butyricum*, *Lactobacillus acidophilus, Bacillus licheniformis*, *Enterococcus faecalis*, and *Saccharomyces cerevisiae*.

4.1 *Bacillus* spp.

From the genus *Bacillus*, different species were tested such as *Bacillus coagulans* [29], *Bacillus cereus* var. Toyoi [30, 31], *Bacillus licheniformis* [32], *Bacillus mesentericus* [33], *Bacillus pumilus* [34], and *Bacillus subtilis* [35]. Even though the strain is a very important factor, not all the strains from the same species have the same effects and, therefore, for the reproduction of the study, not all the studies available in the literature reported the strain tested. In fact, out of eight studies that administrated *B. subtilis* to pig, only four indicate the strain, and in each case, a different strain was reported: *B. subtilis* PB6, C-3102, DSM 5750, and DSM 32540.

B. subtilis is the species for which more studies were found. Data indicate an increase in reproductive performance when probiotic was administrated to sows. Additionally, Zhang *et al*. in 2020 [36] reported an increase of 34% weight of the litter and an increment of 10% of survival in suckling piglets when *B. subtilis* PB6 was administrated to 32 pregnant and lactating sows. On the other hand, Menegat *et al*. in 2019 [35] did not observe an increase in litter size and weight when sows received a dietary supplement with *B. subtilis* C-3102, but they did find a 2.1% higher piglet survival rate in the supplemented sows group and 2% increase in piglet intake compared to the control group. According to Zhang *et al*. [36], *B. subtilis* PB6 can inhibit the growth of pathogenic bacteria in the intestine of the pig as it has survival and germination characteristics in the tract, capable of forming a biofilm and secreting antimicrobial compounds. Likewise, Menegat *et al*. [35] observed that the administration *B. subtilis* C-3102 increased *Lactobacillus* population approximately 2% (1.08 $\log_{10}$ CFU/g) during pregnancy and (0.8 $\log_{10}$ CFU/g) lactation in supplemented sows. Improvement of sow milk quality was also reported, and improvement in fat and protein contents on the milks' sows supplemented *B. subtilis* DSM 5750 and *B. licheniformis* DSM 5749 were 3.28% and 5.81% higher than in the control group. Sow's milk of supplemented animals also increased lactose content, 2% compared to the control group, having a better nutritional value for the piglets [32].

B. subtilis also improved growth performance when it was administrated during piglet weaning phase. Thus, He *et al.* [34] indicated that supplementation with *B. subtilis* DSM 32540 to piglet, at the weaning phase, challenged with F18 enterotoxigenic *E. coli* (ETEC F18) improving their growth performance; the increase was 11% for the daily weight gain and 10% for the average daily feed intake. Results indicate that that supplemented piglets improved metabolization of nutrients and optimized the energy, improving their growth and fighting against infection; however, these finding were not observed when the strain *B. pumilus* DSM 32539 were employed as probiotic for the piglets [34].

At the growing and finishing phase, administration during 104 days of *B. coagulans* (strain no reported) improved a 4% the daily weight gain and the average daily feed intake and modulated the intestinal microbiota, increasing 3% the abundance of Lactobacillus and decreasing a 6% the *E. coli* [29]. Other authors administrated *B. subtillis* combined with *L. acidophilus* and *S. cerevisiae* to 150 pigs, and at the growing and finishing stage, for 10 weeks, the mixture of bacteria showed to increase 1% the *Lactobacillus* population (0.07 $\log_{10}$ CFU/g) and decrease 1% the *E coli* (0.05 $\log_{10}$ CFU/g).

Three different studies reported supplementation with *B. licheniformis*, but only one indicated the strain. Alexopoulos *et al.* [32] supplemented pregnant and lactating sows with *B. licheniformis* DSM 5749 and *B. subtilis* DSM 5750, for 14 days before farrowing and until wean. Supplemented animals lost less body weight during lactation, approximately 3.5 kg. Similar findings were evidenced in litters of supplemented sows, showing 0.1 kg more per piglet in the 14 days postpartum. Possible due to the fact that incidence of diarrhea during the lactation period was significantly lower. Pan *et al*. [37] also reported lower (55%) severity of diarrhea caused by enterotoxigenic

E. coli K88 in piglet to which a mixture of *B. licheniformis* and the yeast *S.* cerevisiae was administrated, 12 days after weaning. In fact, in supplemented animals, *E. coli* concentration decreased 5% and *Lactobacillus* increased 9%. He *et al.* [34] also observed 10% reduction of diarrhea in piglet supplemented with *B. pumilus* DSM 32539, probably due to a reduction of 50% of coliforms. Additionally, in piglets supplemented with a probiotic, mixture of *B. licheniformis* and *S. cerevisiae*, IgA secreted by the intestinal mucosa increased 31%, improving the immune system through the modulation of intestinal microbiota in treated piglets [37].

Regarding sows' serum biochemical parameters, level of cholesterol and total lipid increase 4% in animals treated with *B. subtilis* DSM 5750 and *B. licheniformis* DSM 5749, with beneficial effect in piglets' growth [32]. Piglets growth improvement was reported by Lan *et al*. [38] after the administration of a mixture of *B. coagulance*, *B. licheniformis*, *B. subtilis*, and *C. butyricum*, for 42 days. Average daily gain increased 8 % but not significantly the average daily feed intake possibly due to the improvement of nutrient digestibility that improved 3% due to the use of the probiotic [38].

B. cereus var. Toyoi was investigated by Alexopoulos *et al*. [31], Baum et *al*. [39], Taras *et al*. [40], and Schierack *et al*. [30]. Schierack *et al*. [30] observed that the administration of these bacteria with feed showed to improve blood immune cells of piglets by modulating composition and activities, and the authors indicated that the probiotic also improved the effect of vaccination against influenza and *Mycoplasma*.

4.2 *Clostridium* spp.

C. butyricum was administrated to lactating sows and piglets, and what should be highlighted regarding the application of the bacteria is the fact that most researchers report its administration combined with another bacteria. Hayakawa *et al.* [15] employed *B. mesentericus*, *C.butyricum,* and Enterococcus faecalis; Lan et al. [38] employed *B. coagulance*, *B. lincheniformis*, *B.* subtilis, and *C. butyricum*: *Tsukahara et al.* [33] tested B. mesentericus, *C.butyricum,* and *E. faecalis;* and Wang *et al*. [41] investigated the combination of *C. butyricum* and *E. faecalis.*

Even if the bacteria were suministrated on a different formulation, *Tsukahara et al.* [33] and Wang *et al.* [41] observed similar results, an improvement of serum immunoglobulin (IgA and IgM), and the study of Wang *et al*. [41] was conducted with piglet and the one of Tsukahana *et al*. [33] with pregnant sows. Specifically, Tsukahana *et al.* [33] investigated the probiotic supplementation in unvaccinated and infected sow with porcine epidemic diarrhea virus. The probiotic BIO-THREE PZ (composed of *C. butyricum* TO-A, *Enterococcus* T-110, and *B. mesentericus* TO-A) improved the immune system of unvaccinated, PED-infected sows and their reproductive performance. On the other hand, Wang *et al*. [41] administrated the probiotic to weaned piglets and observed that the probiotic increased the jejunal villus length and jejunal villus height-to-crypt-depth ratio and decreased the jejunal crypt depth. They also reported a relative higher level of *C. butyricum* in supplemented animals and concluded that the mixture can promote growth performance, protect the intestinal pilli morphology, improve immunity, and optimize the intestinal flora in weaned piglets.

When *C. butyricum*, and other bacteria, was administrated to the pregnant and lactating sows and weaned piglets, a modulation occurs not only in the sows but also in the piglets' intestinal microbiota. In sows, *Lactobacillus* increased approximately 2% but *E. coli* decreased 12%, while in piglets *Lactobacillus* increased the same percent than in sows (2%) but E coli decreased much lower (1%) [15]. Li *et al*. [42] also indicated that *C. butyricum* plays an important role in feed addition, and they concluded that *C. butyricum* enhanced intestinal barrier function and inhibited apoptosis-associated speck-like protein-independent NLRP3 inflammasome signaling pathway in weaned piglets after ETEC K88 challenge. Even if beneficial results were observed when *C. butyricum* was supplemented to infected animals, Peeters *et al*. [43] in an assay developed in 2019 did not observe a significant difference in the daily weight gain, serological analysis, or bacteriological analysis when *C. butyricum* alone was supplemented to piglet challenged to *S. typhimurium.* However, this resolves that supplementation should be conducted with the combination of *C. butyricum* and other bacteria to achieve an improvement in swine production.

4.3 *Enterococcus* spp.

E. faecalis and *E. faecium* were supplemented to pigs, and most studies were conducted with their application in combination with other bacteria. Wang *et al*. [41] combined *E. faecalis* with C. *butyricum* and administered the mixture to piglets. Tsukahara *et al*. [33] and Hayakawa et al. [15] administrated a probiotic mixture formulated with three species *E. faecalis*, *C. butyricum*, and *B. mesentericus* to pregnant and lactating sows. Hayakawa *et al*. [15] aimed to reduce diarrhea, while Tsukahara *et al.* [33] aimed to improve the immune systems of sows and piglets, achieving both research groups their objectives.

Regarding *E. faecium*, the study conducted by Scharek *et al*. [44] who administered the strain *E. faecium SF68* to pregnant and lactating sows showed satisfactory results. They observed lower levels of cytotoxic T cells in the jejunal epithelium of piglets of the probiotic group. According to the authors, the difference in T population was not due to the modification of the epithelial cell numbers but due to the reduction of the frequency of β-hemolytic and O141 *E. coli* serovars in the intestinal contents of probiotic piglets. Zhang *et al*. [45] also administrated the bacterial species *E. faecium,* but in combination with *B. subtilis* to piglets. They observed a reduction of diarrhea (16%) that resulted in an increase of 23% of the daily weight gain, certainly due to an improvement of the microbiota composition caused by the administration of the probiotic.

4.4 *Lactobacillus* spp.

Lactobacillus spp. together with *Bifidobacterium* spp. are the genera most used as probiotics in human and veterinary medicine. However, in swine production, there are not many scientific works reporting supplementation with *Lactobacillus*, and generally the species of Lactobacillus are administrated combined with other bacteria to obtain more benefits from the supplementation. Dowarah *et al*. [46], Joysowal *et al.* [47], and Liu *et al*. [6] reported *L. acidophilus* supplementation at growing and finishing stage. Dowarah *et al*. [46] and Joysowal *et al.* [47] combined the strain *L. acidophilus* NCDC-15 and the strain *Pediococcus acidilactici* FT28. The first one administered the mixture to 36 pigs for 180 days. Diarrhea was reduced up to 41%, due to the modulation of the microbiota and an increase in the beneficial genera *Bifidobacterium* (4%) and *Lactobacillus* (7%) and a decrease (10%) in *E. coli* and *Clostridium*, bacterias whose increases are associated with disease. Joysowal et al. [47] supplemented 27 pigs for 90 days and observed, as Dowarah *et al*. [46], an improvement in the microbiota composition and increase of 12% of the final animal weight.

Liu *et al*. [6] tested the probiotic mixture of *L. acidophilus*, *B. subtilis,* and *S. cerevisiae* and reported an improvement of microbiota composition; however, the increase of *Lactobacillus* was only of 1% and the reduction of *E. coli* was only of 1%. The difference in this results between Liu *et al*. [6] and Dowarah *et al*. [46] could be due to various factors, and one of the most important is that in the first case a mixture of three probiotics was used, whereas in the other case only two were employed being *Lactobacillus* the only coincident species, so results can strongly differ. In addition, it is important to highlight that the supplementation was shorter in 2020 and in 2017, 110 days less of treatment with the probiotic mixtures.

4.5 *Pediococcus* spp

Pediococcus is a genus of lactic acid bacteria usually isolated from fermented food, but it can be also isolated from aquatic products, raw animal, plant products, and even human feces. Many species of *Pediococcus* are proven to have links of the human gastrointestinal tract. The species *Pediococcus acidilactici* is one of these species. *P acidilactici* produces bacteriocins, specifically, pediocins with antimicrobial properties. This particular bacterium was employed in pig at the growing and finishing stage and always combined with other bacteria. Supplementation of pigs during 180 days with the strain *P. acidilactici* FT28 combined with *L. acidophilus* NCDC-15 showed a modulation in the animals' intestinal microbiota with a consequently reduction of diarrhea [46]. In another study developed by Joysowal *et al.* [47], pigs were supplemented with the same strain, *P. acidilactici* FT28, alone and compared the results with pigs supplemented with *L. acidophilus* a NCDC-15 and without supplementation. The obtained results showed an improvement in growth performance, feed intake, digestibility of crude protein, and nitrogen retention in *P. acidilactici* FT28-fed group. Authors also reported a better serum albumin/globulin (A/G) ratio and cholesterol and triglyceride levels in the *P. acidilactici* group, compared to the control and *L. acidophilus* supplemented group.

4.6 *Saccharomyces* spp.

Saccharomyces spp. is a live yeast extensively used as probiotic, more specifically *S. boulardii* and *S. cerevisiae*. In human medicine, they have been used for years to prevent the diarrhea associated with antibiotic consumption and as a coadjuvant treatment for *Helicobacter pylori* eradication, so it is expected to have the same effects in pigs. Studies have been carried out with piglets at the post-weaning stage to investigate this objective. Thus, Trevisi *et al*. [48] and Trckova *et al*. [49] evaluated the effect of feeding supplementation with *S. cerevisiae* on diarrhea, and both authors found that supplementation reduced the duration and severity of post-weaning diarrhea caused by ETEC in piglet. In addition, trevisi et al. observed that administration of S. cerevisiae in concomitance with ETEC infections reduced pig illness and mortality.

On the other way, in the growing and finishing stage, production and rentability depend basically on the increment of pig weight. *Saccharomyces* was tested with pigs at this final stage; in 2017, Liu and collaborators [50] administrated, to 100 pigs, *S. cerevisia*e with *B. subtilis*, strain not indicated, for 42 days. The probiotics increased the daily feed intake up to 40 g/day, and consequently the animals daily weight gain was also increased by 57.5 g per day. Later, Liu *et al*. [6] included in the probiotic mixture *L. acidophilus*, and this work was conducted with 150 pigs, and the administration of supplementation was for 70 days. In this case, the incremented in the animal daily weight gain was lower than in 2017, i.e. 28 g/day. This may be due to a lower duration of the treatment or to the strain combination. So, other studies should be developed to confirm these results; however, as none of the studies indicate the strain, data cannot be compared so easily and conclusion cannot be made.

5. Conclusions

In general, all the studies included in this chapter show good results in terms of weight gain, daily intake, reduction of infection, reduction of illness severity, etc. However, in most cases, the studies cannot be compared since they use different strains or combination of probiotics. Since beneficial effects of probiotics are strain-dependent, it is important to highlight that in most of the studies conducted with probiotics, the strain or strains used are not indicated. Therefore, although the use of probiotics seems to be an interesting alternative to the use of antibiotics as prophylactic, more studies and research with solid results which indicate the dose, strain/s, age and health status of animals, type of feed, feed composition, administration mode, duration of treatment period are required. Well-defined studies must be carried out to really determine which strain/strains are effective for a specific objective (diarrhea prevention, increase in weight gain, respiratory pathologies, etc).

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In many parts of the world, the use of antibiotics in food-producing animals is partially or completely banned. This has led to increased research to develop alternatives to antibiotic growth promoters (AGPs). Some of these alternatives include prebiotics, probiotics, and phytoadditives. This book focuses on probiotics, which are the most feasible option to replace AGPs because they directly modulate the gut microbiota and improve gut absorption, which ultimately accelerates growth and production. The book provides recent updates on the use of probiotics in farm animals including poultry, fish, and ruminants as well as pet animals.

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