Antimicrobial growth promoters in feed – possibilities and necessity

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Abstract. The attention of the scientific and professional communities, as well as of food consumers, has always been directed towards finding adequate nutritional strategies that could improve food production and safety. By using antibiotics as feed additives, farmers gained increased profits based on higher growth rates with better feed conversion and lower costs of therapeutic treatment. The quantities of antibiotics used as growth promoters for farm animals have been increasing constantly, but at the same time, the frequency of bacterial resistance to antibiotics and the presence of antibiotic residues in food have become a global problem. To eliminate or minimize these risks, on 1 January 2006, antibiotics were banned from use as additives in animal nutrition in the European Union. Accordingly, there is interest in developing new nutritional strategies that would support the function of the autochthonous microbiota in the animal gastrointestinal tract to control pathogenic bacteria. Probiotics, prebiotics, phytobiotics and feed enzymes are a new generation of growth promoters and largely achieve their effects by using the physiological mechanisms of animals and/or their intestinal microbiomes, enabling animals to completely fulfil their genetic potential with respect to production properties.

1. Introduction

Since the discovery and application of penicillins in the 1940s, antibiotics have had a major role in the prevention, control and treatment of infectious diseases of humans and animals. It has been proven that the use of antibiotics in the animal feed industry is an important means to increase feed efficiency, promote animal growth, and improve the quality of food of animal origin. The rapid diffusion of antibiotics in almost all areas of feed production and processing was initially considered to be progress and modern technology brought about by modern times. Recent studies have shown that the effect of growth-promoting antibiotics correlated with decreased activity of bile salt hydrolase, an intestinal bacterial enzyme [1]. This decreased enzyme activity has a negative effect on the digestion and utilization of feed by the host animals [1]. By adding antibiotics in small quantities, a positive effect is achieved primarily in animals during growth, although there are data on similar effects in different forms of production. Also, the price of foodstuffs of animal origin is about 8% lower compared to the same product obtained from animals fed with antibiotic-free feeds [2].

With continued use of antibiotics as growth promoters, very quickly (by the late 60s) interest grew, not only in their positive, but also in their possible negative, harmful effects. As a negative effect, the development of resistant strains of enterobacteria is a serious problem for disease treatment in veterinary and human medicine. The problem of resistant strains was also multiplied by the emergence of cross resistance as a result of the adaptive ability of microorganisms and the mutagenic effects of antibiotics. However, unreasonable use of antibiotics has led to the fear of the development of resistant bacteria that could transfer, together with its resistance factors, from animals to humans [3]. Specifically, non-therapeutic antimicrobial use is associated with the propagation of multidrug resistance (MDR), including resistance to drugs that have never been used on the farm [4]. The next, common, and certainly more significant problem, is the presence of antibiotic residues in food of animal origin, as well as the possible genotoxic action of antibiotics and their residues that are not immediately apparent, but lead to human genome damage in various degrees of character and intensity.

2. Legislation

Sweden is the leading country in the prohibition of antibiotics used as growth promoters in animal nutrition in Europe, and in 1986, antibiotics in feed were banned in this country. This new approach to animal breeding did not have a greater reverberation and impact on other European countries until the early 1990s, when it began to strengthen due to the consumer movement and consumerism as a way of thinking and marketing [5]. In 1999, the Scientific Steering Committee conducted screening on the use of antimicrobial substances in animal feed in the EU, and limited the use of antibiotics in animal nutrition. The main goal was to minimize the risk of developing resistant bacteria, and to preserve the effectiveness of the antibiotics that are/were being used in human medicine. In Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives used in animal nutrition, antibiotics, other than coccidiostatics and histomonostatics, were allowed to be sold and used as feed additives until 31 December 2005 only, and from 1 January 2006, these substances were deleted from the Register.

However, this ban on the use of antibiotics in feed has caused unwanted effects on the animal industry in the EU, such as an increase in infections in animals and a reduction in animal production. In the meantime, the total amount of antibiotics used in animals has increased, as the use of therapeutic antibiotics and disinfectants has increased significantly due to the high incidence of illness caused by the ban. In order to overcome the increased mortality and morbidity rate due to the ban on antibiotics in food, a number of substitutes have been proposed [6]. Alternatives to antibiotics include antibacterial vaccines, immunomodulatory agents, bacteriophages and their lysi, antimicrobial peptides (AMPs), pro-, pre-, sin- and phytobiotics, and feed enzymes [7]. This paper considers the possibility of developing and applying probiotics, prebiotics, phytobiotics and enzymes that are applicable, effective and affordable in mass livestock production as alternatives to antibiotics.

Prevention of disease is defined as the application of a medicinal product to healthy animals in a situation where the specific and increased risk of disease is present [8]. There are key similarities between growth promotion and disease prevention. Many antibiotic alternatives produce both types of positive effects, growth promotion and disease prevention. In many cases, it is likely that the growth-promoting effect is at least partly due to the ability of the product to inhibit or kill bacteria. At the same time, preventing the animals from diseases can prevent loss of productivity due to illness, whether clinical or sub-clinical [9].

Table 1. Efficiency of non-antibiotic growth promoters, depending on the animal species and the reason for its use [10]

	Cattle					
	Milk-fed calves	Dairy Cows	Beef cattle	Swine	Chicken	Turkeys
Probiotics	GP, DP	GP, DP	GP, DP, dt	GP, dp, dt	GP, DP	GP, DP
Prebiotics	Gp, dp			gp, dp	GP, DP	GP, DP
Organic acids		gp, dp	gp, dp	GP, dp	GP, DP	dp
In-feed enzymes				GP	GP, DP	GP
Antimicrobial peptides	gp, dp	gp, dp, dt	gp	gp, dp	gp, dp	
Phytochemicals (e.g., essential oils)	dp, dp, dt	Gp	gp	gp, dp	GP, dp	gp, dp

Copper, zinc, and other heavy metals		gp, dp	GP, dp	GP, dp	GP	gp
Immune modulators	DP	DP	DP	gp, dp, dt	gp, DP	gp, dp
Vaccines	DP	DP	DP	GP, DP	DP	DP
Bacteriophages, endolysins, lysozyme, and other hydrolases	Dp	dp, dt		gp, dp, dt	dp, dt	gp, dp

GP - Growth promotion, strong scientific evidence for efficacy and commercially used; DP - Disease prevention, strong scientific evidence for efficacy and commercially used; DT - Disease treatment, strong scientific evidence for efficacy and commercially used; gp - Growth promotion, some scientific evidence suggests potential efficacy; dp - Disease prevention, some scientific evidence suggests potential efficacy
dt - Disease treatment, some scientific evidence suggests potential efficacy

3. Feed enzymes

The most encouraging option for promoting growth in animals is the use of enzymes that can be added to feed. These enzymes help animals to digest and absorb plant materials such as cellulose or pectin, which cannot otherwise be used efficiently [11]. Such enzymes (e.g. xylanases and ß-glucanases) are usually added to commercial feed for broilers. The mechanism by which the enzymes fulfil the role of growth promoters are not fully understood, but could include changing the intestinal microbiota, preventing damage caused by non-diseased parts of the plants that cause mechanical friction on the mucous membrane, decomposing larger molecules into compounds with prebiotic activity or influencing the composition of the intestinal content and its digestibility [12].

Enzymes in animal feed also have a role in the prevention of some diseases, such as necrotic enteritis in chickens [13]. The most common enzymes used as additives are mixtures of various glycanases. Recombinant synthetic enzymes such as phytase are commercially produced and sold as feed additives for monogastric animals [14]. Phytase has significant effects on the digestibility of calcium, phosphorus and minerals, as well as on the production of intestinal mucin and endogenous losses, all of which affect the supply of nutrients and ejaculation. The dietary use of encapsulated lysozyme, as a supplement in a pullet feed mixture significantly reduced numbers of *Clostridium perfringens* and gastrointestinal lesions in the ileum [15]. The European Food Safety Authority (EFSA) studied a combination of xylanase and ß-glucanase and concluded that the product is safe and effective as a growth promoter in broilers and turkeys [16]. Based on numerous studies, including systematic reviews and meta-analyses, it has been found that feed enzymes such as xylanases are effective in reducing intestinal lesions, which are a key pre-disposing factor for necrotic enteritis [17]. In pigs, a high level of acidity in the intestines can inactivate enzymes in feed. Enzymes that are stable under such conditions have shown promising results, pointing to the potential for this alternative strategy as growth promoters. Some enzymes, such as phytase, are more effective in improving performance than others [18]. Feed enzymes are not promising growth promoters for ruminants because the conditions in the rumen inactivate all enzymes before reaching the intestine [19]. It is well known that animal reactions to feed enzymes are not completely predictable, and these inconsistencies can be attributed to the type of enzyme, the amount of enzyme used, the enzyme activity, the composition of the mixture, and the individual characteristics [20].

4. Probiotics

Probiotics are live cultures of microorganisms (yeasts, fungi and bacteria) that are used as dietary supplements to improve the balance of the microbiota in the gastrointestinal tract. Probiotics can be divided into defined and undefined. Defined probiotics consist of single strains or mixtures of all-round microorganisms (for example, each microorganism is described at the level of the species, the exact composition of the culture is quantitatively described, and the genomes of particular organisms in the mixture can be completely sequenced to ensure the absence of any antibiotic resistance genes). Undefined probiotics contain microorganisms that are not fully described, usually in mixtures [21].

Generally, undefined probiotics exhibit greater efficacy than defined probiotics, but both strategies are promising approaches to disease prevention, productivity improvement and growth promotion [22].

Numerous scientific studies have quantified the effects of probiotics in growth promotion and disease prevention in broilers. For example, one study showed that probiotics improved bird's productivity and health, and mortality was reduced by more than 20% compared to the control flock [23]. The reduction in mortality was similar to the use of antibiotics [23]. The use of probiotics in laying hens led to a statistically significant increase in productivity [24]. In a study to compare feed enzymes and probiotic strains as additives, both products significantly reduced the mortality of broilers and improved production results compared to animals fed mixtures without additives. Probiotics, however, have shown significantly better results than enzymes in feed. One study has shown there is a wide range of probiotics that can effectively control the clinical symptoms associated with coccidiosis, a potentially destructive disease that is difficult to control without antibiotics [25]. Reviews by the FAO, European Medicines Agency (EMA) and EFSA concluded that probiotics are effective growth promoters in pig breeding and can effectively prevent diarrhoea and reduce mortality due to *Escherichia coli* infections in piglets. Probiotics have also shown efficacy in preventing diarrhoea among young piglets, with a proven reduction in incidence rate of up to 40% [26]. Probiotics have shown efficacy in the prevention of disease in cattle. The use of probiotics increased the efficiency of milk production (measured as kg of milk produced/kg of consumed feed) in dairy cows by 6% [27].

Storage and use of probiotics is a potential challenge. For example, in the production of pelleted feed, the ingredients are usually exposed to high temperatures, which can inactivate probiotics. Although this problem does not exist in other forms of animal feed, probiotics have some common risks, for example potential unwanted and harmful changes in the microbiological balance of the bowel.

5. Prebiotics

Prebiotics are non-viable food ingredients that benefit the host by selectively stimulating growth and/or activity of one or a limited number of bacterial species in the digestive tract, thereby improving the host's health [28]. In addition to local, they can exhibit positive systemic effects after resorption of fermentation products of bacterial metabolism. Prebiotics move directly into the colon, where they undergo selective fermentation, and help maintain eubiosis primarily by supporting the preferred microbiota and by increasing excretion of the unwanted microbiota by faeces. Prebiotics help useful microorganisms to proliferate, but could also have other effects, such as immune system modulation. The efficiency of prebiotics seems to be determined by various factors, including the type of prebiotics, age of animal, health status, zoohygienic conditions and farm management, which must be taken into account when deciding whether to use such a strategy.

Among the numerous feed ingredients, non-digestible carbohydrates (oligo and polysaccharides), certain peptides and proteins, and some lipids are potential prebiotics. Due to their chemical structures, these listed feed components are not subject to enzymatic hydrolysis, nor are they absorbed in the frontal part of the digestive tract, so they can be called "colonal foods", that is, food components that move into the last parts of the digestive tract and serve as a substrate for the bacteria present, providing the host with energy, metabolic substrates and essential microorganisms (17). Lactic acid bacteria and bifidobacteria, considered to be the preferred microbiota in the digestive tract, can use carbohydrates originating from prebiotics for the purposes of their metabolism. However, pathogenic bacteria (*E. coli*, *Salmonella* spp.), and many other gram-negative bacteria do not possess these abilities. These bacteria are eliminated from the intestinal microbial population since the preferred bacterial microbiome has more intensive reproduction capacity [29].

Recent reviews by EMA and EFSA concluded that prebiotics are effective in promoting growth and improving the health status of broilers. In pigs, prebiotics have a positive effect on growth, which increases by 8% in the period after piglets are rejected. In pigs fed with prebiotic supplements, immune responses to intestinal infections such as salmonellosis have been improved with prebiotics [30]. In cattle, prebiotic efficacy appears to be limited to calves. The addition of prebiotics to milk substitutes (galactosyl-lactose) for calves was efficacious [31].

6. Phytobiotics

Phytobiotics are a relatively novel group of additives that have attracted considerable attention in recent years in the feed industry. Phytogenic animal feed additives (phytobiotics or plant drugs) are defined as compounds of plant origin used in animal nutrition to improve animal production results, feed quality, and the quality of the resultant food of animal origin. The said definition is based on the manner of use, while other terms such as herbs, spices, essential oils (EOs) and oily resins can be used in the classification of a wide range of phytogenic compounds. Compared with synthetic antibiotics and inorganic chemicals, these products are obtained from natural sources, are less processed, do not create residues, and could become ideal animal additives and successfully replace antibiotic growth promoters in feed [32]. The basic groups of secondary metabolites in plants include alkaloids, heterosides, saponins, tannins and terpenoids. In the terpenoids, mono- and sesquiterpenes are produced (these form the basis of EOs), while aromatic phenylpropane ingredients are also present in smaller quantities in plants.

Paracelsus von Hohenheim first used the term "essential oil" in the fourteenth century, and the term refered to an effective component of a drug, a "quinta essentia" [33]. Over 3000 types of EOs are known, of which 300 are commercially important and used in industry as aromatic substances. EOs are volatile, scented substances with an oily consistency, produced by plants. The main components of EOs, aldehydes or phenols (cinnamaldehyde, citral, carvacrol, thymol, eugenol), exhibit the highest antibacterial activity, while EOs containing terpenic alcohols have somewhat lower activity. Since EOs are composed of a large number of ingredients, it is presumed that their antimicrobial activity is not related to a specific mechanism of action but is directed at several different targets in the microbial cell. The operating modes of EOs are cell wall degradation, cytoplasmic membrane damage, membrane protein damage, cell cell loss, cytoplasm coagulation, and depletion of the proton gradient [34]. EOs are believed to produce antibacterial effects through two different mechanisms: the first one is related to their hydrophobicity which allows the EO to be imprinted on the phospholipid bilayer cell membrane, while the other relates to the inhibition of bacterial enzymes and receptors by EO binding at specific sites. Thanks to their hydrophobic structure, EOs are then able to destabilize and change the permeability of the bacterial membrane.

7. Conclusion

Various products may be able to replace antibiotics used for the purposes of growth promotion and disease prevention, but implementing these products will require a comprehensive approach ready to consider these alternatives as a part of farm management. All in all, alternatives to antibiotics are promising, as many of them seem to increase animal productivity and simultaneously prevent infection. The attention of the expert and scientific communities is directed towards the replacement of antibiotics in many animal welfare situations. This should allow antibiotics to be preserved for use only when necessary for health protection purposes in humans or animals. Based on numerous examples from on-farm practice and scientific studies, the use of growth promoters other than antibiotics in animal nutrition is medically, nutritionally and economically justified.

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