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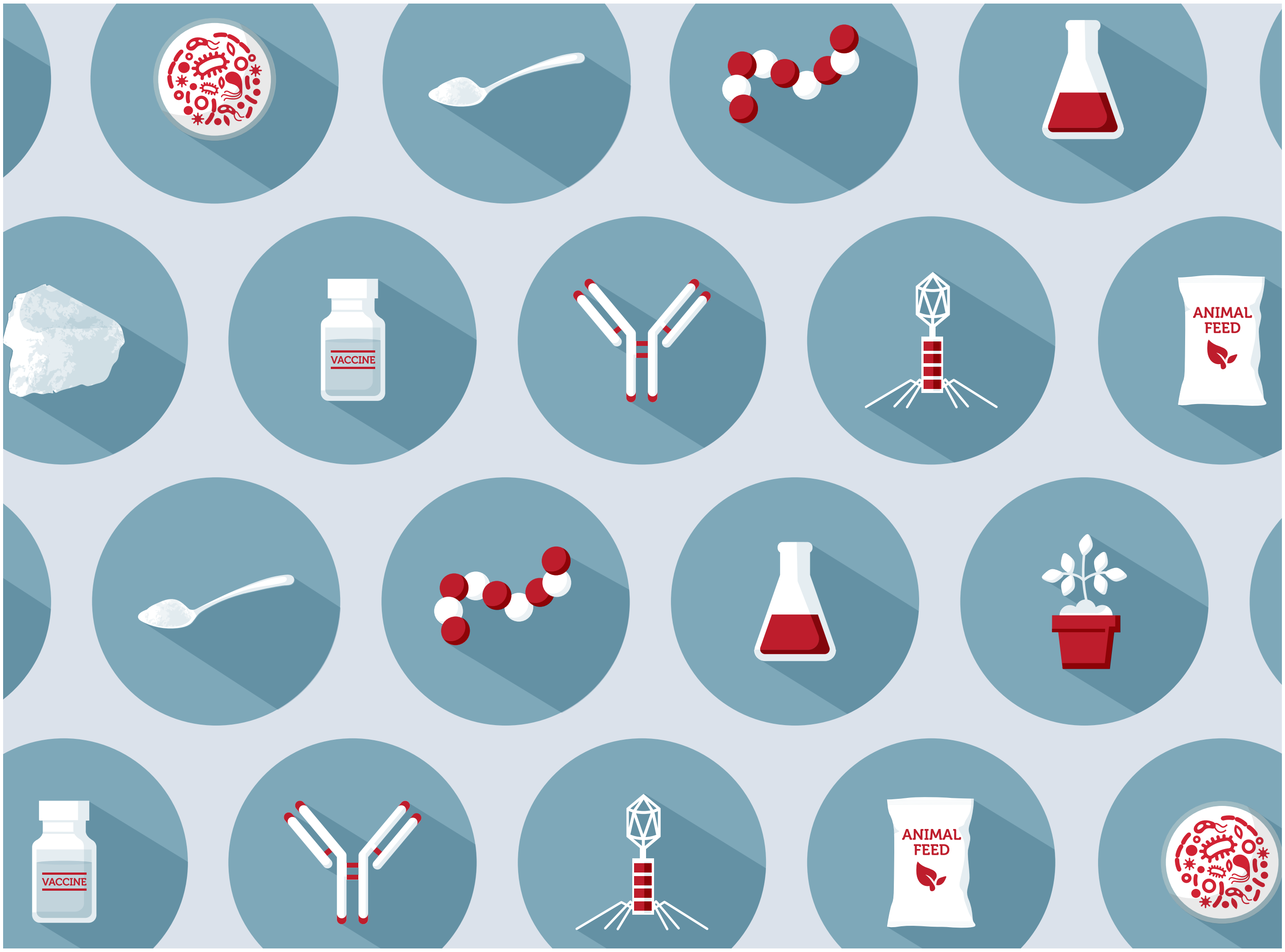

Alternatives to Antibiotics in Animal Agriculture

Vaccines, probiotics, immune modulators, and more can help maintain healthy herds and reduce the need for antibiotics

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Overview

The emergence and spread of antibiotic resistance have created a growing global threat. Because the use of antibiotics in any setting drives resistance expansion everywhere, it is important to minimize the use of these drugs—a goal that depends on eliminating inappropriate uses and finding other means of preventing infections. In human medicine, strategies can include reducing health care-associated infections, limiting the unnecessary use of antibiotics, ensuring the use of those antibiotics effective against a narrow spectrum of bacteria whenever possible, and increasing the use of key vaccines. This report aims to provide an overview of the options available to reduce the need for antibiotics in animal agriculture through the use of non-antibiotic alternative products (such as vaccines or probiotics), with a focus on synthesizing the current body of scientific literature for those products that are already or close to being commercially available, and highlighting key data gaps.

Alternative products play a crucial role in allowing farmers and veterinarians to reduce the use of antibiotics. Vaccines are among the most promising and widely used of these alternatives, but pre- and probiotics and other innovative products are also in use or currently being investigated. Many of these have been shown to simultaneously prevent infection and improve animal performance, such as growth rates or egg production. Today, alternative products are primarily useful for growth promotion and infection prevention, with fewer options available for treatment.

However, the efficacy of alternative products tends to be variable across individual livestock operations and with the disease status of herds, and is often affected by external factors such as weather or feed composition. More research is needed to understand exactly why efficacy is so variable and to ensure optimized use, but this is complicated by the fact that the mechanism of action (i.e., the molecular processes that generate the desired effect) for many alternative products is not well understood.

Alternative products should be considered as one part of a comprehensive herd or flock health management program aimed primarily at the prevention of diseases, rather than curing of infections. An alternative product's efficacy and cost-effectiveness will be central to farmers' decisions about whether to use it, and the sharing of experiences and lessons learned is likely to be as important as formal economic analyses. Therefore public-private partnerships may be a promising approach for understanding how best to integrate alternative products into overall farm management, as they may allow complementary data from experimental studies and actual use data on commercial operations to be combined and contrasted.

Introduction

In the U.S., antibiotics are regulated as animal drugs whereas alternatives to antibiotics may be regulated as animal drugs, biologics, or feed additives. The approval of animal drugs and biologics is contingent upon demonstration of their safety and efficacy; only safety data is required for feed additives.

Antibiotics and their alternatives can be used for treating disease, preventing or controlling infection, or promoting animal productivity and growth (i.e., "growth promotion"). When used for growth promotion, antibiotics are administered to healthy animals to make them grow faster or utilize their feed more efficiently. The use of medically important antibiotics for growth promotion in the U.S. was eliminated effective Jan. 1, 2017.[1] When used for disease prevention, antibiotics are administered to animals without symptoms of disease that have an increased risk of infection, whereas antibiotics used for disease treatment are administered when infection has progressed and disease symptoms are already present in the animal. Antibiotics are used for disease control when a part of the animal group receiving the antibiotic already shows disease symptoms. Many alternative products may simultaneously promote growth and prevent disease, and some products may serve as substitutes for all antibiotic use purposes.

In reality it can be challenging to separate these objectives in terms of actual applications on commercial operations. For instance, many illnesses have negative impacts on animal growth and productivity, and preventing infections can improve farm outputs and protect animal welfare. Similarly, some products may have positive impacts on the general health of the animal—for instance, by boosting the immune system or improving gut health. These products may help a sick animal recover more quickly without specifically treating the infection. In other cases, products may reduce colonization of animals with potentially harmful bacteria and thereby prevent disease.

Alternative products differ in how their use has to be timed to assure effectiveness (Figure 1). Vaccines, for instance, have to be administered well before infection as they rely on the animal developing a protective immune response, which requires time. In contrast, products such as bacteriophages, which are effective because they directly interact with and kill disease-causing bacteria, must be administered around the time of infection; they will work only when bacteria are actually present in abundance and causing infections and, in the absence of bacteria, may be rapidly inactivated in the animal.

Figure 1

Alternative Products Differ in Timing of Administration

Products work through different mechanisms of action

Product type	Mechanism of action	Timing of administration: Prevention long before infection[*]	Timing of administration: Prevention shortly before infection	Timing of administration: Treatment after infection[†]
Hydrolases[‡] Bacteriophages[§]	Targets bacteria		Narrow window around initial infection	
Phytochemicals[‖]	Targets bacteria		Can be applied continuously	
Antimicrobial peptides[#]	Targets bacteria		Narrow window around initial infection	
Organic acids[**]	Targets bacteria		Can be applied continuously	
Probiotics[††]	Improves gut health		Can be applied continuously	
Prebiotics[‡‡]	Improves gut health		Can be applied continuously	
Immune modulators[§§]	Stimulates or enhances host immune response		Narrow window before infection	
Vaccines[‖‖]	Primes host immune response	Applied before infection		

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* Government Accountability Office, "Antibiotic Resistance: Agencies Have Made Limited Progress Addressing Antibiotic Use in Animals" (2011), http://www.gao.gov/new.items/d11801.pdf. According to the report, antibiotics used for disease prevention are administered to animals without symptoms of disease that have an increased risk of infection.

† Ibid. According to the report, antibiotics used for disease treatment are administered when infection has progressed and disease symptoms are already present in the animal.

‡ A. Parisien et al., "Novel Alternatives to Antibiotics: Bacteriophages, Bacterial Cell Wall Hydrolases, and Antimicrobial Peptides," *Journal of Applied Microbiology* 104, no. 1 (2008): 1 -13; Elizabeth M. Ryan et al., "Recent Advances in Bacteriophage Therapy: How Delivery Routes, Formulation, Concentration, and Timing Influence the Success of Phage Therapy," *Journal of Pharmacy and Pharmacology* 63, no. 10 (2011): 1253 -64.

§ Stephen P. Oliver et al., "Asas Centennial Paper: Developments and Future Outlook for Preharvest Food Safety," *Journal of Animal Science* 87, no. 1 (2008), https://www.ncbi.nlm.nih.gov/labs/articles/18708597; Ryan et al., "Recent Advances in Bacteriophage Therapy."

|| Marjorie Murphy Cowan, "Plant Products as Antimicrobial Agents," *Clinical Microbiology Reviews* 12, no. 4 (1999): 564 -82, https://www.ncbi.nlm.nih.gov/pubmed/10515903; Peter K. Mitsch et al., "The Effect of Two Different Blends of Essential Oil Components on the Proliferation of *Clostridium perfringens* in the Intestines of Broiler Chickens," *Poultry Science* 83, no. 4 (2004): 669 -75, https://pdfs.semanticscholar.org/db3b/74f6561d33ae3dfe555dd38142e8cdf50e33.pdf.

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** Gerard Huyghebaert, Richard Ducatelle , and Filip Van Immerseel, "An Update on Alternatives to Antibiotic Growth Promoters for Broilers," *The Veterinary Journal* 187, no. 2 (2011), https://www.ars.usda.gov/alternativestoantibiotics/PDF/publications/09HuyghebaertG.pdf; Andrew D. Wales, Vivien M. Allen, and Robert H. Davies, "Chemical Treatment of Animal Feed and Water for the Control of Salmonella," *Foodborne Pathogens and Disease* 7, no. 1 (2010): 3 -15, http://online.liebertpub.com/doi/abs/10.1089/fpd.2009.0373.

†† Francesca Gaggia, Paola Mattarelli, and Bruno Biavati "Probiotics and Prebiotics in Animal Feeding for Safe Food Production," *International Journal of Food Microbiology* 141, supp. (2010): S15 -28; U.N. Food and Agriculture Organization, "Probiotics in Animal Nutrition: Production, Impact, and Regulation" (2016), http://www.fao.org/3/a-i5933e.pdf.

‡‡ Usha Vyas and Natarajan Ranganathan, "Probiotics, Prebiotics, and Synbiotics: Gut and Beyond," *Gastroenterology Research and Practice* 2012 (2012), https://www.hindawi.com/journals/grp/2012/872716; Saminathan Mookiah et al., "Effects of Dietary Prebiotics, Probiotic, and Synbiotics on Performance, Caecal Bacterial Populations, and Caecal Fermentation Concentrations of Broiler Chickens," *Journal of the Science of Food and Agriculture* 94, no. 2 (2014): 341 -48, https://www.ncbi.nlm.nih.gov/labs/articles/24037967.

§§ James A. Roth and Kevan P. Flaming, "Model Systems to Study Immunomodulation in Domestic Food Animals," *Advances in Veterinary Science and Comparative Medicine* 35 (1990): 21 -41, https://www.researchgate.net/publication/20871077_Model_Systems_to_Study_Immunomodulation_in_Domestic_Food_Animals; Bayer HealthCare LLC, "Zelnate DNA Immunostimulant," accessed June 21, 2017, http://www.zelnate.com/static/documents/Zelnate-ChallengeStudy_Detailer.pdf.

|||| Els N.T. Meeusen et al., "Current Status of Veterinary Vaccines," *Clinical Microbiology Reviews* 20, no. 3 (2007): 489 -510, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1932753; Victor S. Cortese, "Neonatal Immunology," *Veterinary Clinics of North America: Food Animal Practice* 25, no. 1 (2009): 221 -27, http://www.sciencedirect.com/science/article/pii/S0749072008000893?via%3Dihub.

Alternative products may not address all the bacterial pathogens against which a given antibiotic is effective. While this is a limitation, it can also mean fewer side effects. For example, this narrower host range can limit unintended and disruptive consequences on the beneficial microbiota, a problem associated with antibiotics that, for instance, leads to a significantly increased risk of *Clostridium difficile*-associated disease after antibiotic therapy. Moreover, alternative products are typically not affected by antibiotic resistance attributes and may be effective against multidrug-resistant pathogens for which few treatment options otherwise remain.

Some products have been shown to reduce the risk that animals shed foodborne pathogens, such as *Salmonella* or O157:H7 *shigatoxin-producing E. coli*, albeit efficacy as a food safety intervention tends to be more variable and challenging than as an alternative to antibiotics.[2] While such food safety uses are discussed in detail in a separate upcoming report, they again emphasize the fact that the use of an alternative product may simultaneously have multiple benefits.

Conceptually, alternatives to antibiotics can be categorized by the mechanism in which they act. Some products, such as bacteriophages and antibacterial peptides, directly target the pathogen. In contrast, prebiotics and probiotics indirectly inhibit pathogens by favoring beneficial bacteria so that the pathogens are outcompeted. Vaccines and immune modulators follow yet another strategy: They prime the animal's immune system to better control the infection. Management strategies such as biosecurity and feed hygiene further complement the effects by reducing the risk of pathogens being introduced and spreading in the herd or flock.

How an alternative product works is an important consideration in its selection, and can significantly affect compatibility with other products. For example, probiotics can modulate the immune system and enhance the efficacy of certain vaccines, but they may also compete with bacterial vaccine strains and therefore be antagonistic to them.[3] The selection of appropriate alternatives needs to be tailored to a specific animal species, age group, and production class, and should consider other factors such as the attributes of the pathogens of concern. In evaluation of whether an alternative product may be an option to reduce antibiotic use, it is also important to assess its safety for the animal, person administering the product, and end-consumer. Other practical considerations include the ease of administration, cost, variability and unpredictability in effectiveness, need for advanced diagnostics, risk of loss of efficacy due to resistance emergence, and risk of unintended consequences.[4]

Research efforts to date have investigated a very large and diverse group of potential alternatives to antibiotics, often with at least somewhat promising results. However, in some studies efficacy has been evaluated only experimentally, which probably neither reflects real-world husbandry conditions on commercial operations nor the target animals (e.g., studies are often conducted in calves or piglets while the intervention would ultimately be applied to older animals). Potential unintended consequences have generally not been well studied. Typically, cost-effectiveness data are also not available, complicating the evaluation of incentives for implementation.

To optimize the use of scarce public research and development resources, stakeholders must prioritize where to focus. A priority should be placed on areas of greatest need for products that would replace antibiotic use. Two recently developed prioritization schemes, generated by expert groups convened by the World Organisation for Animal Health (OIE) and the U.S. Department of Agriculture (synthesized in Table 1 for broiler chickens as an example), demonstrate the usefulness of a comprehensive, data-driven, and systematic approach for identifying key animal health problems to tackle in order to substantially reduce the need for antibiotics, and the most promising alternative approaches for addressing them. At the same time, the prioritization efforts demonstrate that, in order to permit such prioritization, it is essential to have a comprehensive understanding of animal disease pressures and antibiotic use, emphasizing the need for on-farm antibiotic use data to tailor and prioritize future research efforts.

Some alternatives to antibiotics are already successfully used in commercial food animal production, including segments of the beef cattle, dairy, and poultry industry. For instance, according to data from USDA's National Animal Health Monitoring System (NAHMS), probiotics are used on nearly 30 percent of U.S. feedlots with a capacity of 1,000 cattle or more, with the goal of increasing production efficiency.[5] Similarly, probiotics have been increasingly used on U.S. dairy operations to prevent disease in cows,[6] and are used in young calves to improve productivity and health.[7] Probiotics are also widely used in chicken production to enhance performance and reduce the need for antibiotic use.[8]

Table 1

Prioritization of Research Needs for Alternatives to Antibiotics for Use in Broiler Chickens (Based on Expert Opinion)

Vaccines and other promising alternative approaches can help reduce antibiotic use in animals

	Priority diseases for broiler chickens*			Disease-specific vaccines†			Other promising alternative approaches requiring more research‡
	Disease	Agent	Antibiotic use	Commercial availability	Major constraints	R&D priority	
Enteric diseases	Necrotic enteritis	Bacterial toxin	High	Yes	• Short-lasting and limited immunity • Application inconvenient, no mass application	High	• Phytochemicals • Prebiotics and probiotics • Immune modulators (e.g., egg yolk antibodies) • Antimicrobial peptides • Substances that bind the bacterial toxin (e.g., clays) • Bacteriophages
	Coccidiosis	Parasite, antibiotic use for secondary bacterial infection	High	Yes	• No cross-protection across strains • Current vaccines can cause disease	High	• Essential oils • Other phytochemicals (e.g., saponins)
	Infectious bronchitis	Virus, antibiotic use for secondary bacterial infection	Medium	Yes	• Protection across strains suboptimal • Virus mutates rapidly	Medium	
Generalized infection	*Escherichia coli*	Bacterium, infection possibly secondary to other diseases (e.g., yolk sac infection)	High	Yes	• Protection across strains suboptimal • No vaccine for some primary conditions that predispose for secondary *Escherichia coli*	High	

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Notes:

* As identified by both the OIE ad hoc group on prioritization of diseases for which vaccines could reduce antimicrobial use in animals (http://www.oie.int/standard-setting/specialists-commissions-working-groups/scientific-commission-reports/ad-hoc-groups-reports/) and the Poultry Working Group of the USDA Research Gap Analysis Workshop (https://www.ars.usda.gov/alternativestoantibiotics/Symposium2016/2016%20Working%20Group%20Reports/Poultry%20Working%20Group.pdf); priority diseases identified by only one of the two groups (i.e., infectious bursal disease virus and foodborne pathogens such as *Salmonella* and *Campylobacter)* were not included. Antibiotic use refers to those used in human medicine.

† As identified by the OIE ad hoc group on prioritization of diseases for which vaccines could reduce antimicrobial use in animals (http://www.oie.int/standard-setting/specialists-commissions-working-groups/scientific-commission-reports/ad-hoc-groups-reports/)

‡ As identified by the Poultry Working Group of the USDA Research Gap Analysis Workshop (https://www.ars.usda.gov/alternativestoantibiotics/Symposium2016/2016%20Working%20Group%20Reports/Poultry%20Working%20Group.pdf); data available only for certain diseases.

Alternatives available to reduce the use of antibiotics

It is not a simple task to objectively catalog and then summarize the options available for reducing the need for antibiotics in animal agriculture through the use of non-antibiotic alternatives. As demonstrated in Table 2, the efficacy of alternative products can vary considerably by species and purpose of use. Moreover, some alternative products may be highly effective when used in foot baths or administered directly into the udder, but ineffective after ingestion. Certain products have yielded promising results in experimental studies but are not commonly used on commercial operations. Other products are used commercially even though their efficacy has not been proven. In some instances, the scientific literature yields inconsistent or contradictory results regarding efficacy. Studies differ considerably in how they measure efficacy, and outcomes may not be comparable—for instance, efficacy for disease prevention may be measured in terms of reduction in mortality, reduction in the prevalence of animals with diarrhea, reduction in the severity or duration of diarrhea, reduction in intestinal lesions, or a number of other outcomes. Moreover, few studies directly compare the efficacy of alternatives to that of antibiotics. In some cases, no scientific data evaluating efficacy is available. Finally, not all products in a category (e.g., different probiotic strains or enzymes) may have equal efficacy, and comprehensive data on actual use of alternatives on commercial operations is sparse and not systematically collected.

Table 2 summarizes the available evidence for efficacy in each of the major food producing species based on a comprehensive review of the scientific literature and expert interviews conducted to evaluate commercial use (see appendix for methodological details regarding the literature search and expert interviews). Milk-fed calves are physiologically very dissimilar to older cattle because their digestive tract and immune system are not yet fully formed. At the same time, dairy and beef cattle are managed differently and affected by distinct diseases and conditions. This can have profound impacts on how well individual products work, and efficacy is therefore reported separately for these three groups.

Table 2

Alternatives to Antibiotics for Use in Animal Agriculture

Efficacy of products varies across animal species and reason for use

	Cattle			Swine	Chicken*	Turkey
	Milk-fed calves	Dairy cows	Beef cattle			
Probiotics	● ■	● ■	● ■ △	● □ △	● ■	● ■
Prebiotics	○ □	◌	◌	○ □	● ■	● ■
Organic acids		○ □	○ □	● □	● ■	○ □
In-feed enzymes		◌	◌	● □	● ■	●
Antimicrobial peptides	○ □	○ □† △‡	○	○ □	○ □	
Phytochemicals (e.g., essential oils)	○ □ △	○	○	○ □	● □	○ □
Copper, zinc, and other heavy metals	◌§	○ □**	● □††	● □	●	○
Immune modulators	■	■‡‡	■	○ □ △	○ ■	○ □
Vaccines	■	■	■	§§● ■	■	■
Bacteriophages, endolysins, lysozyme, and other hydrolases	□	□ △		○ □ △	□ △	○ □

● Growth promotion, strong scientific evidence for efficacy and commercially used

○ Growth promotion, some scientific evidence suggests potential efficacy

■ Disease prevention, strong scientific evidence for efficacy and commercially used

□ Disease prevention, some scientific evidence suggests potential efficacy

▲ Disease treatment, strong scientific evidence for efficacy and commercially used

△ Disease treatment, some scientific evidence suggests potential efficacy

◌ Evidence suggesting lack of efficacy

Notes:

Full colors represent strong scientific evidence for efficacy (i.e., based on meta-analysis, systematic review, or review by authoritative organizations such as the Food and Agriculture Organization of the United Nations) and commercially used; also included in this category are products that have market approval as drugs or biologics because efficacy has to be demonstrated as part of the approval process for these products.

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Outlined colors represent some scientific evidence suggesting potential efficacy; in some cases, available scientific evidence may have yielded contradictory results.

* For this table, we focused on broiler chickens and not layers.

† Topical application on teats.

‡ Topical application on teats.

§ Evidence suggesting toxic effects in milk-fed calves.

** Topical application in foot bath.

†† Topical application in foot bath.

‡‡ Approved product for mastitis prevention.

§§ Approved vaccines with growth promotion claim in Australia.

Alternatives to antibiotics for growth promotion

Antibiotics used for growth promotion are typically administered to all animals in a pen, herd, or flock, at a relatively low dosage and over long periods of time. In the U.S., medically important antibiotics are no longer available for growth promotion since Jan. 1, 2017.[9] Therefore, finding alternatives continues to be a priority for the animal industry. Importantly, as shown in Table 2, many alternative products enhance animal productivity and prevent infection at the same time, which could make them particularly attractive for commercial operations. This section—on alternatives to growth promotion—also discusses product efficacy for disease prevention or treatment where applicable, as both considerations are vitally important with regard to commercial usefulness.

There are several challenges to evaluating whether alternative products might substitute for antibiotic growth promoters. First, the mechanism of action by which antibiotics promote growth has not been fully determined,[10] so specific effects on animal and bacterial populations to be replaced by alternatives are not well defined. Moreover, the effectiveness and cost-effectiveness of antibiotic growth promoters are not well understood and may be negatively correlated with the adequacy of farm management practices.[11] Therefore, the minimum effectiveness and cost-effectiveness needed to make alternative products viable substitutes for antibiotic growth promoters are unknown and could change as operations improve management practices. Due to scarcity of on-farm antibiotic use data, it is not clear what are the most pressing health problems driving antibiotic use on the operations looking for replacements of antibiotic growth promoters, and whether these issues may be potentially mitigated by alternative products.

Alternatives such as probiotics are used commercially for growth promotion and occasionally disease prevention. There is a body of scientific studies available that have evaluated the efficacy of different alternatives as growth promoters and, to a more limited extent, for use in disease prevention.[12] These studies, discussed below, have often found highly promising results.

In-feed enzymes

An encouraging option to promote animal growth is enzymes that can be added to animal feed. These help the animals break down and digest plant materials such as cellulose or pectin, which they otherwise cannot utilize effectively.[13] In fact, certain enzymes (e.g., xylanases and beta-glucanases) are already commonly added to commercial feed for broiler chickens.[14] The mechanism behind the effectiveness of in-feed enzymes as growth promoters is not fully understood but may include changes to the gut microbiota, prevention of damage caused by undigested plant parts rubbing against the inner lining of the intestine, breakdown of larger molecules into compounds with prebiotic activity, or impacts on the composition of the intestinal content and its digestibility.[15] In-feed enzymes are also promising interventions for preventing certain diseases such as necrotic enteritis in chickens.[16]

A reasonable amount of research on in-feed enzymes as growth promoters is available, yet efficacy seems to vary greatly by host species. Promising results have been observed in chickens when in-feed enzymes were used for growth promotion and to improve nutrient intake.[17] One study, for instance, found that enzyme supplementation resulted in a 2 to 5 percent improvement in feed efficiency, expressed as the ratio of feed consumption to animal weight gain (i.e., feed-to-gain ratio).[18] Another study of broiler chickens, following their entire 42 days of life until slaughter, reported statistically significant improvements in weight gain as well as improved feed conversion in chicks fed diets containing in-feed enzymes.[19] The European Food Safety Authority (EFSA) has evaluated a combination of xylanases and beta-glucanases and concluded that the product is safe and effective as a growth promoter in chickens and turkeys,[20] and systematic reviews have similarly concluded efficacy of different in-feed enzymes as growth promoters.[21] A number of studies, including systematic reviews and meta-analyses, have also determined that in-feed enzymes such as xylanases are effective at decreasing intestinal lesions and at reducing the risk of necrotic enteritis, for which intestinal lesions are a key predisposing factor.[22]

Results for in-feed enzymes as growth promoters in swine have been variable. The high level of acidity in the swine gut may inactivate in-feed enzymes.[23] Enzymes that are stable under such conditions have shown promising results in swine, indicating the potential for this alternative strategy as a growth promoter in pigs.[24] Some enzymes, such as phytases, generally appear to be more effective at improving performance than others.[25] A meta-analysis recently found evidence of efficacy for growth promotion in swine, but the extent of the growth promoting effect was variable and more data are needed.[26] Some scientific evidence also suggests that in-feed enzymes may reduce the risk of certain diseases such as colibacillosis after piglets are weaned,[27] but more data are needed to further evaluate this application.

In-feed enzymes are not a promising alternative for ruminating animals such as cattle because the rumen inactivates any enzymes before they reach the intestine.[28]

Probiotics

Probiotics are live cultures of microorganisms (e.g., yeast, fungi, and bacteria) that are added to the diet to improve the balance of microbial communities in the gastrointestinal tract.[29] Probiotics can be distinguished as "defined" and "undefined." Defined probiotics consist of single strains or mixtures of comprehensively described microorganisms (e.g., each organism is described to the species level, the exact composition of the culture is quantitatively described, and the genomes of individual organisms in the mixture may have been fully sequenced to assure the absence of any antibiotic resistance genes). Undefined probiotics tend to consist of microbial mixtures that are not completely described.[30] In general, undefined probiotics tend to have higher efficacy than defined probiotics, but both are promising approaches for disease prevention and, in some instances, treatment that may also lead to better production performance and thus growth promotion.[31]

Competitive exclusion products are special types of undefined probiotics, typically given soon after birth or hatching, that help the animals establish a community of beneficial bacteria in the gut before pathogens can colonize there.[32] Competitive exclusion products have in particular shown high efficacy in preventing disease in young animals.[33]

Probiotics are widely used in U.S. poultry operations,[34] and an FAO report has concluded that probiotics can have significant positive effects on the productivity and health of poultry.[35] A number of scientific studies have quantified the efficacy of probiotics for growth promotion and disease prevention in chickens and turkeys. For example, one study reported that probiotics improved productivity and intestinal health in newly hatched birds and reduced mortality by over 20 percent compared with control flocks; the reduction in mortality was similar to that achieved with antibiotics.[36] The use of probiotics in laying hens has resulted in statistically significant increases in productivity, measured in terms of egg production.[37] In an experiment comparing in-feed enzymes to a mixture of probiotic strains, both products significantly reduced broiler mortality and improved production efficiency compared with animals fed a diet that contained neither product. Probiotics, however, showed significantly better results than in-feed enzymes. In fact, a study demonstrated that a wide range of probiotic bacteria can effectively control the clinical symptoms associated with coccidiosis, a potentially devastating poultry disease that tends to be difficult to control without antibiotics. This study compared the efficacy of probiotics to that of ionophores, a class of antibiotics not important for human medicine but used against coccidiosis in birds, and found comparable results, therefore probiotics can significantly decrease the need to use ionophores to prevent diseases associated with coccidiosis.[38]

The use of probiotics in pigs has also shown beneficial effects on productivity and health, and probiotics are already used on commercial swine operations in the U.S.[39] For example, reviews by FAO, the European Medicines Authority (EMA), and EFSA have concluded that probiotics are effective growth promoters in swine, and that they can effectively prevent diarrhea and reduce mortality due to infections with *E. coli* in piglets.[40] A number of scientific studies have quantified the impact of probiotics on productivity as well as on disease rates. Improvements in weight gain of over 7 percent in piglets after weaning and significant increases in feed efficiency in sows have been reported.[41] Probiotics have also shown efficacy in preventing post-weaning diarrhea in young piglets, with demonstrated incidence rate reductions of up to 40 percent.[42] Moreover, one study showed that probiotic use in newborn piglets and calves led to a significant decrease in the prevalence of digestive disorders and mortality rates compared with control animals that received neither probiotics nor antibiotics, comparable to that achieved with antibiotics.[43]

Probiotics have shown promise for disease prevention in cattle,[44] as well as enhancing a variety of production parameters, and probiotics are widely used commercially in cattle. According to recent data, 20 percent of U.S. dairy operations use probiotics to prevent disease in dairy cows, and to improve health and productivity in dairy calves.[45] Similarly, more than 1 in 4 large feedlots with more than 1,000 cattle uses probiotics to prevent disease.[46] An FAO report as well as several meta-analyses, and systematic reviews have concluded that probiotics are effective at enhancing productivity and preventing or treating disease in beef as well as dairy cattle and calves.[47] A number of scientific studies have quantified the impact of probiotics for these purposes. In one study, for instance, probiotic use increased milk production efficiency (measured as kg milk produced/kg feed consumed) in dairy cows by 6 percent.[48] While overall more scientific studies have evaluated the impact of probiotics on growth promotion than on disease prevention in cattle, positive impacts on the latter have also been repeatedly demonstrated.[49]

For all species, storage and administration of probiotics poses a potential challenge. For instance, to create feed pellets, chicken feed is usually exposed to high heat during manufacturing, which may inactivate probiotics, although that problem does not seem to exist in other feed forms.[50] Because live cultures are administered, probiotics have some associated risks, for example potential unintended, undesired, and detrimental changes in the microbial balance of the gut.

Prebiotics

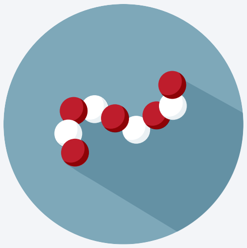
Prebiotics are organic compounds such as certain sugars that, when added to the diet, are indigestible by animals but are broken down by certain beneficial microorganisms in the gut, which selectively stimulates these and other microorganisms' growth.[51] Prebiotics thereby can favor the presence of beneficial microorganisms in the intestine. Both prebiotics and probiotics help beneficial microorganisms to outcompete harmful bacteria but may also have other effects such as modulating the immune system. However, the various ways in which these products work and the diverse biological impacts they can exert—for instance, on the immune systems of animals that ingest them—are not completely understood.

Contrary to the situation for probiotics, the use of prebiotics as growth promoters and for disease prevention has shown inconsistent efficacy. In general, the efficacy of prebiotics seems to be determined by a variety of factors, including the type of prebiotic, animal age and species, animal health status, the housing type, and management practices, all of which have to be considered in the decision whether to use these alternatives.

Prebiotics are used commercially in chickens and turkeys for growth promotion and disease prevention as well as to improve overall gut health, according to expert elicitations.[52] A recent review by EMA and EFSA concluded that prebiotics are effective at promoting growth and reducing disease.[53] Although studies evaluating the efficacy of prebiotics for disease prevention in chickens are fairly limited, significant reductions in the shedding of pathogens and improvements in gut health have been described.[54] However, efficacy appears to be variable,[55] and some products such as fructo-oligosaccharides or mannan appear to be more effective than others.[56]

In pigs, some studies have reported positive growth promoting effects of prebiotics with increases in average daily gains of up to 8 percent in pigs immediately after weaning,[57] but other studies have failed to find a statistically significant impact on growth.[58] In pigs fed a diet containing prebiotics, probiotics can also enhance immune responses against intestinal infections such as salmonellosis.[59]

In cattle, prebiotic efficacy seems to be limited to young calves. The addition of some prebiotics to milk replacers (i.e., the liquid feed given to young calves not nursed by their mothers, primarily on dairy farms) has been shown to promote growth and prevent disease in young dairy calves.[60] In these animals, average body weight gains were significantly greater when fed a diet of milk replacers with a specific type of prebiotic (galactosyl-lactose) than when fed a diet of milk replacer without prebiotic.[61] Even though relatively few studies have evaluated the efficacy of prebiotics for disease prevention in young calves, statistically significant improvements in gut health have been reported.[62] However, young calves differ from older cattle because the rumen, the part of the animal's digestive tract that helps break down complex carbohydrate plant materials such as cellulose, is not fully developed until the calf begins to ingest plant materials. Prebiotics are quickly digested in the fully formed rumen, and thus are rendered ineffective.[63]



Antimicrobial peptides

Antimicrobial peptides are another potentially promising alternative for growth promotion that may aid in disease prevention and possibly treatment. Antimicrobial peptides are short molecules with antibacterial properties that are toxic to certain bacteria.[64] In many cases, these peptides are generated by microorganisms. Antimicrobial peptides also include host defense peptides that are generated by other species including mammals.[65] These host defense peptides are important for innate immune defenses and are therefore discussed further under immune modulators. A variety of antimicrobial peptides have been described, with considerable difference in the types of bacteria they are active against, as well as in their mechanisms of action,[66] which may imply differences in the potential emergence of resistance.[67]

Antimicrobial peptides are promising alternatives for growth promotion as well as disease prevention in chickens. A recent joint opinion issued by EMA and EFSA concluded that such peptides are effective in promoting growth and general gut health in chickens, even though their efficacy in preventing specific diseases is variable.[68] Positive scientific results have been reported in chickens, with increased daily weight gains of up to 7 percent.[69] In vitro studies provide strong circumstantial evidence that the use of antimicrobial peptides in broiler chickens, as well as pigs, improves intestinal health and suppresses harmful bacteria by favoring the growth of beneficial microorganisms.[70] One study under experimental conditions has provided evidence that antimicrobial peptides significantly decrease the prevalence of intestinal pathogens in broiler chickens.[71] Expert interviews conducted for the development of Table 2, however, indicated that these products are not commercially used in the U.S. broiler production. Scientific studies specific to turkey are scarce.

Several scientific studies have demonstrated the potential value of antimicrobial peptides for weight gain and disease prevention in pigs. One study, for instance, evaluated performance in pigs experimentally exposed to *E. coli* after weaning, and reported that pigs given antimicrobial peptides gained significantly more weight than control animals not given these peptides. In fact, weight gains in animals fed antimicrobial peptides were comparable to weight gains in control animals given antibiotics.[72] Other studies have reported statistically significant increases in beneficial bacteria in the guts of pigs or piglets administered antimicrobial peptides, presumably indicating a health-protective effect.[73]

Some studies have evaluated the efficacy of antimicrobial peptides in dairy cattle with potentially promising results for growth promotion as well as the prevention and treatment of udder infections.[74] In fact, nisin, a particular antimicrobial peptide, has been extensively researched for prevention and treatment of udder infections in the time period when dairy cows do not produce milk, and a product for sanitizing the udder before milking has demonstrated significant reductions in udder pathogens in experimental studies.[75]

Notably, there may be ways to combine antimicrobial peptides and probiotics to achieve synergistic effect. Some probiotic strains have been shown to produce bacteriocins, a certain type of antimicrobial peptide.[76] If these probiotic strains can establish themselves in the gut of animals fed the probiotic, they can simultaneously act against harmful gut bacteria in two ways: they can outcompete many of the harmful bacteria and at the same time kill the remaining harmful bacteria through the bacteriocins they produce.

Organic acids

Organic acids, such as citric or acetic acids, are also promising alternatives for growth promotion and disease prevention. Similar to the alternatives previously discussed, the mechanism by which organic acids function as growth promoters when added to feed or drinking water is not well understood. It is likely that an organic acid's ability to kill bacteria contributes to its growth promotion property; in addition, organic acids may affect gut microflora by favoring the growth of certain acid-loving beneficial bacteria, and improve the physiological functions of the stomach by increasing its acidity levels.[77] A recent joint opinion by EMA and EFSA concluded that organic acids are effective growth promoters in chickens and can successfully prevent disease in these animals, even though efficacy is variable.[78] In swine, a meta-analysis concluded that organic acids have demonstrated some, albeit variable, efficacy as growth promoters and a review has concluded that organic acids have positive impacts on disease prevention, measured for instance in the form of reduction in gastro-intestinal illness and diarrhea in piglets.[79] Some studies in cattle have also demonstrated a positive effect of organic acids on performance and the prevention of certain digestive diseases such as rumen acidosis, but more data are needed.[80]

Individual studies have further quantified the impact of organic acids on growth promotion and disease prevention. Adding organic acids* to the diet has been described as exerting direct positive growth effects, with improvements in weight gain in broiler chickens and grain-fed beef cattle of around 17 percent and more than 8 percent, respectively.[81] Promising results have also been described in pigs, although here efficacy may differ by production class and its use may be contraindicated in specific cases, for instance in sows because of potential negative impacts on their milk production.[82] In-feed organic acids also may reduce pathogen survival in the gut.[83] One study, for instance, found that organic acid supplementation in piglets significantly reduced the incidence and severity of post-weaning diarrhea syndrome compared to pigs fed a diet without supplementation of organic acids.[84]

* Studies cited the use of organic acid blend (i.e., orthophosphoric acid, formic acid, propionic acid) in chicken and DL-malate in cattle.

Phytochemicals

Phytochemicals are plant-derived compounds, such as essential oils or tannins that may have antibacterial and growth promoting effects.[85] Different essential oils vary in antibacterial mode of action, which is often not well characterized.[86] Phytochemicals are used on commercial poultry operations for growth promotion as well as disease prevention,[87] and a recent opinion issued jointly by EMA and EFSA concluded that these compounds are effective in promoting growth in chickens but that efficacy depends, at least to some degree, on the part of the plant used.[88] The same conclusion regarding efficacy was reached in a meta-analysis,[89] and some scientific studies have demonstrated that phytochemicals can improve the gastrointestinal health of broiler chickens and reduce levels of coccidian parasites.[90] Some studies have shown positive effects for disease prevention as well as growth promotion in pigs, but others have failed to detect such effects.[91] In adult cattle, a recent meta-analysis concluded that the available data are insufficient to reach a final determination regarding efficacy as growth promoters.[92] Some studies suggest efficacy of phytochemicals for the prevention of diseases in cattle such as diarrhea and to improve digestive health,[93] but more studies are clearly needed. When essential oils are successfully added to feed to increase animal weight gains, they are typically required in high concentrations to achieve antimicrobial effects, which can negatively affect meat quality.[94]

Other alternatives

A variety of other alternative products, such as heavy metals and clay minerals, are also potential substitutes for antibiotic growth promoters, and many may at the same time have disease prevention properties.

Zinc, copper, and other heavy metals

Zinc, copper, and other heavy metals are naturally occurring and necessary trace elements in the diet but are commonly added to the diet in higher concentrations for growth promotion, and occasionally as therapy for enteric disease.[95] The European Commission has concluded that copper is effective at promoting growth in broiler chickens and swine,[96] and a meta-analysis has demonstrated that zinc oxide improved growth in piglets.[97] A meta-analysis has also demonstrated the value of copper as a growth promoter in beef cattle,[98] even though the European Commission has concluded that copper is not known to exert growth promoting effects in any species other than pigs and chickens, and that copper can quickly reach toxic levels in calves.[99] Experimental studies have demonstrated that in chickens, daily gains were significantly improved when broiler feed was supplemented with a combination of inorganic minerals including copper, iron, manganese, and zinc; these inorganic supplements produced a statistically significant increase in broilers' weight gain.[100] Scientific studies of copper have also demonstrated improvements in laying hen performance, and zinc oxide has been shown to reduce the incidence of diarrhea in pigs after weaning.[101] However, concerns about potentially harmful residues of heavy metals in the meat have to be considered carefully.[102] In addition, there is evidence that the use of heavy metals for growth promotion can lead to increased rates of resistance to certain antibiotics, presumably because the genes encoding for resistance to the antibiotic and heavy metals are genetically linked (e.g., present on the same plasmid).[103]

A variety of other substances have been proposed as growth promoters, including clay minerals (e.g., bentonites, zeolites) and rare earth elements (e.g., scandium, lanthanum).[104] Some of these may be effective growth promoters, and may also have efficacy for disease prevention. However, efficacy data are few and often conflicting.[105] In many cases, safety data are also lacking.

Alternatives to antibiotics for disease prevention

Antibiotics and their alternatives can also be used to prevent diseases in healthy animals. Disease prevention uses are defined as the administration of a drug to healthy animals in a situation where a specific and increased disease risk is present.[106] This use is distinct from situations where antibiotics are used to control the spread of diseases in a herd or flock when some animals already show clinical signs of disease.[107] Both uses, however, are aimed at protecting animals from disease during times of increased risk of infection and are grouped under disease prevention for the purpose of this analysis.

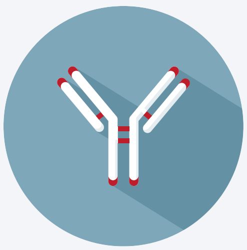
Key similarities exist between growth promotion and disease prevention uses for drugs and alternatives, including the administration to healthy animals and potentially long durations of use. As discussed in the previous section, many antibiotic alternatives are thought to have both positive impacts on preventing disease and promoting growth. In many cases, it is likely that the growth-promoting effect is at least partially due to the product's ability to inhibit or kill bacteria. At the same time, preventing animals from becoming sick can prevent productivity losses due to illness, whether clinical or subclinical in nature.[108]

Vaccines

Vaccines have been widely used in veterinary medicine to prevent diseases caused by viruses or certain bacteria, and they are promising substitutes for some antibiotic uses.[109] Notably, reducing viral infections may lead to decreased antibiotic use because of the risk of misdiagnosis and because antibiotics may be used to prevent or treat secondary bacterial infections.[110] Therefore, vaccines for both viral and bacterial infections are relevant to the discussion around alternatives to antibiotics. Evidence suggests that at least some vaccines may also have positive effects on growth rates and animal performance, even though external factors such as the need to handle animals for vaccine application can impede them.[111] Notably, the current regulatory framework in the U.S. does not permit vaccines to be labeled or marketed for such purposes and, even if these uses were allowed, questions around practicality and cost-effectiveness would have to be resolved.

Vaccines stimulate a protective immune response that is more or less comparable to the effects that follow a natural infection, but generally without the negative impacts caused by the clinical progression of the disease, and vaccines have a long history of successful use in animals. A variety of vaccines are commercially available and actually used on U.S. operations as a management option to prevent and reduce the spread of infectious diseases.[112] For instance, according to recent NAHMS data, more than 70 percent of U.S. operations are estimated to vaccinate very young (i.e., nursery-age) pigs against *Mycoplasma* pneumonia; similarly nearly 60 percent of beef cow-calf operations vaccinate against clostridial diseases caused by *C. chauvoei*.[113] By preventing infection, vaccination can reduce antibiotic use. For example, vaccination against *Lawsonia intracellularis*, a bacterium causing a severe intestinal disease called ileitis, has been shown to reduce the need for oxytetracycline in pigs in Denmark.[114] In the U.S., an estimated 26 percent of breeding pig operations vaccinate against *L. intracellularis*.[115]

Vaccines are among the most promising approaches to disease prevention, but their use is not without challenges. For example, many vaccines have to be given by injection, leading to increased labor costs,[116] and the stress caused by increased handling can affect an animal's immune response[117] and may result in reduced weight gains. Additionally, some vaccines have a narrow range of bacterial or viral strains against which they are effective, and others pose a risk of unintended consequences such as reversion to a pathogenic virus that can cause disease.[118] Research efforts are ongoing to address many of these challenges, such as the potential for mass administration of vaccines or the development of strategies for eliciting more protective immune responses.[119] Therefore, vaccines may become better alternatives to antibiotics in the future.



Immune modulators

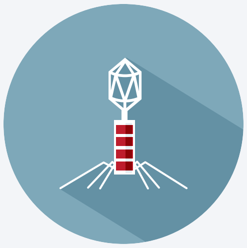
Immune modulators, which as defined here include the transfer of antibodies to elicit passive immune responses, are promising alternatives for disease prevention and potentially for treatment as well. In contrast with vaccines, immune modulators stimulate the immune system in a way that is less dependent on the pathogen causing infection, which makes them effective against a broad range of pathogens.[120] A very broad variety of immune stimulatory substances have been investigated as potential alternatives to antibiotics.[121] These include cytokines (i.e., substances that are secreted by certain immune cells to regulate other parts of the immune system), lipopolysaccharides (i.e., large molecules that are present in the wall of certain bacterial cells and trigger innate immune responses), short segments of bacterial DNA that also stimulate innate immune responses (i.e., CpGs), antibodies derived from egg yolk that provide short-term immunity, and certain plant materials.[122]

In chickens, a meta-analysis showed that egg-yolk antibodies significantly reduce the risk of necrotic enteritis, and several studies have provided promising results for other types of immune modulators.[123] For example, after day-old broiler chickens were intentionally infected with *E. coli*, significantly fewer clinical symptoms were reported in those animals treated with a CpG-based immune modulator than in the control chicks.[124]

In swine, a meta-analysis demonstrated efficacy of egg-yolk antibodies in preventing diarrhea caused by a variety of bacterial and viral pathogens.[125] A systematic review concluded that another type of immune modulator, in the glycans family, failed to demonstrate efficacy in pigs but that the data were scarce.[126] However, individual scientific studies of challenges with bacterial toxins showed highly promising results for vitamin C and glycans in young piglets.[127] Feeding of antibodies derived from egg yolk has also shown promise for the prevention and treatment of diarrhea in young piglets, even though limited stability in the swine gut and narrow host spectrum pose potential challenges, and cost-effectiveness so far remains elusive due to high production costs.[128]

In the U.S., two immune modulators have recently successfully demonstrated safety and efficacy and have been approved for use in cattle. One is for use in dairy cows to prevent udder infections after calving; it is based on a cytokine and recently received animal drug approval from the Food and Drug Administration.[129] Another, based on CpGs, has been approved by USDA as a biologic for use in cattle affected by respiratory disease.[130]

The efficacy of immunostimulants relies on a functioning immune system and therefore may not always be a feasible option; for instance, in very young animals, the immune system is not yet fully functional, and severe stress and disease can also limit the functionality of the immune system.[131] There are also safety concerns about using immunostimulants before the immune system is fully formed because of the potential risk for adverse developmental effects.[132] In addition, the mechanisms of action are rarely well determined.[133]



Bacteriophages, endolysins, and hydrolases

A number of viruses and the enzymes they generate show promise as alternatives for antibiotics that may be used for disease prevention and potentially for treatment, thereby also potentially indirectly affecting production performance.

Bacteriophages

Bacteriophages are viruses that infect and kill bacteria.[134] Most bacteriophages have a narrow range of bacterial strains they can infect, which in extreme cases can be restricted to a single strain of a bacterium.[135] Bacteriophages can therefore be used in a highly targeted way with minimal unintended impacts on other bacteria and the host.[136] In addition, antibiotic resistance typically does not interfere with the bacteriophage's ability to infect and kill the bacterium, which may make them one of few treatment options for infections with multidrug-resistant bacteria.[137] In addition, because the bacteriophages multiply in the bacteria they infect, a reasonably broad dosage range can be effective.[138] However, bacteria can become resistant to bacteriophages; bacteriophages may rapidly degrade in the environment; and there is some risk that certain bacteriophages may have the ability to spread antibiotic resistance genes.[139] Overall, bacteriophage therapy tends to be extremely time-sensitive. For example, phage therapy had limited efficacy when administered more than 16 hours after experimental infection.[140] Notably, bacteriophages are actually naturally occurring and common in the environment.[141]

Bacteriophages have been used for disease prevention and treatment,[142] with promising results. For example, they have protected chickens from respiratory disease after experimental infection with *E. coli*.[143] Similarly, *Salmonella* infection in day-old broiler chicks was successfully treated by a phage cocktail containing bacteriophages specific to *Salmonella enteritidis*.[144] Bacteriophages have also been evaluated as treatments for colibacillosis in chickens, and mortality was comparable to the comparison group that received the antibiotic enrofloxacin.[145]

Phage therapy has also shown promising results in piglets and calves, where bacteriophages significantly reduced the prevalence of diarrhea caused by *E. coli* and successfully treated them in piglets.[146] However, the major obstacles to using bacteriophages for disease treatment in animals include the lack of rapid and accurate diagnostics—which are necessary because the phages typically are effective only against a very narrow range of bacterial strains—the risk of phage inactivation via the host immune response, and rapid emergence of resistant bacterial strains.[147] Phage cocktails that contain several different bacteriophage strains can help address these limitations, but to date, efficacy for treatment of pathogenic organisms has remained limited.

Endolysins and lysozymes

Endolysins and lysozymes are hydrolases. Hydrolases are enzymes that degrade peptidoglycans, the main building block of the bacterial cell wall, and thereby kill bacteria. The hydrolases can be derived from a number of different sources, including bacteriophages, as well as animals, plants, bacteria, and insects, with varying specificity for target bacteria.[148]

Endolysins

Endolysins, also commonly referred to as virolysins, are generated by bacteriophages.[149] Bacteriophages generate endolysins at specific stages of their life cycle, shortly before the virus destroys the bacterial cell. In that process, endolysins aid in the release of the newly generated bacteriophages.[150] Endolysins tend to have a relatively narrow spectrum of bacteria against which they are effective[151] and are highly thermostable. In experiments at 100 degrees Celsius, some retained over 70 percent of their activity against *Staphylococcus aureus*. Such heat stability can be important to assure product integrity, as some feed is processed at high temperatures.[152] The mechanism by which endolysins target and eliminate pathogenic bacteria has been fully described and depends on two distinct functions: binding to specific sites in the bacteria cell wall and cleaving the bonds between the peptidoglycans in the cell wall.[153]

Endolysins are tentatively promising enzymes for the prevention and treatment of certain bacterial infections. In part this is because it is believed to be more difficult for bacteria to develop resistance against them, and in part because it may be possible to specifically engineer endolysins with the desired host spectrum.[154] However, concerns about potential adverse immune responses and the downsides of a relatively narrow host spectrum have to be considered. Yet, although efficacy data specific for the use of endolysins in food-producing animals have so far remained scarce, endolysins have shown promising results against a relatively broad range of bacteria.[155] It should be noted that endolysins are not effective against all bacteria. Because of differences in the bacterial cell wall, endolysins tend to have limited efficacy against Gram-negative bacteria.[156]

Lysozymes and autolysins

Lysozymes and autolysins are hydrolases generated by eukaryotic organisms (i.e., animals and plants) and bacteria, respectively. In humans, lysozymes are an important component of the innate immune system and naturally present in the skin and secreted into saliva, urine, milk, and other bodily fluids.[157] Lysozymes in particular tend to have activity against a broad spectrum of bacteria and are known to effectively break down the carbohydrate component of peptidoglycan layer of bacteria. They are also known to be effective against viruses and other pathogens.[158] Lysozymes and autolysins are promising alternatives to antibiotics, although they share many of the limitations discussed under endolysins.

Other disease prevention alternatives

A variety of other approaches for disease prevention have been proposed, including biofilm inhibitors and quorum-sensing inhibitors (i.e., substances that disrupt biofilm formation, a bacterial communication system that plays an important part in the infection process).[159] While these approaches may offer innovative alternatives to antibiotics, data on safety and efficacy are to date largely lacking. In addition, their impact on production performance for growth promotion purposes replacing antibiotics remains largely unknown. One class of specific and particularly promising products is virulence inhibitors: molecules that directly affect the harmful microbes and block key functions they need in order to survive and infect. For example, they may prevent bacteria from forming pili, structures that allow them to adhere to animal cells.[160] Experimental data for inhibitors remain limited, so the safety and efficacy of these approaches are unclear; however, such novel approaches represent a new path, one that does not attempt to directly kill bacteria but rather tries to restrain some of their pathogenic activities. This approach may for instance be less likely to disrupt the healthy balance in the gut.

Farm management and biosecurity

While a detailed analysis is beyond the scope of this paper, biosecurity and management practices are an important part of disease prevention that can improve overall animal health and significantly reduce the risk of pathogen introduction into the herd or flock.[161] Notably, a comprehensive approach that includes alternative products and improved management practices is likely to be more effective than relying on a single alternative product or approach to manage health and prevent disease.[162] In fact, improvements in biosecurity have been widely accepted as an effective means of preventing the introduction of diseases into herds or flocks.[163] This concept applies widely across species, production systems, and pathogens. It addresses the risk of animal disease outbreaks such as avian or swine influenza while reducing the risk for introducing certain foodborne pathogens such as *Campylobacter*. In many cases, biosecurity is regarded as a prerequisite for successful herd or flock management.

Alternatives to antibiotics for disease treatment

Compared with disease prevention and growth promotion alternatives, fewer alternatives to antibiotics exist for the treatment of disease. As discussed above, potentially promising approaches include probiotics, antibacterial peptides, and immune modulators as well as bacteriophages and endolysins. While far from commercial use, other alternative approaches currently being explored include predatory bacteria and Cas9.

Predatory bacteria

Predatory bacteria such as the Gram-negative bacteria *Bdellovibrio* spp. and *Micavibrio* spp. possess the ability to attack and kill certain pathogenic bacteria, for example multidrug-resistant *E. coli* and *Klebsiella* strains; in vitro studies have provided some encouraging results.[164]

Cas9

Cas9 and similar products work by reprogramming parts of the bacterial immune system (i.e., Cas9, a nuclease in the type II CRISPR system of bacteria) to selectively target specific parts of the bacterial genome (i.e., virulence factors), thereby selectively inactivating harmful bacteria that possess these virulence genes. In vitro studies have shown some promising results.[165]

In addition, nanoparticle-stabilized liposomes, certain metals such as silver, and other substances have also shown promising antibiotic efficacy in vitro.[166] These approaches are very promising; however, none of these innovative approaches is likely to be available for use in livestock species in the foreseeable future.

Conclusion

A variety of products and management practices may eventually be able to replace a substantive proportion of current antibiotic use for prevention and growth promotion purposes, but this effort will require a comprehensive approach that considers alternatives as one part of a herd health management program.

Overall, alternatives to antibiotics are promising, as many appear to simultaneously enhance animal productivity and prevent infection, both of which hold much appeal to food animal producers. However, in several instances, efficacy has been evaluated only experimentally, which probably neither reflects real-world husbandry conditions on commercial operations nor the target animals (e.g., studies are often conducted in calves or piglets while the intervention would ultimately be applied to older animals). In other cases, the approach might be broad and indirect but effective, such as biosecurity measures. Potential unintended consequences have generally not been well studied. Typically, cost-effectiveness data are also not available, complicating the evaluation of incentives for implementation.

Nonetheless, some commercial food animal producers are already successfully using available alternatives for growth promotion and disease prevention, including probiotics and vaccines.[167] More information on these uses could complement experimental data from academic research studies. Such data could be shared through public-private partnerships, and findings could be more widely disseminated through extension services. This could prove instrumental to the successful use of these interventions as part of herd- or flock-health management plans.

A variety of other alternatives for growth promotion and/or disease prevention have been proposed, and early results were found to be positive, but more data under realistic conditions are urgently needed, as are data on potential interactions among alternatives. A variety of factors may hinder the commercial development of these approaches, including regulatory requirements* and concerns about market size, particularly if antibiotics remain available to producers and veterinarians. To optimize the use of scarce public research and development resources, a priority should be placed on areas of greatest need for products to replace antibiotic use. However, as demonstrated in Table 1, to develop an evidence-based prioritization, a comprehensive understanding of animal disease conditions that necessitate antibiotic use and the mechanism of action and roles antibiotic alternatives play is crucial. Emphasis needs to be given to on-farm antibiotic use data to tailor and prioritize future research efforts. Alternatives have the potential to replace antibiotics in many situations. This can reduce antibiotic use in animal agriculture, and allow these lifesaving drugs to be preserved for use when absolutely needed to protect human or animal health. Focused research and development will help bring promising technologies to the veterinary market and guide their use. That, in turn, will help reduce antibiotic use in animal agriculture without endangering animal health, productivity, and welfare.

Appendix: Methodology of literature review and expert interviews

Comprehensive review

Literature searches were conducted in early 2017 using the search engines Google Scholar, Google, and PubMed and were based on a predetermined set of search terms (available on request). In addition, the literature cited in selected studies was reviewed to keep additional relevant studies. For the first 20 pages of results per search, all abstracts were reviewed to determine whether they met the inclusion criteria. Relevant full-text articles were reviewed to ensure that the studies focused on clear endpoints such as increased production for growth promotion and animal health outcomes for disease prevention and treatment. Excluded from the search results were studies that pertain exclusively to the following foodborne pathogens: *Campylobacter*, *Salmonella*, and enterotoxigenic *E. coli*, unless those strains were evaluated with regard to clinical outcomes in food animals.

Expert elicitation

Experts used to provide feedback were independent from the report's external peer reviewers. Academic veterinarians and food-animal experts with species-specific experience in clinical and extension work were identified through review of the pertinent literature and a peer-nomination process. Experts were consulted to provide feedback on the use of alternative products in the commercial setting. In addition, experts were asked to confirm the lack of scientific studies in those situations where the literature search failed to uncover relevant data.

The full list of literature references and expert opinions on which Table 2 is based is available on request.

* Regulatory requirements associated with alternatives to antibiotics in animal agriculture are outside the scope of this report.

Endnotes

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