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Global restriction of using antibiotic growth promoters and alternative strategies in poultry production

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ABSTRACT

A growing global concern of antibiotic use in poultry diets due to its potential adverse effects on birds and human health, food safety and the environment has led to a complete ban or restricted use in some countries, and, at the same time, expanding options for the use of alternative feed additives. Multiple, rather than a single additive may replace antibiotic growth promoters (AGPs) in poultry. Blending of feeding additives and hygienic farm management, vaccination and biosecurity may help achieve good intestinal health, stabilise enteric ecosystems and result in sustainable and cost effective production performance of birds. Moreover, controlling unsolicited ingredients at the production level must have the support of different markets responsible for the supply of safe and quality poultry products for consumers. This requires the further increase and diversification of value added poultry products and the expansion of their markets through strategic planning and gradual limitation of live bird markets. More research is warranted in order to explore suitable, reliable and cost effective alternatives to AGPs for commercial use, and strategic poultry value chain development.

Keywords: *AGPs, global trends, alternatives, poultry*

1. Introduction

The use of antibiotics in animal and poultry feed has been a global practice since the middle of the last century. Antibiotics are used both at therapeutic and subtherapeutic levels to promote growth and feed efficiency. However, judicial use of antibiotics in food animals for growth promotion and disease prevention has been controversial for a long time due to the development of antibiotic resistant bacteria in animals and humans[1], resulting in treatment failure when needed[2]. This problem has also been gradually increasing due to the misuse of antibiotics in animal and poultry feed. Evidence shows that antibiotic resistant genes can be transmitted from animal microbiota to human microbiota[3]. As a result, every year there is huge economic loss due to increased medical costs of less effective antibiotics for human health. Very recently, a report from the European Union (EU) indicated that about 25,000 patients die each year from infections by drug resistant bacteria, which is equivalent to €1.5 billion in hospital costs[4]. This report indicates the seriousness of the problem across the globe. Moreover, about 90% of antibiotics given to livestock are excreted into the environment and may be a source of pollution[2]. It is documented that antibiotic resistant bacteria can transmit directly, and indirectly through food chains, air, water, and soil. In addition, some antibiotic drugs have carcinogenic and genotoxic effects on human health[5]. As a consequence of concern for public health safety, several countries have banned or restricted the use of human health related antibiotics in feed.

The World Health Organization (WHO) suggested that the use of antibiotic growth promoters (AGPs) that are also used in human medicine be terminated or rapidly phased out by legislation, until risk assessments are carried out[6]. The EU imposed a complete ban of all AGPs in animal feed in January 2006. But, a few groups of people heavily criticised the total ban of AGPs in farm animals and argued that such a ban follows 'precautionary principles' rather than scientific facts[7]. However, the movement towards restricted use of AGPs in farm animals appears to be an inevitable start towards a future global ban.

Currently, a number of possible alternatives to AGPs are used. Some of the alternatives include significant changes in husbandry practices, and the strategic use of enteric microflora modifiers, including acidifiers, probiotics, enzymes, algae and herbal products, microflora enhancers, and immuno-modulators. However, every alternative has some limitations and no one individually acts as an AGP alternative for sustainable growth and production of birds. As a result, the poultry industry is now facing a great challenge to maintain production performance of birds due to increased feed costs, and the need for alternative supplements to replace antibiotics used in feed. Therefore, the objective of this paper is to review restrictions on the use of AGPs in animal and poultry feed accrued over time globally, its impacts, and the efficacy of feeding available alternatives of AGPs on poultry production.

2. Current status of restriction on antibiotic use in animal and poultry feed

The restriction of antibiotic use in animal feed is a controversial global issue. A recent survey of 128 countries conducted by Alltech reviewed the growing restrictions on the use of antibiotics as growth promoters. This survey focused on the 59 countries where restrictions exist or are likely to be implemented, including 28 from the EU and the top seven countries in terms of livestock production. Sweden was the first country in the world and South Korea was the first country in Asia where antibiotic use in feed was completely banned.

In addition, the USA, Canada, Mexico, Japan, Hong Kong, China and India have limited the use of antimicrobials in feed. The USA is not only limiting antibiotic use but is also moving more towards a significant reduction of antibiotics in food animal production. As of 2017, under FDA regulations, antibiotics can no longer be used for growth promoting purposes across the USA. Companies including McDonald's, A&W, Chipotle and Panera have already begun promoting their chickens raised without antibiotics. In October 2010, Bangladesh also imposed a complete ban of AGPs in animal feed through the Fish and Animal Feed Act 2010 and Animal Feed Rules 2013. Very recently, the Consumer Association of Penang has urged the agri-based industry ministries in Malaysia to ban the use of antibiotics in animal feed where they claim to have reached alarming levels. Some other countries have limited requirements to obtain veterinary prescriptions for using antibiotics in food animals. Among these countries, Australia, Brazil and Ukraine do not have any formal national restrictions on antimicrobial use for the purposes of growth promotion.

It is clear that restriction on the use of AGPs in food animal production is expanding and some countries have been observing the situation and are looking for alternatives to AGPs. Consequently, more research is needed to find reliable and cost effective alternatives in animal agriculture. However, strong monitoring, supervision and quality control systems must be imposed on the industry, the market and at different levels between field and market to ensure AGP-free animal feed. Moreover, an increase and diversification of value added poultry products in the market requires expanded biosecurity and food safety measures. A summary of the impact of AGP restriction on production performance of poultry is shown in Table 1.

3. Antibiotics and their mode of action in animals

3.1 Dietary antibiotics in poultry

The use of antibiotics in animal feed has several benefits. It improves animal welfare and food safety by maintaining animal health and reducing certain pathogens and it reduces animal production costs. Economic benefits are shared over the food production and value added chains. Most of the cost savings of antibiotics are attributed to improved feed conversion, and this response is the highest in fast-growing genetically improved animals reared in intensive production systems. Other cost savings come from faster growth rate, reduced mortality, a high resistance to disease challenge, improved reproductive performance, and improved manure and litter quality.

Rosen[8] reviewed a total of 12,153 feeding trials conducted on animals fed AGPs and concluded that 72% of AGPs gave a positive response on animal production performance. The magnitude of responses was dependent upon the type of animal management, disinfection procedures, age of the farm buildings, and quality of the feed. Finally, the use of AGPs has a positive impact on two important issues of animal agriculture, animal welfare and environmental stewardship. Animal welfare is definitely improved in animals that are healthier due to the disease-suppressing

Table 1 *Summary of the impacts of AGP restriction on production performance of poultry*[a]

Countries[b] (First action was taken)	Implementation process	Production performance	References
EU (2006)	Requires veterinary prescription to use antimicrobials in food animals but it allows 27 member states to grant exemptions in certain cases. They are also using AGP alternatives and finding alternative strategies.	Production performance was normal with minimal economic repercussions for food animal producers.	Refs[132,133]
The Netherlands (1997)	Monitoring AMR in food animals; requires veterinary prescription; uses AGP alternatives.	Sale of antibiotics licensed declined by 32%; production performance was normal.	Refs[5,133]
Germany (1996)	Implemented the NARS for AMR monitoring; data sharing on resistance issue and collection of data on pharmaceutical use by the producers; reduced AGP use through prevention of infectious diseases; monitoring the use of antibiotics by veterinarians or by animals, requires veterinary prescription, uses AGP alternatives.	Reduced the use of antimicrobials but production performance was normal.	Ref.[132]
Denmark (2000)	Poultry industry voluntarily stopped AGP, requires veterinary prescription. Altered production practices, such as improved farm biosecurity and diets; lower stocking densities; uses AGP alternatives in animal diets	Antibiotic use in food animals dropped by 25%; reduced *Salmonella* infections; negatively impacted poultry production to some extent; occurrence of resistant *E. faecium* dropped.	Refs[132–134]
Sweden (1986)	A full ban of AGPs without veterinary prescription. The ban was not uniform across the animal species. Using AGP alternatives and searching for viable alternatives.	No clinical or economic impact in egg production was found; broiler chicken producers were still able to use antimicrobials to prevent and treat infections, but therapeutic uses of antimicrobials increased to control necrotic enteritis; no negative clinical effects on turkey production.	Refs[132,133,135]

Table 1 *Continued*

Countries[b] (First action was taken)	Implementation process	Production performance	References
Taiwan (2005)	Requires veterinary prescription. Trying to impose complete ban using AGP alternatives.	Production performance was normal.	Ref.[132]
South Korea (2011)	Requires veterinary prescription, improved biosecurity of farm management, and the use of AGP alternatives and searching for new alternatives.	Production performance was normal.	Refs[132,133]
Bangladesh (2010)	Provide license to the industry to ensure AGP free feed; supervision and monitoring the feed factory at central and regional levels; provide feed analysis services to ensure quality control of feed; using feed alternatives and finding new cost effective alternatives.	Reduction of the use of AGPs in feed and increased use of alternatives, but production performance was normal.	Ref.[133]

[a] Impact was reported from the pooled data of the article.
[b] EU, European Union; AMR, antimicrobial resistance; NARS, national antibiotic resistance strategy.

effects of antibiotics. The improved utilisation of dietary nutrients by supplemental antibiotics results in a significant reduction in nitrogen, phosphorus, and other nutrients excreted into the environment[8].

3.2 The mode of action of antibiotics

Antibiotics are natural metabolites of fungi that inhibit the growth of bacteria. The mode of action of antibiotics is classified on the basis of drug-target interaction and whether the resultant inhibition of cellular function is lethal to bacteria[9]. It has been shown that three major classes of bactericidal antibiotics, regardless of drug-target interaction, stimulate the production of highly deleterious hydroxyl radicals in Gram-negative and Gram-positive bacteria, which ultimately contribute to cell death. Some antibiotics interfere with the building and maintenance of cell walls, while others interrupt proper protein translation at the ribosomal level. Because of their elevated rate of growth and proliferation, bacteria are vulnerable to antibiotics that target active cellular metabolism. This enables the host to grow and perform better than that achieved under normal growth conditions[10].

Antibiotics limit the growth and colonisation of numerous pathogenic and non-pathogenic species of bacteria in the gut[11]. Antibiotics reduce the production of antagonistic microbial metabolites, such as ammonia[12], which adversely affects the physiology of host animals. However, subtherapeutic levels of antibiotics in diets may reduce the weight and length of the intestines. It is documented that, a thinner intestinal epithelium in antibiotic-fed animals may enhance nutrient absorption[13] and reduce the metabolic demands of the gastrointestinal system. The reduction of gastrointestinal bacteria may also alleviate the competition for vital nutrients between the host bird and microbes[14]. Lee *et al.*[15] indicated that the dietary medication programmes of coccidiostats plus antibiotics modulate various parameters of immunity in chickens and regulate cytokine/chemokine mRNA levels in gut epithelial and spleen cells. In addition, antibiotics may reduce the adverse effects of immunological stress on growth performance by lowering the enteric microbial load[16]. A summary of the mode of action of AGPs on animal health is presented in Table 2.

Table 2 *Summary of mode of actions of AGPs and their impact on animal health*[a]

Mode of action	Impact on animal health	References
Alters bacterial metabolic processes	Destroys harmful bacteria in poultry; minimises the adverse effects of dietary changes; prevents multiplication of common pathogenic bacteria; reduces the incidence of enteritis.	Refs[11,17,136]
Limits growth and proliferation of certain bacteria	Impairs the growth and proliferation of microorganisms; prevents diseases; improves animal welfare; limits the growth and colonisation of numerous non-pathogenic bacteria in the gut, including *Lactobacilli*, *Bifidobacteria*, *Bacteroides*, and *Enterococci*.	Refs[2,11,137]

Table 2 *Continued*

Mode of action	Impact on animal health	References
Inhibits the production of various toxins and thinning the intestinal wall	Bird growth and performance increases; improves absorption of feed nutrients, feed efficiency and growth performance; increases egg production of laying hens and hatchability of fertile eggs; reduces weight and length of the intestines.	Refs[138,139]
Stimulates body defence	Reduces stress and mortality of chicks; improves immunity.	Refs[139,140]
Modifies intestinal microflora	Helps improve bird performance and health status; reduces the microbial use of nutrients.	Refs[14,137]
Reduces antagonistic microbial metabolites and enzymes	Increases amino acid levels in the gut and improves nitrogen balance.	Ref.[139]

[a] Impact was reported from the pooled data of the article.

4. Alternative strategies to AGPs in poultry production

4.1 Feed management practices in the absence of AGPs

Numerous feed additives have been proposed as viable alternatives to AGPs in poultry diets. A summary of impacts of different alternatives is shown in Table 3. The use of compounds that have antimicrobial effects is one way to improve intestinal health, immune response, and bird performance in the absence of AGPs. Antibiotics work by decreasing the microbial load in the intestinal tract, resulting in a reduction of energy and protein required for maintaining and nourishing the intestinal tissues; thus, more nutrients are partitioned for growth and production. By contrast, most natural feed additives do not reduce overall microbial loads, but, they alter the intestinal microflora profile by limiting the colonisation of unfavourable bacteria and promote the activity or growth of more favourable bacteria. Antibiotic alternatives modulate gut health *via* several possible mechanisms: altering intestinal pH; maintaining protective intestinal mucins; promoting beneficial intestinal organisms, and/or inhibiting pathogens; enhancing the production of volatile short-chain fatty acids; enhancing nutrient uptake; and increasing the humeral immune response[17].

Although there is growing scientific evidence for many of these antibiotic replacements, the claim of efficacy is in many cases inadequately substantiated[18]. The search has been for a single intervention to replace antibiotics, and this has proven to be less efficient than a multi-factorial approach[19]. In general, a number of options are available for enhancing the performance of poultry in the absence of AGPs. The principal mode of action of these supplements can be divided into four basic strategies: (*i*) direct reduction of pathogens; (*ii*) stimulation or introduction of beneficial bacteria; (*iii*) improvement of nutrient utilisation by the host; and

Table 3 *Summary of the impact of different alternatives for AGPs and their effects on poultry*[a]

Alternatives	Effects on poultry	References
Organic acids and acidifiers	Lowers the pH of the intestine; improves weight gain and feed efficiency and meat quality; increases the immune characteristics and number of lactic acid bacteria in the intestine; decreases the number of coliform bacteria.	Refs[26,28,29, 141–143]
Direct fed microbials or probiotics	Increases the plasma immunoglobulin levels, decreases *Escherichia coli*, and improves gut health; increases beneficial microorganisms and decreases pathogenic organisms; improves feed intake and efficiency and weight gain; improves egg quality and production; reduces chick mortality and stimulates immunity.	Refs[31,36,37,41, 144–147]
Prebiotics	Stimulates the growth of non-pathogenic bacteria; provides substrates for the bacterial fermentation in the lower gut of the host; stimulates immunity and neutralises toxins; inhibits colonisation of pathogenic bacteria; provides energy and other limiting nutrients for intestinal mucosa; enhances growth and feed efficiency.	Refs[37,43–45, 49,105, 148–150]
Enzymes	Improves fibre and non-starch polysaccharide digestion and utilisation; increases intestinal viscosity; increases endogenous nitrogen flow and bacterial fermentation in the GI tract; improves growth and feed efficiency.	Refs[68,151–156]
Essential oils and plant extracts	Improves weight gain, immune characteristics, and the colonisation of *Lactobacillus* in the intestines; improves digestive tract health and growth performance; reduces concentration of *Clostridium perfringens* in the intestine; inhibits growth of pathogenic microorganisms like *E. coli, Salmonella* spp. and/or *Clostridium* spp.	Refs[21,76–81, 84,101,143, 157,158]
Bacteriophages	Improves egg production; improves body weight and feed efficiency; prevents collibacilosis.	Refs[99–102]
Antimicrobial peptides and fermented protein	Improves live performance and gut health; decreases bacterial count in the intestine; enhances immunity and nutrient utilisation.	Refs[11,105,107, 110]
Hyperimmune egg yolk antibodies (IgY)	Increases immunoglobulin levels; lowers faecal shedding and *Salmonella enteritidis* in the gut; reduces *Salmonella* contaminated eggs.	Refs[116,120,123, 124,159]
Vitamins, minerals, electrolytes and other supplements	Improves feed utilisation and immune response; reduces stress; acts as an antioxidant and influences intestinal microflora; improves growth and carcass quality of broiler chickens.	Refs[86,89,95, 160–163]

[a] Effects were reported from the pooled data of the article.

(*iv*) stimulation or modulation of the immune system of the bird. Within these general categories there are hundreds of commercial products available claiming to be effective in improving growth performance and health of poultry. However, an alternative strategy must yield comparable economic return and sustainable production efficiency, if it is to be accepted for commercial use.

4.1.1 Organic acids and acidifiers

Organic acids have strong bacteriostatic effects and they have been proposed as *Salmonella* control agents in feed and water supplies for livestock and poultry[20]. Some acidifiers and organic acids have shown antimicrobial activity[21]. The antibacterial activity is related to the reduction of pH, as well as their ability to dissociate and easily enter the microbial cell by both passive and carrier-mediated transport mechanisms. After entry into the cell, the organic acid releases the proton H^+ in the more alkaline environment, resulting in a decrease of intracellular pH. This hinders microbial metabolism by inhibiting the action of important microbial enzymes and forces the bacterial cell to use energy to export the excess of protons H^+, resulting in death by starvation. In addition, protons can denature the bacterial acid sensitive proteins and DNA[22]. Furthermore, organic acids reduce the buffering capacity of the feed, resulting in increased levels of hydrochloric acid in the stomach and improved nutrient digestibility. The reduction in pH also increases pepsin activity and stimulates the secretion of pancreatic enzymes, which also improves nutrient digestibility[23].

The most common organic acids used in poultry feed are citric acid, propionic acid, butyric acid, fumaric acid, formic acid, phenyllactic acid, benzoic acid and lactic acid. Generally, lactic acid bacteria are able to grow at a relatively low pH, which means that they are more resistant to organic acids than more pathogenic species. However, the use of organic acids has not gained as much attention in poultry production because limited positive responses in weight gain and feed efficiency have been achieved[24]. By contrast, Fascina *et al.*[25] reported a positive influence on either feed efficiency or growth performance by dietary supplementation of lactic acid, benzoic acid, formic acid, citric acid and acetic acid in broiler diets. In addition, supplementation of coated sodium butyrate was also found to enhance growth performance of broiler chickens, which may be attributed to better mucosal development[26]. Therefore, dietary organic acids may improve bird performance by increasing the absorption of available nutrients, reducing the toxic bacterial metabolites and the incidence of subclinical infections and increasing secretion of immune mediators[27].

Organic acids have also been used as food preservatives to prevent the growth of microorganisms and extend the shelf life of processed food. In addition to preservation, various organic acids are used for different technical purposes in the animal feed and human food industries such as: acidifiers, antifungal agents, antioxidants, flavour and pH modifiers. However, some organic acids are becoming increasingly resistant to some organic acids similar to antibiotic resistance. Therefore, in broiler diets, it is wise to use new organic acids which have antimicrobial and immunomodulatory activity, and they should be safe for human health[28]. Kim *et al.*[29] reported that dietary supplementation of phenyllactic acid (PLA) improved growth and feed efficiency of broiler chickens. They also found that PLA supplementation increased the immune characteristics and the number of lactic acid bacteria, decreased the number of coliform bacteria, and improved the meat quality attributes of broiler chickens. Finally, they concluded that dietary PLA could be a viable alternative to antibiotics in broiler diets[29].

4.1.2 Direct-fed microbials

Direct-fed microbials (DFM) (probiotic), a source of live beneficial microorganisms, have been used as an effective alternative to antibiotics in the animal feed industry over the last few decades due to their diversified function on animal health and productivity. In general, *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus*, *Lactococcus*, *Streptococcus* and *Saccharomyces cerevisae* are frequently used as DFM in the poultry feed industry. These microorganisms may influence the intestinal microbiota as well as host health and welfare in different ways, such as competitive exclusion of pathogenic bacteria, lowering gut pH, competing for mucosal attachment and nutrients, producing bacteriocins, stimulating the immune system, increasing production of short-chain fatty acids, increasing epithelial integrity, and stimulating intraepithelial lymphocytes[16,30,31]. The mechanism of action associated with the beneficial effects of probiotics is 'competitive exclusion'[32]. Competitive exclusion is the physical blocking of opportunistic pathogen colonisation by probiotic bacteria *via* their ability to physically colonise environmental niches within the intestinal villi and colonic crypts which are the favourite colonisation sites of enteric pathogens[33]. In addition, Chichlowski *et al.*[34] reported that probiotic organisms may also selectively colonise areas around the opening to goblet cells. However, more research is needed to fully define the mechanisms of probiotic effects on the body before they can be used in the feed industry in a consistently efficacious and cost effective manner. Additionally, detailed molecular and cellular mechanisms that govern the multiple interactions between the intestinal microflora, pathogenic bacteria, and the host immune system need to be addressed before DFM is used in food animal production[35].

Currently, supplemental DFM has received special attention from the broiler industry to promote the balance and quality of the intestinal microflora for the host, but the efficacy of these products varies according to their production procedure and practical application. Several researchers reported that feeding DFM had improved the growth performance of broiler chickens[36,37] and egg production of laying hens[38]. By contrast, other researchers did not find any positive effects of using dietary DFM on growth performance of broiler chickens[31,35] and pigs[39]. The inconsistent results may be explained by variations in the number of species of microorganism added to poultry diets as DFM. It is hypothesised that the potential benefit of DFM depends upon the microbial species, strain, concentration, production techniques, and storage condition. There is evidence to show that better performance has been achieved by the use of a mixture of microorganisms with different species rather than a single microbial species or strain[40]. In a recent study, Salim *et al.*[41] found that the dietary supplementation of DFM increased the growth performance of birds at an early age, stimulated the immune response, decreased the number of *E. coli*, and improved the ileal morphology of broiler chickens. They concluded that DFM that contained a mixture of several beneficial microorganisms could be a viable alternative to antibiotics in broiler diets[41].

4.1.3 Prebiotics

Dietary prebiotic components are not digested by the host, but they benefit the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the gut, predominantly those that produce short-chain fatty acids[42]. Prebiotics have several advantages over probiotics, one being that they are organic compounds and culture viability need not to be maintained as for probiotics. Prebiotics have been shown to stimulate enteric colonisation of unculturable bacteria[43,44] and this discourages the colonisation of enteric pathogens. Furthermore, they have the advantage of being more stable to the heat and pressure incurred during feed processing. Any feed ingredient that enters the large intestine is a potential prebiotic, but it must be fermented by microorganisms to benefit the host[45]. Most current successes have been derived from using non-digestible oligosaccharides; especially, those containing fructose, xylose, galactose, glucose and mannose[42]. The oligosaccharides and polysaccharides are preferentially utilisable by *Bifidobacteria* and other lactic acid-producing bacteria that modulate gut associated immune function[46]. In addition, oligosaccharides originating from β-glucans of yeast cell walls are thought to stimulate performance of the animals due to their immunomodulatory functions. Their main action is to enhance phagocytosis and proliferation of monocytes and macrophages[47]. The interaction of glucans with macrophages can have huge effects in the host, because macrophages play a crucial role in immunomodulation. Several studies have documented significant health benefits by using immune modulating β-1,3 or 1,6-glucan as a feed ingredient to protect animals against microorganisms[48].

Some structural carbohydrate components of non-starch polysaccharides (NSP) have also been used and studied as potential prebiotics in poultry diets. Galactooligosaccharides can modify the colonic microflora by lowering some Gram-negative bacteria, such as coliforms, and increasing potentially health-promoting bacteria, such as *Bifidobacteria* and *Lactobacillus*[49]. It has been documented that the inclusion of various levels of mannan oligosaccharide to broiler diets significantly increased their body weight and feed efficiency[50,51], villi height[52], intestinal immunity[53], altered jejunal gene expression[54] and intestinal microbiota[55]. Likewise, in other studies using frouctooligosaccharide and isomaltooligosaccharide as an antibiotic alternative, live performance and feed efficiency of broiler chickens was significantly improved[56,57]. Dietary galactomannans can also suppress the colonisation of *Salmonella typhimurium in vitro* and *Salmonella enteritidis* in laying hens[58]. In addition to its effect on microbial fermentation, arabinoxylan has been shown to activate a macrophage cell line in the intestine and thus, decrease enteric pathogen colonisation in broiler chickens[59]. Prebiotics therefore have potential microbiota modulating and immunomodulatory functions, enhancing the performance of birds, which make them a viable AGP alternative feed additive for poultry[60].

4.1.4 Enzyme supplementation

Dietary enzyme supplementation has become a standard practice in the poultry industry to reduce feed costs and to increase dietary phosphorus, energy, and protein utilisation. Supplemental enzymes in the feed are used to achieve the following objectives: (*i*) increase the supply of enzymes in the gut[61]; (*ii*) alleviate the adverse effects of anti-nutritional factors, such as arabinoxylans, *β*-glucans, *etc*.; (*iii*) increase the availability of certain nutrients for absorption and enhance the energy value of feed ingredients[62,63]; and (*iv*) modulate intestinal microflora to a healthier state[64]. The major enzymes used in animal feed are hydrolytic protease, amylase, lipase, phytase, NSP-degrading enzymes, and cellulase. The enzymes with proven efficacies for animal husbandry include xylanase, arabinoxylanase, *β*-glucanase, cellulase, phytase, and mannanase[65]. Amylase and lipase are commonly used in corn–soybean meal based diets to supplement endogenous enzymes of the animal, and thus improve nutrient digestibility and growth performance of birds[66]. It is documented that the supplementation of poultry diets with enzyme mixtures, has produced significant improvements in the growth performance of chickens[67,68]. Greenwood *et al*.[69] reported that supplementing a corn–soybean meal based broiler starter diet with an enzyme preparation containing a mixture of xylanase, protease, and amylase increased the growth performance of birds. The potential beneficial effects of dietary enzyme supplementation however, are sometimes inconsistent due to the enzyme types, sources, levels, diet composition, and species used: these should be considered during diet formulation without AGPs[70].

Fischer and Classen[71] reported that the bacterial count from the small intestine of broilers fed wheat-based diets was lower in xylanase-supplemented birds than the control. Because enzyme supplementation reduces the microbial population in the small intestine, it changed the entire intestinal ecosystem of the bird[72], and resulted in the decrease of the adverse effects of microbial fermentation. Some of the adverse effects of active microbial fermentation include: deconjugation of bile salts reducing fat digestion; competition between the host and the microbiota for nutrients; atrophy of the intestinal villi; and enlargement of digestive organs[73,24]. In addition, Santos[74] reported that supplemental NSP-degrading enzymes reduces the adverse effects of dietary NSP on nutrient digestibility, and increases the variety of non-starch oligosaccharides. These non-starch oligosaccharides work as substrate for a more diverse microbiota, thus augmenting the positive effect of NSP on microbial ecosystem stability and discouraging *Salmonella* colonisation in turkeys[74]. However, in a comprehensive review, Rosen[75] concluded that the effect of enzymes was nearly equivalent to the effects of antibiotics on the growth and feed efficiency of chickens. Therefore, enzyme supplementation in poultry diets seems to be capable of limiting the performance losses associated with the removal of AGPs.

4.1.5 Essential oils and plant extracts

Essential oils are volatile compounds extracted from specific plants or plant extracts. In animal nutrition, they offer varying degrees of performance, environmental and nutritional benefits, depending on blend and strength. The use of a wide range of plant extracts, such as sea algae, essentials oils and other natural substances to enhance animal health and performance, has been documented for a long time due to their anti-inflammatory, immunomodulatory, antioxidant and antibacterial activities. Among these products, essential oils have long been recognised for their anti-microbial activity, and they have gained much attention for their potential as alternatives to antibiotics[76]. In an early study, Lee and Ahn[77] found that cinnamaldehyde strongly inhibits *Clostridium perfringens* and *Bacteroides fragilis* and moderately inhibits *Bifidobacterium longum* and *Lactobacillus acidophilus* isolated from humans. Although the exact anti-microbial mechanism of essential oils is not clear, it may be associated with their lipophilic property and chemical structure[78]. In addition, essential oils from oregano are showing the greatest potential as an alternative to AGPs. An *in vivo* and *in vitro* study in chickens fed garlic metabolites (propyl thiosulphinate and propyl thiosulphinate oxide) changed the immunologic and genomic parameters which improved the resistance to *Eimeria acervulina* infections[79]. Later, Kim *et al.*[80] observed that dietary *Capsicum* and *Curcuma longa* oleoresins regulate susceptibility to experimental avian necrotic enteritis (NE) and alter the gut microbiota of commercial broiler chickens. Similarly, Kim *et al.*[81] also found that dietary supplementation of *Curcuma longa* enhanced resistance against cocciodiosis infections in chickens. They concluded that dietary phytonutrients exert beneficial effects on gut health by reducing the negative consequences of NE, while nutratherapeutic mechanism may alter gut microbial communities in chickens. Therefore, essential oils and several herbal plants can modify the gut microflora and reduce the microbial load by suppressing bacteria proliferation as do antibiotics[82]. However, the processing techniques, availability and cost of these products are the main challenge for use as an antibiotic alternative in the feed industry of poultry.

Yoshizawa *et al.*[83] reported that algae extract activated the macrophages and increased the proinflammatory cytokine production of laboratory animals. It has been reported that the supplementation of *Chlorella* in human and animal diets resulted in numerous biochemical and physiological functions, such as, growth promoting, antioxidant function, and immunomodulation. In addition, antimicrobial properties of *Chlorella* are considered to be an effective alternative to AGPs in diets to maintain optimum health and productivity of the animal. Kang *et al.*[84] concluded that dietary supplementation of fresh liquid *Chlorella* improved bodyweight gain, immune characteristics, and the production of *Lactobacillus* bacteria in the intestinal microflora of broiler chickens. To be as effective as growth promoters, these herbal antimicrobial compounds must be supplemented in the feed in a more concentrated form than what is found in their natural state. Additionally, most of these plant extracts need further processing before being used in poultry diets, which may increase the usage costs.

4.1.6 Vitamins and trace minerals

Vitamins and several trace minerals have been used to improve feed utilisation as well as growth of birds and thereby help to yield a better return of production. Vitamin C has essential roles for improving feed utilisation and reducing various stresses in the farming system resulting in enhanced performance of birds[85]. Vitamin C and E also function as antioxidants that can reduce production stress and improve the carcass quality of the animal. A recent meta-analysis indicated that meat quality and immune response of male broiler chickens could be improved by dietary vitamin E supplementation[86]. However, Lin and Chang[87] suggested that moderate supplementation of vitamin E enhanced immune responses to selective antigens but excessive vitamin E depressed specific immune response in cockerels fed a corn–soyabean meal-based diet.

It has also been documented that zinc has bactericidal properties that may lead to microbial load reduction in the intestine and influence gut health of the animal[88]. In a review article[89], it was shown that zinc is critical to maintain growth, reproductive efficiency, bone and glandular development and to develop a strong immune system in broiler chickens. Similarly, Lu *et al.*[90] showed that dietary supplementation of copper chloride or copper sulfate significantly increased growth performance and carcass weight in broiler chickens. The growth promoting effect of the dietary zinc and copper can be attributed to their antimicrobial properties and immune competence activities[91,92]. In addition to zinc and copper, other trace minerals such as chromium and selenium have potential beneficial effects on broiler chickens raised under stress conditions[93,94]. However, Visca *et al.*[95] reported that dietary supplementation of iron has a dual role as a growth inhibitor as well as a growth promoter in chickens. Therefore, it is necessary to know the modes of action of the minerals before using them in poultry diets in replacement of AGPs.

4.1.7 Bacteriophages

Bacteriophages have enormous potential to be used in a variety of applications as an alternative to antibiotics and disinfectants. Bacteriophages are viruses that naturally infect and kill bacteria. They are safe for animal production as they have no harmful effects on animal body cells. Therefore, it would appear possible to use bacteriophages to prevent bacterial diseases of animals[96]. Several researchers throughout the world are researching bacteriophages as alternatives to antibiotics. Huff *et al.*[97,98] suggested that bacteriophages could be developed as an effective alternative to antibiotics to prevent and treat bacterial diseases in poultry. Bacteriophages may be sprayed on birds at the hatchery to prevent the early onset of colibacillosis. It might also be sprayed in a house with a severe outbreak of colibacillosis to prevent bird-to-bird transmission[99]. Several studies have demonstrated the effects of supplemental diets with bacteriophages on production and growth performance of animals. Zhao *et al.*[100] reported that dietary supplementation of bacteriophages significantly improved egg production of laying hens. In addition, improved body weight and feed efficiency were reported in broilers fed diets supplemented with various levels of bacteriophages[101,102]. However, further research is needed to develop practical and cost effective bacteriophage products for use in poultry production systems.

4.1.8 Antimicrobial peptides and fermented protein

Antimicrobial peptides (AMPs) are small biological molecules isolated from living organisms. They are an attractive candidate for the design of new antimicrobial agents and immune modulation for birds[103]. Antimicrobial peptides provide immediately effective, non-specific defences against infections due to their role as important components of the innate immune system[104]. The beneficial effects of AMPs on growth performance are mostly due to their antimicrobial and immunomodulating activity, thereby promoting nutrient digestibility and health of poultry[105]. Previous researchers found that antimicrobial peptides interact with the surface membrane of bacteria either by forming discrete pores or by disrupting the membrane leading to cell leakage and resulting cell death[106]. Apart from directly attacking microbes, AMPs can confer protection by alternative mechanisms, such as maintenance of normal gut homeostasis, and modulation of host inflammatory responses[107]. The properties of AMPs include responding to microbial infections by acting on host targets rather than microbial targets which would be an advantage over traditional antibiotic use in birds. Moreover, inhibition of the synthesis of nucleic acids, proteins, cell wall components and essential enzymatic activities has been reported by Nguyen and co-workers[108].

Recently, several synthetic peptides have risen to provenance as AGP alternatives in poultry although these mostly focused on their protective potential against several pathogens causing infectious diseases rather than growth promoting activities in birds. Lee *et al.*[109] suggested that the cNK-2, a synthetic AMP, is a novel anti-infective peptide that can be used for protection against avian coccidiosis during commercial poultry production. In a recent study, Kim *et al.*[110] reported that cNK-2 is a potential immunomodulating agent rather than an anti-microbial agent. Additionally, Wen and He[111] found improved weight gain, feed efficiency and intestinal villus height, and decreased aerobic bacterial counts in both jejunal and caecal contents of chickens fed chimeric peptides derived from insects and yeast. Similarly, Choi *et al.*[112] reported beneficial effects of the performance of chicken fed diets supplemented with a chemically synthesised AMP on their nutrient retention, intestinal morphology and microflora contents. In addition, several researchers[113,114] investigated the effects of naturally synthesised AMPs on chicken and they reported that the birds fed naturally synthesised AMPs had improved growth performance, gut morphology, mucosal immune characteristics and serum IgA levels compared to unsupplemented birds. In conclusion, AMPs have several beneficial effects on growth performance, nutrient digestibility, intestinal morphology and gut microbiota in animals[37,105]. Therefore, AMPs may be used as a viable alternative to AGPs in the poultry industry, if available on the market.

Recently several fermented proteins such as, fermented rapeseed, fermented seaweed or fermented soybeans have played a significant role to reduce antibiotic use in animal feed industry. A Danish firm named 'European Protein' said their strategy of reducing the incidence of bacteria and associated diseases in animals was through the introduction of lactic-acid fermented feedstuffs, as an alternative to antibiotics. They also reported that about 30 pig farmers in Denmark and one in Poland used fermented protein and managed to bring their antibiotic use on their farm below 10% (see ref.[115]). However, further research is needed to find appropriate fermented products and quantities for different farming situations.

4.1.9 Hyperimmune antibodies

Hyperimmune egg yolk antibodies (IgY) are produced by a hen that has been vaccinated against certain infectious diseases, and have been used in the prevention and treatment of various intestinal diseases. They are also used for general stimulation of the immune system, especially the immunoglobulin levels of birds[116]. Generally three immunoglobulin classes, IgA, IgM and IgY, are found in birds, and IgY has potential as an alternative to AGPs in poultry production[82]. However, limited research has been conducted on the use of egg yolk antibodies as viable and cost effective alternatives to AGPs for improving the growth performance of poultry[117]. Previous studies reported that the generation of egg antibodies in breeding hens could be passively transferred to the progeny and improve their productivity. Pimentel *et al.*[118] showed that progeny from hens injected with jack bean urease had improved live weight at three weeks of age. They suggested that urease antibodies maternally transferred to the progeny decreased ammonia production in the intestinal tract by inhibiting bacterial urease enzymes and improving growth performance in chicken. Other important concerns are the bactericidal effect and stability of these antibodies in the gastrointestinal tract when they are fed to the animal. It has been documented that the microencapsulation may be an effective method for protecting IgY from gastrointestinal inactivation[119]. However, the antibacterial properties of these components need to be justified by further investigation[120].

In recent years, IgY have attracted considerable attention as an alternative to antibiotics to maintain animal health and performance. It has been documented that oral administration of IgY acts as potential AGPs for controlling diarrhoea and exerting growth-promoting activity in pigs[121]. A number of studies conducted in chickens, indicated that this technology had the potential to be used as a disease control strategy in the poultry industry[122]. Rahimi *et al.*[123] examined the effect of *Salmonella enteritidis*-specific IgY administration on 3-day old experimentally infected birds and found lower faecal shedding and *S. enteritidis* concentration in the caecal content. They also found a lower isolation of *S. enteritidis* from the liver, spleen and ileum of birds. By contrast, Wilkie[124] studied the effectiveness of chicken egg antibody administration on *Campylobacter jejuni* and *S. enteritidis* colonisation in the gastrointestinal tract of broiler chickens and observed that IgY activity *in vivo* and *in vitro* was unable to demonstrate any significant reduction in the intestinal colonisation by either *S. enteritidis* or *C. jejuni*. However, the mechanism of action of orally applied IgY for pathogen reduction is still unknown. The mode of action is obviously the binding of antibodies to certain specific components on the bacterial surface. It is hypothesised that these cell surface components can easily be recognised and bound by antibodies. This may lead to the impairment of the biological functions of those components that play a vital role in the bacterial growth and attachment to the intestinal cells[125]. In this way, the antibodies protect against adhesion of bacteria at the intestinal cells and prevent invasion into epithelial cells[126]. Another important concern is stability of these antibodies in the gastrointestinal tract when they are fed to poultry. Therefore, with the global restriction on subtherapeutic antibiotic uses and the enforcement of strict legislation on food safety issues, passive immunisation by oral administration of pathogen-specific IgY may be a useful alternative to AGPs in poultry diets[120]. However, more research is needed into using viable and cost effective egg antibodies in the growth promotion of poultry.

4.2 Farm management practices in the absence of AGPs

Although alternative feed supplements may compensate for the reduction or elimination of AGPs in feed, some changes in poultry husbandry practices may also be important[82]. Considerable evidence shows that the application of AGPs or alternatives in feed is most effective when given to animals raised in unsanitary environmental conditions. Good barn sanitation, proper vaccination, pest control, maintaining effective quarantine, biosecurity practices and waste management are necessary to reduce pathogen load and exposure, and minimise the need for antimicrobial therapy[127]. Appropriate environmental temperatures and lighting, and maintenance of appropriate ventilation rate in poultry houses are important since pathogens may be spread through the air and the environment. Water must be clean and drinkers, feeders and other equipment must be properly maintained to minimise spoilage and prevent a bloom of pathogens in the litter and environment of the birds. Proper selection of feed ingredients and feeding practices may also affect the spread of diseases. Automated watering and feeding systems are associated with a decrease in the risk of infection with *Salmonella* compared to trough feeding. In addition, stocking density in the barn should be maintained according to standard management practices of the breed, strain or species of bird. It has also been documented that the implementation of a good sanitation programme is usually much less costly than any disease treatment[17].

4.3 Immunisation in the absence of AGPs

The immune system of an animal is the primary mechanism to fight against infectious diseases. Boosting both humoral and cellular immunity will increase an animal's ability to resist diseases, and this may be done through appropriate feeding[128]. A proinflammatory innate immune response is associated with the mobilisation of nutrients away from growth and suppression of feed intake. Thus, dietary immunomodulators or vaccines enhance humoral immunity and minimise immunological stress that will affect health and live performance of animals.

Vaccines prevent bacterial infections and protect against both antibiotic-resistant and antibiotic-susceptible strains[129]. They would have two positive effects from the perspective of resistance: (*i*) preventing infection by drug-resistant bacteria, which may be hard or impossible to treat with current therapeutics; and (*ii*) reducing the overall number of bacterial infections[130]. Thus, proper vaccination may replace the use of antibiotics. A vaccine is a biological preparation that provides active acquired immunity against a particular disease. The agent used in the vaccine stimulates the body's immune system to recognise the agent and destroy the microorganisms associated with that agent. Vaccination is the most effective method of preventing infectious diseases, especially viral diseases[131]. Some bacterial diseases may also be prevented through vaccination like that of *Salmonella* spp. Therefore, antibiotic uses and AMR may be reduced or avoided by ensuring strict routine vaccination and maintaining a vaccination schedule.

5. Conclusions and recommendations

Global restrictions on AGP use in poultry feed varies across countries. However, social and consumer concerns on quality and safety of food may lead to the use of AGP-free poultry feed in the near future. A variable number of feed additives are used or have been proposed as viable alternatives to AGPs in poultry diets. Their efficacy is dependent on the understanding of their mode of action, and their influence on gut health and growth performance. A combination of strategic feeding and types of feed additives may help achieve good intestinal health, stable enteric ecosystems and sustainable production performance of birds. However, in addition to strategic use of feed and feed alternatives, good hygienic farm practices, vaccination and strict biosecurity are necessary. Further research is required to explore suitable, reliable and cost effective alternatives to AGPs for commercial use. Moreover, an increase in and diversification of value added poultry products through gradual limitations of live bird markets, especially in a country like Bangladesh, may help achieve the goal of safe food production.

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