Review Article

Antimicrobial peptides as a feed additive alternative to animal production, food safety and public health implications: An overview

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Abstract

In the last few years, feed additives have been used in animal nutrition to improve nutrient utilization, health parameters and animal performance. However, the use of antibiotics as feed additives has allowed the occurrence of antimicrobial resistance (AMR), which can bring as a consequence, an increase in the morbidity and mortality of diseases that were previously treatable with antibiotics. In this context, antimicrobial peptides (AMP) have appeared as a promising strategy because they have multiple biological activities and represent a powerful strategy to prevent the development of resistant microorganisms. Despite the small number of studies applied in vivo, AMP appear as a potent alternative to the use of antibiotics in animal nutrition, due to an increase in feed efficiency and the prevention/treatment of some animal diseases. This review discusses the problems associated with antimicrobial resistance and the use of AMP as a strong candidate to replace conventional antibiotics, mainly in the animal industry.

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1. Introduction

According to the Food Outlook 2020, disclosed by The Food and Agricultural Organization (FAO, 2020), world meat demand increased 119 million metric tons. However, a reduction in pig meat production is anticipated due to the impact of African swine fever disease. In contrast, the same report shows an expansion of poultry and ovine meat production due to the increased demand. In the dairy sector, milk production is expected to experience an improvement of 1.4%, producing 860 million metric tons of milk per year.

Brazilian companies lead the world beef market, which moves more than 7 million metric tons per year between exports and imports. Since 2005, this country has internationalized the sector, purchasing large processing plants abroad. In 2018, Brazil became the major exporter, trading more than 2 million metric tons of meat, and was followed by Australia, which exported approximately 1.5 million metric tons (ABIEC, 2019). Therefore, countries need to improve the feed safety, health and process certification and quality of origin (traceability) aspects of the herd to avoid the possible serious risk of losing the positive results already achieved in international markets (Morgan et al., 2016; Conchon and Lopes, 2012).

The production of poultry meat in the world is led by the USA, China, and Brazil, which is responsible for a high economic value (FAO, 2020). In recent years, consumers have changed their perspectives when purchasing food products, focusing mainly on food safety (Heneghan, 2015). This factor is related to nutritionally adequate usage and safe foods (Coleman-Jensen et al., 2020), with safe foods being those that do not affect consumer health (Chassy, 2010).

Various techniques have been employed in animal production to maximize food production. To achieve this, research needed to change from being solely focused on animal nutrition, replacing food nutritional value studies with an understanding of animal physiological processes and the factors that affect them (Wallace, 1994). In recent years, research has sought to manipulate and
improve fermentative patterns and ruminal metabolism with additives in the diet (Meyer et al., 2009; Moya et al., 2009; Possenti et al., 2008), aiming to improve animal feed efficiency.

However, institutes such as the World Health Organization (WHO) and FAO have demonstrated concerns about the use of antibiotic additives in some situations, among them animal nutrition. Because of that, this research has been undertaken with the aim of finding some replacements for the usual additives.

2. Antimicrobial resistance (AMR)

AMR is considered to be one of the great challenges of the human health system. Every year, about 700,000 people die from antimicrobial resistance. Because of that, this research has been undertaken with the aim of finding some replacements for the usual additives.

As a consequence of AMR problems, international organizations, countries, academia, productive and technological sectors have been mobilizing on different levels of performance to tackle this obstacle. In 2015, the “Global Action Plan to Combat Antimicrobial Resistance” was launched because of a partnership between the FAO and the WHO, with the aim of keeping the treatment of infectious diseases effective and safe. In the fields of agriculture and animal production, the use of antibiotics to accelerate animal growth for human consumption, and their possible consequences of AMR, is a matter of great relevance due to its high economic impact. However, since 2016, the use of antimicrobial compounds as growth promoters has been banned by the European Union, considered the largest food importer in the world. Therefore, the recent call to combat AMR has led some countries to outline strategies for developing new and effectiveness antimicrobial molecules.

3. Antimicrobial peptides (AMP)

AMP are molecules with low molecular weight, belonging to a diverse and abundant group of biomolecules. AMP are produced in several types of animal and plant cells, with vast biological activity against bacteria, viruses, and fungi (Lai and Gallo, 2009). AMP are part of the natural innate immune system of animals and, in plants, form a defense system similar to the innate immunity observed in animals, protecting them from pathogens and pests (Lehrer et al., 1993; Gabay, 1994; Boman, 1995; Hultmark 1993; Shewry and Lucas, 1997; Gallo et al., 2002). AMP often share a common feature: the presence of even-numbered cysteine residues connected by disulfide bridges, which gives them high stability (Broekaert et al., 1997). In addition, their amino acid composition, amphipathicity, helicity, cationicity and size make them able to become inserted into lipid membranes, leading the target microorganism to death (Lorenzen et al., 2012; Brogden, 2005; Vicente et al., 2013; Izadpanah and Gallo, 2005). The continuous increase of multidrug resistant microorganisms’ appearance has led research to shed light on these molecules, allowing the development of new therapeutic agents (Duin and Paterson, 2016; Gallo et al., 2002). Appreciation of the therapeutic potential of AMP is due to their ability to rapidly kill many microorganisms such as fungi, viruses, and bacteria, mainly those which have become resistant to multiple conventional drugs.

Despite conventional antibiotics that usually have the mechanism of action through a high affinity for a defined target in the microorganism, AMP perform multiple antimicrobial functions, which acts as a powerful strategy to prevent the development of resistance by microorganisms (Peschel and Sahl, 2006). Further, they can act on different targets in the cells such as DNA, RNA, regulatory enzymes, and other proteins (Maria-Neto et al., 2015). Specifically, their main advantage is the property to still kill multidrug-resistant bacteria. Zhang and Sack (2012) demonstrated that AMP can inhibit the methicillin-resistant bacteria Strep- tococcus aureus and P. aeruginosa, the latter being resistant to conventional antimicrobials, causing severe hospital infections. Unlike direct attacks against microorganisms, AMP may offer protection by...
different mechanisms, such as attacking unspcific targets (i.e., plasmatic membranes), maintaining normal gut homeostasis or modulating host inflammatory responses (Wang et al., 2015; Cespedes et al., 2012; Lai and Gallo, 2009). Current studies are not enough to fully support the synergies between conventional antibi-otics and AMP, although insights could be revealed by exploring different AMP together (Magana et al., 2020). In human medicine, scientists already have published studies testing the capacity of AMP to reverse drug resistance. Teng et al. (2020) tested some AMP as anticancer drugs to reverse the cells resistance to regular drugs that have been used. Interestingly, they have found, for the first time, an antimicrobial peptide that could reverse cell resistance.

3.1. AMP activity

The most studied classes of AMP are those with antibacterial activity. Most AMP can interact with bacterial membranes and there are at least 9 hypotheses of mechanisms of action: 1) electro- troporation; 2) carpet model, 3) membrane thinning or thickening, 4) non-lytic membrane depolarization, 5) toroidal pore, 6) oxidized lipid targeting, 7) barrel stave, 8) disordered toroidal pore, and 9) non-bilayer intermediate (Magana et al., 2020). There are several databases where most AMP are compiled and registered. The Antimicrobial Peptide Database (APD - http://aps.unmc.edu/AP/main.php) has 3,201 AMP from 6 kingdoms (357 bacteriocins/peptide antibiotics from bacteria, 5 from archaea, 8 from protists, 20 from fungi, 352 from plants, and 2,377 from ani-mals, including some synthetic peptides) with a large variety of activity (Wang et al, 2009, 2016c; Wang and Wang 2004). A second database is the Data Repository of AntiMicrobial Peptides (DRAMP - http://dramp.cpu-bioinfo.org/), an open-access and manually curated database harboring diverse annotations of AMP including sequences, structures, activities, physicochemical, patent, clinical and reference information with 20,434 entries, 5,619 of which are general AMP (containing natural and synthetic AMP), 14,739 AMP patents and 76 AMP in drug development (Kang et al, 2019; Liu et al, 2017, 2018; Fan et al, 2016). Finally, the DBAASP (acronym for Database of Antimicrobial Activity and Structure of Peptides - https://dbaasp.org/) was developed to provide information and analytical resources for the scientific community, to help in developing antimicrobial compounds with a high therapeutic index (Pirtskhalava et al., 2016). To date, there are many other databases and resources for AMP research described elsewhere (Magana et al., 2020).

Antimicrobial peptides isolated from insects are the largest group of known AMP (Wang et al., 2016a). Among the antibacterial peptides, cecropins were extensively studied and represent an important component of insect defense systems against bacterial infection (Hoffmann, 1995). Synthetic cecropins exhibit a powerful inhibitory efficacy against Escherichia coli, P. aeruginosa, Bacillus megatherium and Micrococcus luteus (Andreou et al., 1985) and these classes of peptides act to destroy the bacterial membrane integrity (Silvestro et al., 2000). Another highlighted insect AMP group is the defensins (Hoffmann and Hetru, 1992), which act against Gram-positive bacteria and participate in the antibacterial defense re-actions in insects (Wang et al., 2016a).

Another group of animals that presents a rich arsenal of AMP to defend against noxious microorganisms are the amphibians (Simmaco et al., 2004). The magainins were isolated from an African frog's skin and its synthetic peptide form displayed antibacte-rial activity against numerous Gram-positive and Gram-negative bacteria such as E. coli, S. aureus and Klebsiella pneumonia. The synthetic limmocharin, a peptide from amphibians' skin, showed antimicrobial activity against Gram-positive and negative bacteria (Wang et al., 2016b). Also, the synthetic blycanins, an amphibian AMP extracted from an Oriental frog, showed antibacterial activity against E. coli and S. aureus (Lin et al., 2014). Finally, the hyalins and ceratin-Ha (Ctxt-Ha) are cytolytic peptides isolated from the arboreal South American frog Hylidaeinae abequans in that present a broad biological activity against bacteria and fungi (Castro et al., 2009; Cespedes et al., 2012; Vicente et al., 2013). From mammals, defensins and cathelicidins (CATH) are the main classes of AMP that have been identified. Defensins show a broad range of antimicrob-ial activity against bacteria that have demonstrated resistance to antibiotic treatments (Verma et al., 2007) and CATH exert antibacterial activity against Gram-positiv- and Gram-negative bacte-ria via electrostatic interactions with the bacterial cell membrane (Dean et al., 2011).

One of the interesting types of AMP studied are bacteriocins (Russell and Mantovani, 2005), which are ribosomal peptides released into the extracellular medium by Gram-positive and Gram-negative microorganisms and which have specific bacteri-cial or bacteriostatic action (Collins et al., 2010; De Vuyst and Leroy, 2007). The first bacteriocin was initially identified as an antimicrobial protein produced by E. coli, called colicin (Gratia, 1925).

In 1969, studies in bacteriocins produced by lactic acid bacteria aroused more interest in nisin, the first commercially applied bacteriocin. Nisin was added to the European list of food additives in 1983 and authorized for use in processed cheeses by the Food and Drug Administration (FDA) in 1988. Since then, the research field on this biomolecule has extensively increased, allowing the discovery and detailed characterization of many bacteriocins in recent decades (Rohlson et al., 2019; Hwanhlem et al., 2017; Collins et al., 2010). Recently, this compound has received great attention due to its high potential for application in the food industry — being used as natural preservatives — as well as being suggested to reduce the indiscriminate use of antibiotics in food products for humans and animals (Sabo et al., 2014). The production of bacteriocins oc- curs initially as a response mechanism to stimuli or environmental stress generated by microbial competition. They are usually syn-thesized as inactive pre-peptides with a precursor sequence in the N-terminal region (Xie and van Der Donk, 2004) and transported to the cell surface during the exponential phase and catalyzed into the active form (Aucher et al., 2005).

Bacteriocins are also widely used in the clinical field, e.g., in the treatment of topical dermatitis (Valenta et al., 1996), stomach ulcers and colon infections and respiratory tract infections (Dicks et al., 2018). Nisin, combined with conventional antibiotics, effectively helped the membrane permeabilization of an enteric multidrug-resistant Salmonella strain (Singh et al., 2013). This combination of antibiotics with AMP represents a way to decrease the use of conventional antibiotics in medical applications and help to reduce resistant bacteria (Naghmouchi et al., 2011). Kamarajan et al. (2015) demonstrated that nisin ZP (a naturally occurring variant) reduced tumorigenesis in vivo. In addition, a long-term treatment with nisin ZP extended survival and induced apoptosis dose-dependently in human umbilical vein endothelial cells (HUVEC), with concomitant decrease in vascular sprout formation in vitro and reduction of intratumoral microvessel density in vivo.

Over 70,000 distinct fungi have been identified and some of these can cause serious damage to human health (Li et al., 2012). Since the AMP have pleiotropic functions, i.e., a single molecule has several characteristics that are often unrelated, they can exert strong antifungal activity and could be useful in addressing fungal infections. In addition, many AMP are viral inhibitors (Jenssen et al., 2006). This antiviral activity can be related to the direct interaction with the virus or is a result of an indirect effect through interactions with potential target cells. Moreover, there are some studies that show the effects of some AMP against influenza virus and human
immunodeficiency virus (HIV) (Tripathi et al., 2013; Barlow et al., 2011).

4. AMP utilization in animal feed

Antibiotics have been used in the animal industry for more than 50 years (Xiao et al., 2015). Although its use brings benefits, the misuse has caused some problems, including the emergence of bacteria resistant to antibiotics and drug residues in meat products (Bacani and Basaran, 2019).

4.1. Swine

In the swine industry, post weaning diarrhea is a serious problem for production, due to increased mortality and reduced growth performance. Approximately, 50% of piglet mortality due to diarrhea is caused by enterotoxigenic E. coli (ETEC) (Cutler et al., 2007). According to United States Department of Agriculture (USDA, 2018), 95.5% of swine farms in United States include subtherapeutic concentrations of antibiotics in young pigs’ diets. Despite that, farmers still reported a large occurrence of diarrhea, an unsurprising fact since very often they have seen a broad spectrum of antibiotic resistance among ETEC strains (Lanz et al., 2003; Maynard et al., 2004).

A potential alternative to conventional antibiotics is the colicins, a class of bacteriocins produced by and effective against E. coli, killing bacteria by disrupting the ionic gradient (FAO, 2020). Recent studies have demonstrated that colicins are effective against ETEC strains from pigs in vitro (Cutler et al., 2007) and against a wide range of E. coli strains (Murinda et al., 1996; Lanz et al., 2003). The colicins are not related to any antibiotic used in human medicine and do not leave any kind of traces in the animals (Cutler et al., 2007). Cutler et al. (2007) tested 2 doses of colicins (11.5 and 16.5 mg of colicin/kg of diet) on weaning pigs and observed a 40% higher weight gain and a 7% lower feed efficiency in the higher dose group compared to the control group (no colicin) showing that the animals have a better performance with colicin even when a small dose was used. Also, the authors did not observe any signs of post weaning diarrhea in the pigs in the higher dose group.

Other types of AMP that have been used are the synthetic AMP A3 and P5 (Yoon et al., 2012 a;b; 2014), Yoon et al. (2012a) evaluated different increasing levels of AMP A3, which were 0, 60 and 90 mg/kg of diet, as an alternative to conventional antibiotics. The study analyzed the growth performance, coefficient of total tract apparent digestibility (CTTAD) of nutrients and intestinal aspects of weaning pigs. A linear improvement on final body weight and an average daily gain, with no effect on average daily feed intake and feed efficiency were found. In addition, they observed a linear improvement on CTTAD of dry matter and crude protein, and no apparent digestibility (CTTAD) of nutrients and intestinal aspects of weanling pigs as an alternative to conventional antibiotics for this reason, in recent years, studies in rumen manipulation have widely increased. Antibiotics can increment the rumen efficiency; however, as occurred in swine industry, long-term usage may not be beneficial for ruminants and consumers (Cheema et al., 2011). Therefore, attempts have been made to replace them with better and safer alternatives.

As can be seen, there are some proven strategies with AMP to replace the use of antibiotics as growth promoters. However, further studies are needed to identify the precise in vivo mechanism of the action of these AMP to allow for safer utilization in animal nutrition.

4.2. Cattle

The rumen is an essential organ for nutrient fermentation due its capacity to produce end-products, particularly short chain fatty acids (SCFA) and microbial protein, the major energy and protein source to ruminants, respectively (Kristensen et al., 2005). Therefore, the more efficient the rumen is, the more end-products are synthetized. For this reason, in recent years, studies in rumen manipulation have widely increased. Antibiotics can increment the rumen efficiency; however, as occurred in swine industry, long-term usage may not be beneficial for ruminants and consumers (Cheema et al., 2011). Therefore, attempts have been made to replace them with better and safer alternatives.

Ruminants, as other animals, produce many AMP that act as natural innate barriers, limiting microbial infectious diseases and, therefore, have different sizes and mechanism of actions (Brogden et al., 2003). Ruminants present 2 AMP groups, available in different tissues, macrophages, mucosal epithelial cells and polymorphonuclear leukocytes, as follows: 1) anionic peptides – a small group rich in aspartic and glutamic acids, with activity against Gram-negative and Gram-positive bacteria, and 2) cationic peptides – rich in proline and cysteine, with activity against Gram-positive and Gram-negative bacteria (Brogden et al., 1996, 1997). These peptides have also antiviral and antifungal activities (Jenssen et al., 2009; Lee-Huang et al., 1999; Arnold et al., 1980).

Some ruminal Gram-positive bacteria can produce bacteriocins, an inhibitor of other bacteria species (Kalmokoff et al., 1996). A lantibiotic, bovicin HCS, was identified and isolated from Streptococcus bovis HC5 by Mantovani et al. (2001) with a large antibacterial activity and no bacterial adaptation demonstrated (Mantovani et al., 2001, 2002). Lee et al. (2002) tested the effects of bovicin HCS on ruminal methane production in vitro and demonstrated that it can inhibit methane production at pH 6.7. Another study found that bovicin HCS is even more effective in 5.5 pH, showing that the molecule can be more effective in animals fed grain rather than forage (Mantovani et al., 2002). The same study also tested the capacity of bovicin HCS to inhibit methane production in a successive fashion. In addition, it was found that microorganisms, which received bovicin HCS, gradually lose their activity, i.e., the methanogen archaea did not adapt to the molecule. Lima et al. (2009) investigated if bovicin HCS could inhibit the
deamination of mixed ruminal bacteria and evaluated if bovicin HCS and monensin affected the same types of ammonia producing ruminal bacteria. Interestingly, they found that bovicin HCS and monensin have activity against the same types of bacteria.

4.3. Broilers and layers

In the poultry industry, the greatest problem raised is based on the presence of Salmonella sp., which also affects human public health (Narushin et al., 2020). The defense mechanisms of both broilers and laying hens have been studied for several years. As a result, it has been possible to identify AMP and classify them into 2 large families, defensins and CATH (Akbari et al., 2008).

Defensins are composed of between 18 and 45 amino acids, predominately comprised of cystines, cationic and hydrophobic residues. They present antimicrobial activity against bacteria, some fungi, protozoa, and even viruses (Sugiarto and Yu, 2004), immuno-modulatory, anti-inflammatory, and intermediaries in regeneration of skin wounds (Xiao et al., 2020; Akbari et al., 2008). They are subdivided into α, β, and γ, which are differentiated by the disulfide bridge position, size (kDa), and structure (β-sheet dimer or cyclic). Furthermore, in premature ovule the AvD induced the chicks, as well as cattle, pigs and humans have β-defensins, but not α- or γ-defensins (Sugiarto and Yu, 2004). Avian β-defensins (AvD) are produced by the activation of encoded genes in response to an external or environmental factor and thus achieve homeostasis. These genes remain inactivated when chickens are not strained or under an external or environmental factor and thus achieve homeostasis.

In laying hens, white eggs have been demonstrated to possess antimicrobial proteins and β-defensins. Also, a wide variety of cationically active AMP such as gallinacins, which can interact with pathogens cell membrane, have also been found. In addition, many of these AMP were identified as anti-biofilms that could eliminate microorganisms (Arena et al., 2020). A large list of AMP and other peptides related to anticancer, anti-inflammatory, antihypertensive and antiviral effects were also identified in chicken yolk plasma (Arena and Scalon, 2018). Gallinacin 11 has demonstrated an effective response against Salmonella enteritidis and Listeria monocytogenes in chickens (Higgs et al., 2005).

Some ceratotoxins, such as the cationic AMP, Ctx (Ile21)-Ha are a promising peptide applied against S. enteritidis and S. typhimurium, it also showed great activity against multidrug-resistant bacteria (Roque-Borda et al., 2021a). Innovative systems such as microparticles were successfully applied to control systemic infection caused by Salmonella (Roque-Borda et al., 2021b). Likewise, it was reported that these microparticles would be able to reduce the mortality rate of the chicks during the first days of post-infection life and increase their weight with the passing of days (Roque-Borda et al., 2021c).

A previous study has shown that the regulation of AMP was influenced by the presence of probiotics in chickens, because their relationship was inversely proportional (Ma et al., 2020). The gene expression of AMP decreased as the probiotic concentration increased, which indicates that AMP may not be entirely necessary in the presence of probiotics. However, there are still gaps to be studied in this relationship (Schlee et al., 2008; Akbari et al., 2008; Wehkmamp et al., 2004). Another important regulator could be yeast culture (YC), where the expression of genes encoding AMP is increased, such as those expressing the AvD 1,4 and 7 peptides and CATH 1 and 3. These molecules presented beneficial results for aged laying hens, demonstrating how beneficial this combination supplemented with YC can be, and the impact it has with the age of the chickens (Zhang et al., 2020).

In recent published studies on broilers, the AMP Microrn J25 was used against Salmonella sp. and E. coli. This peptide, showing antimicrobial activity, promoted animal growth performance, which improved the fecal flora composition and intestinal structure, as well as an induction of an efficient immune response (Wang et al., 2020). Another important agent used was potato proteins, which reduced the presence of coliform bacteria and improved broiler production performance (Ohh et al., 2009). Lactobacillins (Xu et al., 2020), which are polypeptides with antimicrobial activity produced by lactic acid bacteria (LAB), were also studied. Lactobacillus bacteriocin plantaricin K (Plnk) is part of this group and was expressed in vitro by genetic engineering. This peptide is particularly selective because it does not eliminate Gram-positive bacteria and exclusively eliminates pathogenic bacteria from the body. In addition to not altering the intestinal flora diversity, this peptide is a specific promoter of intestinal microbial control and has become an option for studies of selective AMP (Xu et al., 2020). A recombinant peptide, plectasin, complements the positive results already mentioned, and has exhibited an increase in duodenal lipase yield and trypsin activity, which are important for the digestion process in broilers (Ma et al., 2020).

Some studies based on broilers have also integrated the use of suberin from Bacillus subtilis 168, which was shown to have a great biological effect against Clostridium perfringens, S. aureus and P aeruginosa, also helping the proliferation and count of LAB (Ozcan et al., 2019). This recombinant AMP has also been shown to generate immunological memory (e.g., humoral, and cellular) by increasing the lymphocyte T content (CD4- to-CD8+ ratio), and Newcastle disease antibodies. Therefore, due to these results, a highly effective vaccine could be generated using this AMP as immunity stimulators (Liu et al., 2019).

In addition, it has also been shown that mixed-formulations of AMP and other compounds could have promising effects, such as those that use marine algae-cecropin, when Laminaria japonica powders were used as a feed supplement, showing a high growth performance and immune protection (Bai et al., 2019); This mixed effect could be key to the development of new antimicrobial additives as a food source for chickens.

There are other AMP such as magainin (Wang et al., 2016b), nisin (O’Connor et al., 2020), camel lactoferrin “36” and “chimera” (Daneshmand et al., 2019a,b) that are described in literature. These molecules have shown results like those already mentioned in this section. For all these reasons, AMP are essential for the survival and animal protection and become an interesting and valuable alternative for poultry production improvement.

5. Food preservatives

AMP have been applied in food preservation for more than 25 years. The most studied AMP were the bacteriocins produced by the LAB family, as they have potential application in food preservation (as shown in their application in broilers) (Kareem and Razavi, 2020). Among the most studied, plantaricins showed a high rate of microbial inhibition and activity against S. enteritidis, E. coli, L. monocytogenes, C. perfringens and B. cereus, among others (Thakur, 2017). Regarding the plantaricin family, some molecules have special features, such as: plantaricin A, inducer of production of other plantaricins (E/F and J/K); plantaricin C, applied in conservation and preservation of cheese and plantaricin S, extracted from fermented green olives (Kareem and Razavi, 2020).

The use of natural preservatives in replacement of chemical agents is an important strategy to increase shelf life of minimally processed fruits and vegetables (da Costa et al., 2019; Przybylski et al., 2016). In Brazil, with the approval of nisin in 1996, the first AMP/bacteriocin was used commercially by the Health Department
as a preservative. In addition, pediocin peptide, isolated from *Pediococcus* spp., is mainly used as a preservative for meat products, which protects from heat treatment due to its stability over a wide pH range (2 to 8) and high temperatures (maximum at 121°C) (da Costa et al., 2019).

A new peptide recently isolated from broccoli, known as broccoli napin, has revealed a protective action against pathogenic fungi, such as *Fusarium culmorum* and *Penicillium expansum*, being one of the most problematic pathogens in the agricultural sector. Moreover, the molecule has shown thermal stability, anti-tumor activity, and presented no hemolysis, protecting mainly crops and cereal-based products (Thery et al., 2020).

The x137–141 peptide was obtained from hemoglobin hydrolysis of blood residues of slaughterhouses, a residual by-product of beef production. This peptide has shown a high rate of microbial inhibition and potential antioxidant capacity. Thus, these functions together are interesting for their application in the food industry (Przybylski et al., 2020). Other AMP, such as those isolated from the house fly (*Musca domestica pupae*) (Md-AMP), which are used to preserve pork (chilled pork), have been found to destabilize the cell membrane and bind to DNA (Dang et al., 2020). The application of some AMP in the food industry has been tested through pilot trials of mono-organ meats, such as polyphenolase terephthylate with the mitochondrial-targeted peptide 1. This molecule was evaluated in meat and dairy foods, demonstrating satisfactory results, corroborating a great progress and efficiency of AMP (Gogliettino et al., 2020). Therefore, these recent discoveries could lead to a promising alternative for food additives.

6. Cattle disease treatments with AMP

As mentioned, AMP have been considered a new source of biomolecules in several fields of scientific research, given their potential against many pathogenic microorganisms. In addition, the increase of microorganisms’ resistance to antibiotics and the inability to discern the mechanisms of inhibition of these microorganisms have become a matter of concern, receiving immediate attention from the pharmaceutical industry to governmental and academic institutions (Lima et al., 2009). Many pathologies associated with animal production are related to the presence of bacteria, fungi, and viruses. However, they have already been evaluated as a method of prevention and treatment. In this section, AMP applications with successful results in cases of diseases caused by several types of microorganisms in cattle production are described.

*Trichomonas foetus* and *T. vaginalis* are parasites that generate enormous economic problems due to their high morbidity rates. These microorganisms can also be transmitted by humans through sexual contact. To overcome this problem, the D-hecate peptide was evaluated, proving to be highly effective against these microorganisms at a concentration of 10 μmol/L, by promoting a serious rupture in plasma membrane (Mutwiri et al., 2000). It also exhibited a great activity in cancer cells, appearing as an alternative in this type of disease in cattle production (Leuschner and Hansel, 2004). Some food additives, such as sodium bicarbonate, helped tritrpticin, also an AMP with high biological effectiveness, against *T. vaginalis* (Infante et al., 2011).

The bovine respiratory syncytial virus is considered a serious risk disease due to economic losses. This virus was counteracted by CATH LL-37 peptide, which is exceptionally attractive for monclonal antibodies, due to their specificity and ability to induce immumity (Caskey et al., 2019). In vitro studies were carried out with this AMP in human cells and the molecule induced direct damage to the viral envelope, modifying the viral structure and decreasing the binding and interaction with epithelial cells. In addition, studies with murines indicated that an exogenous application would help

in the eradication of post-infection disease (Armiento et al., 2020; Currie et al., 2016). Therefore, it is possible to use AMP with anti-viral activity when applied in cattle, with possible efficient results.

Bovine respiratory disease complex (BRDC) is one of the most common illnesses in animal production caused by *Mannheimia haemolytica*, *Histophilus somni* and *Pasteurella multocida*. Bovines have as a natural defense mechanism, the tracheal antimicrobial peptide (TAP), that is affected and inhibited due to the stress generated by hormonal regulation in animal production (Siracusa, 2018; Vulikh et al., 2019). Synthetic TAP was used to evaluate its direct dosage effect via the nasal route. However, results showed no effects and no variance, explained by the amount of mucosa present in the nasal passage, which could be related to peptide instability and degradation (Vulikh et al., 2019). In this way, the current challenge is to use carriers or formulations for peptide interaction with the target pathogenic bacteria, with no destabilization or degradation. Another study evidenced the antimicrobial activity of bovine NK-lysin (natural killer cell origin and lytic properties) derived peptides, such as NK1, NK2A, NK2B and NK2C exhibiting activity against BRDC, which demonstrated excellent results, modifying the structure of the cell membrane, and causing a cytosolic effusion (Dassanayake et al., 2017).

In summary, these studies indicate that the use of peptides in animal models or in vitro studies could be taken as a reference for the development of novel and safer molecules for the improvement of livestock production.

7. Public health implications and future implications

The discovery of antibiotics in 1928, by Alexander Fleming, helped to improve the life expectancy of humankind (Hu et al., 2020). Following the increasing use of antibiotics over time, antimicrobial resistance became a severe public health problem. In recent years, antibiotics used against some diseases such as *K. pneumoniae* and *E. coli* are no longer effective in at least 50% of treated people (Peleg and Hooper, 2010; Shaikh et al., 2015). Moreover, the last option for gonorrhea medical treatment has failed in at least 10 countries (World Health Organization (WHO), 2019). The lack of policies to control the use of antibiotics, their misuse by humans and in livestock production have helped to achieve this threatening scenario.

Since 2015, AMR has become a worldwide priority. The WHO created the “Global Action Plan on Antimicrobial Resistance” to encourage a wise use of antibiotics and some strategies to reduce their consumption. This plan of action identified that some common medical conditions, such as tuberculosis, HIV, malaria, sexually transmitted diseases, urinary tract infections, pneumonia, blood-stream infections, and food poisoning have become resistant to a large number of conventional antibiotics (Roque-Borda et al., 2021d). Consequently, this fact has forced medical practitioners to use so-called “last-resort” drugs, which are expensive and mostly unavailable in poor countries.

Therefore, the urgent need for the development of new antimicrobials, mainly natural ones, is a powerful strategy to minimize the indiscriminate use of conventional antibiotics and could help treat and overcome some diseases, thus improving our quality of life.

8. Conclusions

Advances in animal production have demanded the increase of use of additives for productivity and to avoid mortality by pathogens. However, the increasing application of antibiotics and other growth promoters has revealed unfortunate “side effects”: the appearance of AMR, multdrug resistant bacteria, and hazardous
risks for human health. To tackle this broad-spectrum problem at different levels, this review presents and discusses an interesting alternative among several researched molecules: the AMP. These compounds, which have been proven to be efficient and promising, have shown biological activity and applicability against several microorganisms, mainly in the areas of animal production, as well as being suitable for food preservation. The multiple properties of AMP make them optimistic and powerful candidates to replace conventional drugs. Moreover, the development of new natural peptide-based antimicrobials for livestock, swine and poultry can help to reduce the antimicrobial problem without affecting either animal production or human health, nor leaving any pharmacological residues that generate environmental impact issues.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that might appropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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