Antimicrobial peptides as promising alternatives to antibiotics in weanling pigs - a review

Thi Hoan Tran[1*], Dinh Hai Nguyen[2*]

[1] Faculty of Veterinary Medicine and Animal Science, Thai Nguyen University of Agriculture and Forestry, Thai Nguyen, Vietnam
[2] CJ Bio Vietnam

*These authors contributed equally to this work

Abstract

Antimicrobial peptides are relatively small peptides (< 10kDa), cationic and amphipathic peptides of variable length sequence and structure which are an important component and widely existing in nature as the first line defence in wide range of organisms. Antimicrobial peptides have the widespread distribution of potent, broad spectrum antimicrobial peptides in multicellular organisms that have been suggested to use to resist a wide range of microbes, including bacteria, fungi, viruses, and protozoa. Due to the emergence of spreading antibiotic resistance in the environment, and the presence of chemical residues in animal products which results in the development of antimicrobial peptides, which have a good application prospect in animal husbandry and aquaculture, especially in young animal. The available documents to date has shown that antimicrobial peptides have the potential to prevent the growth of pathogens, improve the intestinal mucosal function, the immune system, digestion, and absorption capacity, as well as useful intestinal flora, and decrease diarrhea rate, keep the weanling pig health, and eventually improve the growth performance in weaning pigs, which is a potential alternatives to replace antibiotics in the diets of weaning pigs. This review will give an overview of (i) the source of antimicrobial peptides, (ii) mode of antimicrobial actions, and (iii) application of antimicrobial peptides on weaning pigs and systematically.

Keywords: antimicrobial peptides; antibiotic, weaning pigs

Introduction

Weaning is a stressful experience for the piglets involving psychological, nutritional, microbiological, and immunological, and environmental stresses because during this weaning transition period piglets have to face with abrupt separation from their dam, mixing with other litters, moving to new environment and switch from highly digestible feed (milk) to a less digestible commercial feed (Lalles, 2008), which could result in economic losses due to decreased growth rate, feed efficiency, diarrhoea, intestinal disturbances, and piglets' health.

Antibiotics have been used very successfully to weanling pigs for the diarrhoea prevention or treatment and growth performance improvement in the worldwide. However, the use of antibiotics as growth promoters in animal feed has been banned in many countries including European Union since 2006 as well as South Korea since 2011 (Nguyen et al., 2018) due to the concern of antibiotic resistance in pathogens and antibiotic residues in animal products. Therefore, massive efforts have been made to find different ways to maintain animal health and performance, many researchers have suggested that the use of organic acids, organic minerals, bacteriophages, probiotics, and prebiotics as potential alternatives (Nguyen et al., 2020). Among a variety of candidates for the replacement of antibiotic growth promoters, antimicrobial peptides (AMPs) are promising alternatives. Antimicrobial peptides are an important component of the first line defence in various animal species, the natural defences of most living organisms against invading pathogens with the widespread distribution of potent, broad spectrum AMPs in multicellular organisms that have been suggested to use to resist a wide range of microbes, including bacteria, fungi, viruses, and protozoa (Reddy et al., 2004; Leeson, 2001).

Unlike conventional antibiotics, which usually function through a defined high-affinity antimicrobial target and which can induce resistance in microorganisms, AMPs exert multiple antimicrobial activities that might provide a strategy to prevent bacteria from developing resistance (Peschel and Sahl, 2006). 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 (Lai and Gallo,

2009; Wang et al., 2015). Administrating of various AMPs has been illustrated to improve growth performance, nutrient digestibility, intestinal microflora, intestinal morphology and immune system in pigs (Tang et al., 2008; Yoo et al., 2013). It is therefore this article provides an overview of their common sources, mechanism of action. The response of weanling pigs to antibacterial peptides of the previous reports pigs is also reviewed.

Antimicrobial peptides

Since the identification of the first antibacterial protein family, thionins, during the early 1970s, more than 750 different AMPs have been identified in various organism ranging from insects to plants to animals as well as in humans (Schnapp et al., 1998; Leeson, 2001). Antimicrobial peptides are relatively small (<10kDa), cationic and amphipathic peptides of variable length sequence and structure. These peptides have been grouped based on their primary structure, amino acid composition and their size (Ravichandran et al., 2010).

The diversity of natural AMPs causes difficulty in their classification. According to Huan et al. (2020), AMPs are classified based on (1) sources, (2) activity, (3) structural characteristics, and (4) amino acid-rich species. However, in this work we will summary the classification of AMPs based on the sources.

Source of antimicrobial peptides

Mammalian sources

Mammalian antimicrobial peptides are found in human, sheep, cattle, and other vertebrates. They have common features such size, cationic charge, and an amphipathic nature. Defensins and cathelicidins are the main antimicrobial based on such features in mammals. Defensins can be divided into α-, β-, and θ-defensins depending on the position of disulfide bonds (Reddy et al., 2004). Table 1 further summarizes discovery of various AMPs from variety of mammal.

Table 1. Antimicrobial peptides derived from mammal (Boparai and Sharma, 2020)

Peptide name	Source	Amino acid number	Antimicrobial activity	References
Cathelicidins	Human neutrophils	30	F, G^-, G^+	Sheehan et al., 2018
A Defensins	Human neutrophils	12 - 80	F, G^-, G^+	Schaal et al., 2018
Human Histatin 8	*Homo sapiens*	12	F, G^-, G^+	Khurshid et al., 2017
LL37	Neutrophils *(Homo sapiens)*	37	F, G^-, G^+	Baxter et al., 2017
Androctonin	*Androctonus australis*	25	F, G^-, G^+	V Panteleev et al., 2017
Bactenecin	Bovine Neutrophils	12	G^-, G^+	Young-Speirs et al., 2018
Brevinin	*Rana brevipora porsa*	24	G^-, G^+	Savelyeva et al., 2014
Buforin II	*Bufo bufo gargarizans*	21	F, G^-, G^+	Sun et al., 2015
Cupiennin	*Cupiennius salei*	35	G^-, G^+	Upadhyay, 2018
Dermaseptin S1	*Phyllomedusa sauvagii*	34	G^-, G^+	Belmadani et al., 2018
Lycotoxin	*Lycosa carolinensis*	27	G^-, G^+	Tahir et al., 2018
Tachyplesins	*Tachypleus tridentatus*	17	G^-	Kuzmin et al., 2017

Note: F, Fungus; G-, Gram negative; G+, Gram positive

Plant sources

Plant AMPs have evolved differently from AMPs from other life forms. Most plant AMPs involves host plant resistance to pathogens such as fungi, viruses, and bacteria, whereas a few plant AMPs from the cyclotide family carry insecticidal functions (Noonan, 2017). Ranging size from 2 to 9 kDa, all plant AMPs are globular, compact, and cysteine-rich peptides (Leeson, 2001). Thionins were the first plant AMPs to be described. Subsequently, the antimicrobial activities of various defensins, lipid transfer proteins, hevein-and knottin-like peptides, including MBP-1 from maize, IbAMP from the seeds of impatiens, snakins from potatoes, and shepherdins from roots of shepherd's purse have been identified (Garcıa-Olmedo, 2015; Tam, 2015). Table 2 further summarizes discovery of various AMPs from variety of plant.

Table 2. Antimicrobial peptides derived from plant (Shwaik et al., 2021)

Peptide name	Source	Antimicrobial activity	References
Defensin			
Rs-AFP1; Rs-AFP2	*Raphanus sativus*	F, Y	Shwaiki et al., 2020a
IbAMP1	*Impatiens balsamina*	F, B	Wu et al., 2013
Cp-thionin II	*Vigna unguiculata*	F, B	Schmidt et al., 2019
MsDef1; MtDef4	*Medicago sativa*	F	Sagaram et al., 2011
Thionins			
Tu-AMP 1; Tu-AMP 2	*Tulipa gesneriana*	F, B	Fujimura et al., 2004
Wheat β-Purothionins	*Triticum aestivum*	B	Mak and Jones, 1976
Thionin 2.4	*Arabidopsis thaliana*	F	Asano et al., 2013
Snakin/ GASA			
St-SN1	*Solanum tuberosum*	F, Y, B	Shwaiki et al., 2020b
MsSN1	*Medicago sativa*	F, B	García et al., 2014
Snakin-Z	*Ziziphus jujuba*	F, B	Daneshmand et al., 2013
Cyclotides			
Cycloviolacin O2	*Viola odorata*	F, B	Zarrabi et al., 2013
Cycloviolacin O8	*Viola odorata*	F	Parsley et al., 2018
Knottin type			
PAFP-S	*Phytolacca americana*	F	Shao et al., 1999
Mj-AMP1; Mj-AMP2	*Mirabilis jalapa*	F	Cammue et al., 1992
Hevein-type			
Ee-CBP	*Euonymus europaeus*	F	Van Den Bergh et al., 2002
SmAMP3	*Stellaria media*	F	Rogozhin et al., 2015
EAFP1	*Eucommia ulmoides*	F	Huang et al., 2002
EAFP2			
Lipid Transfer Protein (LTP)			
Ca-LTP1	*Capsicum annuum*	F	Diz et al., 2011
Mung bean nsLTP	*Phaseolus mungo*	F, B	Lin et al., 2005
2S albumin proteins			
Pe-AFP1	*Passiflora edulis*	F, Y	Pelegrini et al., 2006
CW-1	*Malva parviflora*	F	Wang and Bunkers, 2000

Note: B, Bacteria; F, Fungus; Y, Yeast

Insect sources

It is well known that insects are extremely resistant to bacterial infections. Antimicrobial peptides are mainly synthesized in fat bodies and blood cells of insects, which is one of the main reasons for insects' strong adaptability to survival (Vilcinskas, 2013). The cecropinis is the first and most famous family of antimicrobial peptides from insect that was identified in the 1980 from the pupae of Hyalophora cecropia (Hultmark et al., 1980). Most insect AMPs are cationic molecules due to the presence of basic residues with activities against bacteria. According to their amino acid sequences and structures, antimicrobial peptides can be classifed in four diferent groups: cysteinerich peptides (e.g. defensins), the α-helical peptides (e.g. cecropins), glycine (Gly) -rich proteins (e.g. attacins), and proline-rich peptides (e.g. drosocins) (Bulet and Stocklin, 2005; Makarova et al., 2018). Table 3 further summarizes discovery of various AMPs from variety of insects.

Table 3. Antimicrobial peptides derived from insect (Boparai et al., 2020)

Peptide name	Source	Amino acid number	Antimicrobial activity	References
Acaloleptin	*Acalolepta luxuriosa*	71	G^-, G^+	Vogel et al., 2014
Andropin	*Drosophila melanogaster*	34	G^+	Abry et al., 2017
Apidaecin IA	*Apis mellifera*	18	G^-	Farouk et al., 2017
Cecropin	*Hyalophora cecropia*	37	G^-	Lee and Lee, 2015
Defensin- α	*Aedes aegypti*	40	G^-, G^+	Price et al., 2015
Drosomycin	*Drosophila melanogaster*	44	F	Allocca et al., 2018
Holotricin	*Holotrichia diomphalia*	43	G^-, G^+	Thiyonila et al., 2018
Sapecin- α	*Sarcophaga peregrine*	40	G^-, G^+	Manabe et al., 2017
Tenicin 1	*Tenebrio molitor*	43	G^-, G^+	Yang et al., 2018
Thanatin	*Podisus maculiventris*	21	G^-, G^+	Duwadi et al., 2018

Note: F, Fungus; G-, Gram negative; G+, Gram positive

Amphibian sources

Antimicrobial peptides from amphibians play an important role in the protection of amphibians from the pathogens that have

induced the global amphibian population decline (Rollins-Smith, 2009). Frogs are the main source of amphibian AMPs and the most famous AMP from frogs is magainin; the skin secretions of frogs from genera Xenopus, Silurana, Hymenochirus, and Pseudhymenochirus under the Pipidae family are rich in AMPs (Conlon and Mechkarska, 2014). Cancrin which had 19 amino acids has been reported as the first AMP from the sea amphibian Rana cancrivora (Lu et al., 2008). This marks a broader source of AMPs of amphibians. Table 4 further summarizes discovery of various AMPs from variety of frogs.

Table 4. Antimicrobial peptides derived from frogs (Patocka et al., 2019)

Peptide name	Source	Antimicrobial activity	References
Esculentin-1	Rana esculenta	46	Simmaco et al., 1993
Esculentin-1a	Rana esculenta	46	Simmaco et al., 1994
Esculentin-1b	Rana saharica	46	Marenah et al., 2006
Esculentin-1c	Rana esculenta	45	Kang et al., 2010
Esculentin-2a	Rana esculenta	37	Simmaco et al., 1993
Esculentin-1SEa	Dusky gopher	46	Graham et al., 2006
Esculentin-2SE	Dusky gopher	37	Graham et al., 2006
Esculentin-2- OG15	Amolops soloensis	78	Wang et al., 2010
Esculentin-2- OG11	Amolops soloensis	78	Wang et al., 2010

Microorganism sources

Antimicrobial peptides can be also obtained from microorganisms like bacteria and fungi, and some famous peptides are nisin, gramicidin from Lactococcus lactis, Bacillus subtilis, and Bacillus brevis (Cao et al., 2018). Microorganism-derived AMPs have long been used as food preservatives. Recently, peptides produced by members of the genus Bacillus were shown to have a broad spectrum of antimicrobial activity against pathogenic microbes. Bacillus-derived AMPs can be synthesized both ribosomally and nonribosomally and can be classified according to peptide biosynthesis, structure, and molecular weight. Table 5 further summarizes discovery of various AMPs from variety of microorganism.

Table 5. Antimicrobial peptides derived from microorganism (Boparai et al., 2020)

Peptide name	Source	Amino acid number	Antimicrobial activity	References
Nisin	*Lactococcus lactis*	34	G^+	Mills et al., 2017
Enterocin	*Enterococcus*	70	G^+, G^-	Braïek et al., 2018
Ericin S	*Bacillus subtilis*	32	G^+	Sharma et al., 2018
Plantaricin A	*Lactobacillus plantarum*	26	G^+, G^-	Jiang et al., 2018
Leucocin A	*Leuconostoc pseudomesenteroides*	37	G^+, G^-	Chen et al., 2018
Subtilin	*Bacillus subtilis*	32	G^+	Singh et al., 2017
Microcin J25	*Escherichia coli AY25*	21	G^+	Zhao et al., 2016
Gramicidin A	*Bacillus brevis*	15	G^+, G^-	Muhammad et al., 2016
Streptin 1	*Bacillus subtilis A1/3*	23	G^+	Bosma and LanthioPep, 2017
Gassericin A	*Lactobacillus gasseri LA39*	58	G^+, G^-	Maldonado-Barragán et al., 2016
Circularin A	*Clostridium beijerinckii ATCC 25752*	69	G^+, G^-	Perez et al., 2016
Plantaricin C19	*Lactobacillus plantarum C19*	37	G^+	Wang et al., 2018
Enterocin P	*Enterococcus faecium P13*	44	G^+	Le et al., 2014
Subtilosin A	*Bacillus subtilis*	35	G^+, G^-	Venturina et al., 2016
Plantaricin; ASM1	*Lactobacillus plantarum A-1*	43	G^+	Wang et al., 2018
Lichenin	*Bacillus licheniformis*	12	G^+, G^-	Bhat, 2018

Note: G-, Gram-, G+, Gram+

Aqua sources

Fishes are one of the organisms that have managed to survive in a milieu of pathogenic organisms. The primary interference of fish with their environment happens through a mucous layer that covers its entire body. Marine fishes possess antimicrobial peptides as a part of their defense system, which are mainly present in the mucous layer indicating that they eliminate the pathogenic bacteria before they enter the skin barrier (Ravichandran et al., 2010). Based on both structural features and origins, fish AMPs have classified into four classes: (i) AMPs exhibiting an α-helical structure (pleurocidins, moronecidins and piscidins); (ii) β-sheet structured AMPs containing 4 disulfide bonds (hepcidins); (iii) proteolytic fragments of structural or functional proteins; (iv) AMPs produced by fish-associated bacteria (Desriac et al., 2013). Table 6 further summarizes discovery of various AMPs from variety of fishes.

Table 6. Antimicrobial peptides derived from fishes (Desriac et al., 2013)

Peptide name	Source	Antimicrobial activity	Antimicrobial activity	References
Pleurocidins				
WF1	*P. americanus*	25	G-, G+	Cole et al., 1997
WF2	*P. americanus*	26	F, G-, G+	Douglas et al., 2003
WF1L	*Pp. americanus*	24	F, G-, G+	Douglas et al., 2003
WFX	*Pp. americanus*	21	F, G-, G+	Douglas et al., 2003
WFY	*Pp. americanus*	21	F, G-, G+	Douglas et al., 2003
WF3	*P. americanus*	23	F, G-, G+	Douglas et al., 2001
WF4	*P. americanus*	25	F, G-, G+	Douglas et al., 2001
Moronecidins				
Moronecidin	*Morone chrysops*	22	F, G-, G+	Lauth et al., 2002
Moronecidin	*Morone saxatilis*	22	F, G-, G+	Lauth et al., 2002
Dicentracin	*Dicentrarchus labrax*	22		Salerno et al., 2007
Piscidins				
Piscidin-1	*Morone saxatilis*	22	F, G-, G+	Lauth et al., 2002
Piscidin-2	*Morone saxatilis*	22	V, F, G-, G+	Campagna et al., 2007
Piscidin-3	*Morone chrysops x*	22	G-, G+	Silphaduang and Noga, 2001
	Morone saxatilis			
Epinecidin				
Epinecidin-1	*Epinephelus coioides*	25	G-, G+	Pan et al., 2007
Grammistins				
Grammistin Pp2b	*Pogonoperca punctata*	13	H, G-, G+	Sugiyama et al., 2005
Grammistin Pp2a	*Pogonoperca punctata*	13	G-, G+	Kaji et al., 2006
Grammistin Pp4b	*Pogonoperca punctata*	24	H, G-, G+	Kaji et al., 2006
Grammistin Pp4a	*Pogonoperca punctata*	24	H, G-, G+	Kaji et al., 2006
Grammistin Pp3	*Pogonoperca punctata*	25	H, G-, G+	Sugiyama et al., 2005
Grammistin GsG	*Grammistes sexlineatus*	24	H, G-, G+	Sugiyama et al., 2005
Grammistin GsF	*Grammistes sexlineatus*	25	H, G-, G+	Sugiyama et al., 2005
GrammistinGsC	*Grammistes sexlineatus*	26	G-, G+	Sugiyama et al., 2005
Grammistin GsA	*Grammistes sexlineatus*	28	G-, G+	Sugiyama et al., 2005
GrammistinGsE	*Grammistes sexlineatus*	13	H, G-, G+	Kaji et al., 2006
Grammistin Gs D	*Grammistes sexlineatus*	13	H, G-, G+	Kaji et al., 2006
Grammistin Gs B	*Grammistes sexlineatus*	12	H, G-, G+	Sugiyama et al., 2005
Chrysophsins				
Chrysophsin-1	*Pagrus major*	25	G-, G+	Iijima et al., 2003
Chrysophsin-2	*Pagrus major*	25	G-, G+	Iijima et al., 2003
Chrysophsin-3	*Pagrus major*	20	G-, G+	Iijima et al., 2003

Note: F, Fungus; G-, Gram-, G+, Gram+; H, Hemolytic; V, Viruses

Modes of antimicrobial peptides actions

The primary sequences of the different classes and sources of antimicrobial peptides show little homology, differing in peptide length, amino acid composition, charge, hydrophobicity, and secondary structure. Nevertheless, most AMPs are cationic and amphipathic. Indeed, these two structural features play key roles in the antimicrobial actions exerted by these peptides (Leeson, 2001).

Membrane interaction

According to the report of Leeson (2001), before reaching the phospholipid membrane, peptides must traverse the negatively charged outer wall of Gram-negative bacteria, which contains lipopolysaccharides, or through the outer cell wall of Gram-positive bacteria which contains acidic polysaccharides (teichoic acids) (Vorland et al., 1999). In this mechanism, the cationic peptides initially interact with the surface lipopolysaccharides, competitively displacing divalent cations that bridge and partly neutralize the lipopolysaccharides. This causes disruption of the outer membrane that appears as blebs when observed under the microscope (Ulvatne et al., 2001). Studies with several antimicrobial peptides of different lengths hydrophobicities and structures revealed that these blebs are formed only below the minimal inhibitory concentration. At or above the minimal inhibitory concentration, however, bacteria are partially lysed and disintegrated (Ramanathan et al., 2002).

Membrane penetration

Basing on the report of Leeson (2001), the initial interaction of cationic antimicrobial peptides with the cytoplasmic membrane involves the insertion of the peptides parallel to the membrane surface into the interface between the phospholipid head groups and fatty acid chains of the outer monolayer of this membrane. Consequently, the membrane can be rendered permeable through

the formation of transmembrane pores, causing cell lysis and leading cell death (Benitez et al., 2003). Two major models, the barrel-stave "model and the"carpet-like"model, have been proposed to describe how the peptides interact with the membrane (Huang, 2000; Shai and Oren, 2001).

In the barrel-stave "model, as few as three membrane-bound peptides (amphipathic α-helix, hydrophobic α-helix, β-sheet, or both α-helix, β-sheet structures) recognize each other on the membrane surface, oligomerize, insert themselves into the hydrophilic membrane bilayer, and form transmembrane pores. Hence, amphipathic peptides align perpendicular to the membrane to form the staves of a transient barrel of various sizes, forming a hydrophilic pore in its center traversing the cytoplasmic membrane. This would then lead to leakage of the cytoplasmic contents and, subsequently, death (Huang, 2000).

In the carpet-like "model, antimicrobial peptides penetrate into the membrane using the following sequence: (1) binding of peptide monomers to the phospholipid head groups and alignment of the positively charged amino acids of the peptide monomers on the surface of the membrane so that their hydrophilic surface is facing the phospholipid headgroups or water molecules; (2) rotation of the molecule leading to reorientation of the hydrophobic residues toward the hydrophobic core of the membrane; and (3) once a threshold con- centration is reached, the bilayer curvature is disrupted, leading to permeability and disintegration of the membrane (Shai and Oren, 2001). Unlike the "barrel-stave "model, no specific peptide structure is needed in the "carpet-like "model.

Interaction with cellular components

To acting at the cell membrane, antimicrobial peptides may also exhibit their activity against multiple potential targets such as cell division, DNA, ribonucleic acid (RNA) for protein synthesis, and autolysin activation (Cudic and Otvos, 2002).

Application of antimicrobial peptides in weaning pigs

Antimicrobial peptides usage in swine to improve performance

Weaned pigs often face post-weaning challenges, including diarrhea, impaired growth rate, low feed intake, and mortality (Yin et al., 2010). Antimicrobial peptides are considered as feed additive to maintain the performance and health status of weaned piglets. It is indicated that the growth-promoting effects of the AMPs were thanks to the improvements in the intestinal morphology (Wang et al., 2006; Wang et al., 2007; Yoon et al., 2013; Xiong et al., 2014), PR-39 and protegrin-1 gene expression to some extent, that promotes the nutrition digestion and absorption, regulates and enhances the immunity of mucosa (Wang et al., 2006). In addition, the digestibility of nutrients in pigs fed diets supplemented with the AMPs might be due to modulation of gut environment, improvement of intestine microbial balance (Wang et al., 2007; Jin et al., 2008a,b; Tang et al., 2008).

Other researchers also demonstrated positive influence of the dietary AMPs administration on the growth performance and nutrient digestibility in weaning pigs (Table 7).

Table 7. Effect of antimicrobial peptides on growth performance and nutrient digestibility in weaning pigs.

Composition of AMPs	Dose (g/kg feed)	Growth performance			References
		ADG	ADFI	G:F	
Lactoferrin, cecropin, defensin, and plectasin	0.2; 0.3	*	*	*	Xiong et al., 2014
Lactoferrin	1	*	*	*	Wang et al., 2006
Lactoferrin	1	*	*	*	Wang et al., 2007
AMP-P5	0.04; 0.06	*	*	*	Yoon et al., 2012
AMP-A3	0.06; 0.09	*	Ns	Ns	Yoon et al., 2013
Refined potato protein	0.2; 0.4; 0.6	*	Ns	*	Jin et al., 2009
Antibacterial peptide	0.01	*	*	Ns	Wang and Feng, 2011
Lactoferricin–lactoferrampin	0.1	*	*	Ns	Tang et al., 2008
Niacin	0.04	*	-	-	Feng et al., 2021
WK3	0.05	*	*	Ns	Cao et al., 2021
WK3	2 mg/kg BW	*	*	Ns	Zhang et al., 2021
Buforin II	5 mL	*	*	*	Tang et al., 2013
M. domestica and porcine defensin	0.4	*	Ns	-	Shi et al., 2018
Cathelicidin-BF	0.6 mg/kg BW	*	*	Ns	Feng et al., 2020
Composition of antimicrobial peptides	**Dose (g/kg)**	**Nutrient digestibility**			**References**
		DM	CP	GE	
AMP-P5	0.04; 0.06	*	*	*	Yoon et al., 2013
AMP-A3	0.06; 0.09	*	*	-	Yoon et al., 2012
M. domestica and porcine defensin	0.4	*	Ns	Ns	Shi et al., 2018

Note: ADG, average daily gain; ADFI, average daily feed intake; AMPs, Antimicrobial peptides; BW, body weight; CP, crude protein; DM, dry matter; GE, gross energy; G:F, gain to feed ratio; -, no measurement.

Antimicrobial peptides usage in weaning pigs to improve intestinal morphology

Pigs' intestine is home for a dynamic microbial population that forms a complex ecosystem and has a symbiotic relationship with the host. The population of gut microbes, or microbiota, plays key roles in maintaining nutritional, physiological, and immunological functions of the pigs (Lee and Mazmanian, 2010; Brestoff and Artis, 2013). A toxin produced by pathogenic bacteria in the gut can cause inflammation of the intestinal mucosa and diarrhea associated with morphological changes in the small intestine, such as shortening of the villi and an increase in crypt depth (Xiao et al., 2015), and these issues normally happens in piglets after weaning and lead to the reduce of feed intake and increase risk of disease. Previous studies reported that the antibacterial action of AMPs provides an effective support for normal intestinal morphology and gut microflora of animal (Table 8).

Table 8. Effects of antimicrobial peptides on intestinal morphology and gut microflora in weaning pigs.

Composition of AMPs	Dose (g/kg feed)	Intestinal morphology		Gut microflora	References
		Villus height	Crypt depth		
Lactoferrin	1	*	*	-	Wang et al., 2014
Lactoferrin	1	*	*	Reduced the total viable counts of E. coli and Salmonella in the small intestine Increased the colonic Lactobacillus and Bifidobacterium	Wang et al., 2007
AMP-P5	0.04	-	-	Decreased the total anaerobic bacteria, coliforms	Yoon et al., 2013
	0.06				
AMP-A3	0.06	*	*	Decreased the total anaerobic bacteria, coliforms and Clostridium spp.	Yoon et al., 2012
	0.09				
Refined potato protein	0.2	-	-	Decreased total bacteria, Coliform bacteria, Staphylococcus spp.	Jin et al., 2009
	0.4				
	0.6				
lactoferricin–lactoferrampin	0.1	*	Ns	Decreased the concentration of E.coli Increased the concentration of Lactobacilli and Bafidobacteria	Tang et al., 2008
Niacin	0.04	*	-	-	Feng et al., 2021
WK3	0.05	*	*	Decreased the Enterobacterium spp. concentration Increased the concentration of Lactobacillus spp. and Bifidobacteria spp.	Cao et al., 2021
WK3	2 mg/kg BW	*	Ns	Decreased the Enterocossus spp. concentration	Zhang et al., 2021
Buforin II	5 mL	*	Ns	-	Tang et al., 2013
M. domestica and porcine defensin	0.4	-	-	Decreased the E.coli concentration Increased the concentration of Lactobacillus spp. and Bifidobacteria spp.	Shi et al., 2018
Cathelicidin-BF	0.6 mg/kg BW	*	Ns	-	Feng et al., 2020

Note: AMPs, Antimicrobial peptides; BW, body weight; Ns, no significant; -, no measurement.

Antimicrobial peptides usage in weaning pigs to enhance the immune

Antimicrobial peptides are gene-encoded natural antibiotics with potent and broad antimicrobial capabilities that function as a first line of defense in the innate immunity of the host (Ganz, 2002; Lehrer and Ganz, 2002). The action of cationic AMPs is not limited to their effect on microorganism. Antimicrobial peptides may serve as a bridge between the innate and adaptive immune systems (Gudmundsson and Agerberth, 1999; Hancock and Diamond, 2000; Hancock, 2001). More detailed studies are performed in weaning pigs in Table 9.

Table 9. Effects of antimicrobial peptides on immune response in weaning pigs.

Composition of AMPs	Dose (g/kg feed)	Immune response	References
Lactoferricin–lactoferrampin	0.1	Increased serum IgA, IgG and IgM levels	Tang et al., 2008
AMP-A3	0.06	Had no effect on serum immunoglobulins (IgG, IgA and IgM) concentration	Yoon et al., 2012
	0.09		
Lactoferrin		Increased in serum IgG, IgA, (d 15 and 30) and IgM (d 15) concentration	Shan et al., 2007
Cecropin A and Cecropin D		Increased levels of secretory IgA in jejunum and serum IgA, IgG, interleukin-1β and interleukin-6	Wu et al., 2012
WK3	0.05	Inhibited intestinal inflammation by down regulating the mRNA expression of IL-1α and TLR-4 in the jejunal mucosa	Cao et al., 2021
Cathelicidin-BF	0.6 mg/kg BW	Decreases the expression of IL-6, IL-8 and IL-22	Yi et al., 2015
APB	0.25	Enhanced the proliferation of T blood T cell subsets (the percentages of CD3+, CD3+CD4+, and CD3+CD8+	Ren et al., 2015
	0.5	Decreased the percentages of apoptotic spleen cells significantly	
	1		
WK3	2 mg/kg BW	Downregulated the mRNA expression of IL-1α, TLR-4 and MyD88 in the jejunal mucosa	Zhang et al., 2021
APB	0.25; 0.5;	Increased on the immunoglobulin levels (IgG, IgM, and IgA)	Yuan et al., 2015
	1		
Buforin II	5 mL	Increased goblet cell amount, and the expression	Tang et al., 2013
		level of HGF, Reg-3γ, TGF-β1 and TFF-3 in the jejunum and the ileum	
Cathelicidin-BF	0.6 mg/kg BW	Increased in serum IgG, IgA concentration and the expression levels of ZO-1, Occludin and Claudin-1 in the jejunum and colon.	Feng et al., 2020
		Decreased serum TNF-a, IL-6, IL-8, IL-10 and TGF-β production	

Note: AMPs, Antimicrobial peptides; BW, body weight.

Conclusions

From the above studies it is understood that, antimicrobial peptides are an important component of the first line defence in wide range of organisms, from insects to plants to bacteria, animals as well as in humans in order to against invading pathogens thanks to the widespread distribution of potent, broad spectrum antimicrobial peptides in multicellular organisms that have been suggested to use to resist a wide range of microbes, including bacteria, fungi, viruses, and protozoa. However, the effect of antimicrobial peptides in practice is not always consistent due to the wide variety of available antimicrobial peptides, the type composition, dosage, animal species, animal age, and health status of animals. The available documents to date in feeding such compounds to weaning pigs seem to justify the assumption that antimicrobial peptides have the potential to prevent the growth of pathogens, improve the intestinal mucosal function, the immune system, digestion, and absorption capacity, as well as useful intestinal flora, and decrease animal diarrhea rate, keep the weanling pig health, and eventually improve the growth performance, and potential alternatives to replace antibiotics in the diets of animals.

List of abbreviations

ADG: Average daily gain
ADFI: Average daily feed intake
AMPs: Antimicrobial peptides
B: Bacteria
BW: body weight
F: Fungus
FI: Feed intake
G-: Gram negative
G+: Gram positive
GE: Gross energy
G:F: Gain to feed ratio
H: Hemolytic
Ns: no significance
V: Viruses
Y: Yeast

REFERENCES

1. Bera, A., Singh, S., Nagaraj, R. and Vaidya, T. 2003. Induction of autophagic cell death in Leishmania donovani by antimicrobial peptides. Mol. Biochem. Prasitol. 127, 23-35.
2. Boman, H.G., Agerberth, B. and Boman, A., 1993. Mechanisms of action on Escherichia coli of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. Infect. Immun. 61, 2978-2984.
3. Boparai, J.K. and Sharma, P.K., 2020. Mini review on antimicrobial peptides, sources, mechanism and recent applications. Pept. Sci. Lett. 27, 4-16.
4. Brestoff, J.R. and Artis, D., 2013. Commensal bacteria at the interface of host metabolism and the immune system. Nat. Immunol. 14, 676-684.
5. Bulet, P. and Stocklin, R., 2005. Insect antimicrobial peptides: structures, properties and gene regulation. Pept. Sci. Lett. 12, 3-11.
6. Cao, J., de la Fuente-Nunez, C., Ou, R.W., Torres, M.D.T., Pande, S.G., Sinskey, A.J. and Lu, T.K., 2018. Yeast-based synthetic biology platform for antimicrobial peptide production. ACS synthetic biology. 7, 896-902.
7. Charp, P.A., Rice, W.G., Raynor, R.L., Reimund, E., Kinkade Jr, J.M., Ganz, T., Selsted, M.E., Lehrer, R.I. and Kuo, J.F., 1988. Inhibition of protein kinase C by defensins, antibiotic peptides from human neutrophils. Biochem. Pharmacol. 37, 951-956.
8. Conlon, J.M. and Mechkarska, M., 2014. Host-defense peptides with therapeutic potential from skin secretions of frogs from the family pipidae. Pharmaceuticals. 7, 58-77.
9. Cudic, M. and Otvos Jr, L., 2002. Intracellular targets of antibacterial peptides. Curr. Drug. Targets. 3, 101-106.
10. Desriac, F., Jégou, C., Brillet, B., Le Chevalier, P. and Fleury, Y., 2013. Antimicrobial peptides from fish. Utilization of Fish Waste. 106-141.
11. Falla, T.J., Karunaratne, D.N. and Hancock, R.E., 1996. Mode of action of the antimicrobial peptide indolicidin. J. Biol. Chem. 271, 19298-19303.
12. Friedrich, C.L., Rozek, A., Patrzykat, A. and Hancock, R.E., 2001. Structure and mechanism of action of an indolicidin peptide derivative with improved activity against gram-positive bacteria. J. Biol. Chem. 276, 24015-24022.
13. Ganz, T., 2002. Antimicrobial polypeptides in host defense of the respiratory tract. Eur. J. Clin. Invest. 109, 693-697.
14. Gudmundsson, G.H. and Agerberth, B., 1999. Neutrophil antibacterial peptides, multifunctional effector molecules in the mammalian immune system. J. Immunol. Methods. 232, 45-54.
15. Hancock, R.E. and Diamond, G., 2000. The role of cationic antimicrobial peptides in innate host defences. Trends Immunol. 8, 402-410.
16. Hancock, R.E., 2001. Cationic peptides: effectors in innate immunity and novel antimicrobials. Lancet. Infect. Dis. 1, 156-164.
17. Huang, H.W., 2000. Action of antimicrobial peptides: two-state model. Biochem. 39, 8347-8352.
18. Huan, Y., Kong, Q., Mou, H. and Yi, H., 2020. Antimicrobial peptides: classification, design, application and research progress in multiple fields. Front. Microbiol. 11, 2559.
19. Hultmark, D., Steiner, H., Rasmuson, T. and Boman, H.G., 1980. Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of Hyalophora cecropia. Eur. j. biochem. 106, 7-16.
20. Lai, Y. and Gallo, R.L., 2009. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends. Immunol. 30, 131-141.
21. Lalles, J.P., 2008. Nutrition and gut health of the young pig around weaning: what news. Archiva Zootechnica, 11, 5-15.
22. Le, T.N., Do, T.H., Nguyen, T.N., Tran, N.T., Enfors, S.O. and Truong, H., 2014. Expression and simple purification strategy for the generation of anti-microbial active Enterocin P from Enterococcus faecium expressed in Escherichia coli ER2566. Iran. J. Biotechnol. 12, 17-25.
23. Leeson, S., 2001. Nutrition of the chicken (No. 04; SF494, L4 2001).
24. Lehrer, R.I. and Ganz, T., 2002. Defensins of vertebrate animals. Curr. Opin. Immunol. 14, 96-102.
25. Lu, Y., Ma, Y., Wang, X., Liang, J., Zhang, C., Zhang, K., Lin, G. and Lai, R., 2008. The first antimicrobial peptide from sea amphibian. Mol. Immunol. 45, 678-681.
26. Makarova, O., Johnston, P., Rodriguez-Rojas, A., El Shazely, B., Morales, J.M. and Rolff, J., 2018. Genomics of experimental adaptation of Staphylococcus aureus to a natural combination of insect antimicrobial peptides. Sci. Rep. 8, 1-8.
27. Nguyen, D.H., Lee, K.Y., Mohammadigheisar, M. and Kim, I.H., 2018. Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers. Poult. Sci. 97, 4351-4358.
28. Nguyen, D.H., Seok, W.J. and Kim, I.H., 2020. Organic acids mixture as a dietary additive for pigs-a review. Animals, 10, 952.
29. Noonan, J., Williams, W.P. and Shan, X., 2017. Investigation of antimicrobial peptide genes associated with fungus and insect resistance in maize. Int. J. Mol. Sci. 18, 1938.
30. Patocka, J., Nepovimova, E., Klimova, B., Wu, Q. and Kuca, K., 2019. Antimicrobial peptides: Amphibian host defense peptides. Current medicinal chemistry, 26(32), pp.5924-5946.
31. Peschel, A. and Sahl, H.G., 2006. The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nat. Rev. Microbio., 4, 529-536.
32. Ramanathan, B., Davis, E.G., Ross, C.R. and Blecha, F., 2002. Cathelicidins: microbicidal activity, mechanisms of action, and roles in innate immunity. Microbes and infection, 4(3), pp.361-372.
33. Ravichandran, S., Kumaravel, K., Rameshkumar, G. and Ajithkumar, T.T., 2010. Antimicrobial peptides from the marine fishes. Res. J. Immunol. 3, 146-156.
34. Reddy, K.V.R., Yedery, R.D. and Aranha, C., 2004. Antimicrobial peptides: premises and promises. Int. J. Antimicrob. Agents. 24, 536-547.

35. Risso, A., Zanetti, M. and Gennaro, R., 1998. Cytotoxicity and apoptosis mediated by two peptides of innate immunity. Cell. Immunol. 189, 107-115.
36. Rollins-Smith, L.A., 2009. The role of amphibian antimicrobial peptides in protection of amphibians from pathogens linked to global amphibian declines. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1788, 1593-1599.
37. Schnapp, D., Reid, C.J. and Harris, A., 1998. Localization of expression of human beta defensin-1 in the pancreas and kidney. J. Path. 186, 99-103.
38. Shai, Y. and Oren, Z., 2001. From "carpet" mechanism to de-novo designed diastereomeric cell-selective antimicrobial peptides. Peptides, 22, 1629-1641.
39. Shi, J., Zhang, P., Xu, M.M., Fang, Z., Lin, Y., Che, L., Feng, B., Li, J., Li, G., Wu, D. and Xu, S., 2018. Effects of composite antimicrobial peptide on growth performance and health in weaned piglets. Anim. Sci. J. 89, 397-403.
40. Tam, J.P., Wang, S., Wong, K.H. and Tan, W.L., 2015. Antimicrobial peptides from plants. Pharmaceuticals, 8, 711-757.
41. Tang, Z., Yin, Y., Zhang, Y., Huang, R., Sun, Z., Li, T., Chu, W., Kong, X., Li, L., Geng, M. and Tu, Q., 2008. Effects of dietary supplementation with an expressed fusion peptide bovine lactoferricin–lactoferrampin on performance, immune function and intestinal mucosal morphology in piglets weaned at age 21 d. Br. J. Nutr. 101, 998-1005.
42. Tang, Z.R., Deng, H., Zhang, X.L., Zen, Y., Xiao, D.F., Sun, W.Z. and Zhang, Z., 2013. Effects of orally administering the antimicrobial peptide buforin II on small intestinal mucosal membrane integrity, the expression of tight junction proteins and protective factors in weaned piglets challenged by enterotoxigenic Escherichia coli. Anim. Feed Sci. Technol. 186, 177-185.
43. Ulvatne, H., Haukland, H.H., Olsvik, Ø. and Vorland, L.H., 2001. Lactoferricin B causes depolarization of the cytoplasmic membrane of Escherichia coli ATCC 25922 and fusion of negatively charged liposomes. FEBS letters. 492, 62-65.
44. Vilcinskas, A., 2013. Evolutionary plasticity of insect immunity. J. Insect. Phys. 59, 123-129.
45. Vorland, L.H., Ulvatne, H., Rekdal, Ø. and Svendsen, J.S., 1999. Initial binding sites of antimicrobial peptides in Staphylococcus aureus and Escherichia coli. Scand. J. Infect. Dis. 31, 467-473.
46. Wang, Y., Shan, T., Xu, Z., Liu, J. and Feng, J., 2006. Effect of lactoferrin on the growth performance, intestinal morphology, and expression of PR-39 and protegrin-1 genes in weaned piglets. J. Anim. Sci. 84, 2636-2641.
47. Wang, Y.Z., Shan, T.Z., Xu, Z.R., Feng, J. and Wang, Z.Q., 2007. Effects of the lactoferrin (LF) on the growth performance, intestinal microflora and morphology of weanling pigs. Anim. Feed Sci. Technol. 135, 263-272.
48. Wang, J.H., Wu, C.C. and Feng, J., 2011. Effect of dietary antibacterial peptide and zinc-methionine on performance and serum biochemical parameters in piglets. Czech. J. Anim. Sci. 56, 30-36.
49. Wang, S., A Thacker, P., Watford, M. and Qiao, S., 2015. Functions of antimicrobial peptides in gut homeostasis. Curr. Protein Pept. Sci. 16, 582-591.
50. Xiao, H., Shao, F., Wu, M., Ren, W., Xiong, X., Tan, B. and Yin, Y., 2015. The application of antimicrobial peptides as growth and health promoters for swine. J. Anim. Sci. Biotechnol. 6, 1-6.
51. Xiong, X., Yang, H.S., Li, L., Wang, Y.F., Huang, R.L., Li, F.N., Wang, S.P. and Qiu, W., 2014. Effects of antimicrobial peptides in nursery diets on growth performance of pigs reared on five different farms. Livest. Sci. 167, 206-210.
52. Yi, H., Yu, C., Zhang, H., Song, D., Jiang, D., Du, H. and Wang, Y., 2015. Cathelicidin-BF suppresses intestinal inflammation by inhibiting the nuclear factor-κB signaling pathway and enhancing the phagocytosis of immune cells via STAT-1 in weanling piglets. Int. Immunopharmacol. 28, 61-69.
53. Yin, Y., Yao, K., Liu, Z., Gong, M., Ruan, Z., Deng, D., Tan, B., Liu, Z. and Wu, G., 2010. Supplementing L-leucine to a low-protein diet increases tissue protein synthesis in weanling pigs. Amino acids, 39, 1477-1486.
54. Yoon, J.H., Ingale, S.L., Kim, J.S., Kim, K.H., Lee, S.H., Park, Y.K., Kwon, I.K. and Chae, B.J., 2012. Effects of dietary supplementation of antimicrobial peptide-A3 on growth performance, nutrient digestibility, intestinal and fecal microflora and intestinal morphology in weanling pigs. Anim. Feed Sci. Technol. 177, 98-107.
55. Yoon, J.H., Ingale, S.L., Kim, J.S., Kim, K.H., Lohakare, J., Park, Y.K., Park, J.C., Kwon, I.K. and Chae, B.J., 2013. Effects of dietary supplementation with antimicrobial peptide-P5 on growth performance, apparent total tract digestibility, faecal and intestinal microflora and intestinal morphology of weanling pigs. J. Sci. Food. Agric. 93, 587-592.
56. Yuan, W., Jin, H.T., Ren, Z.H., Deng, J.L., Zuo, Z.C., Wang, Y., Deng, H.D. and Deng, Y.T., 2015. Effects of antibacterial peptide on humoral immunity in weaned piglets. Food. Agric. Immunol, 26, 682-689.
57. Zhang, L., Guo, T., Zhan, N., Sun, T. and Shan, A., 2021. The Effects of the antimicrobial peptide WK3 on diarrhea, growth performance and intestinal health of weaned piglets challenged with enterotoxigenic Escherichia coli K88. Food. Nutr. Res.