Antimicrobial peptides as novel alternatives to antibiotics

Xueqin Zhao1,2, Hanna Fotina1, Lei Wang2, Jianhe Hu2

1Sumy National Agrarian University, Sumy, Ukraine
2College of Animal Science and Veterinary Medicine, Henan Institute of Science and Technology, Xinxiang, China

Zhao, X., Fotina, H., Wang, L., & Hu, J. (2020). Antimicrobial peptides as novel alternatives to antibiotics. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Veterinary sciences, 22(98), 74–78. doi: 10.32718/nvlvet9813

Introduction

Since the discovery of antibiotics, people have opened the golden age of antibiotic. At present, the World Health Organization has stated that antibiotics are harmful to human and animal health, and animal husbandry has confirmed that the unreasonable use of antibiotics are closely related to antibiotic resistance (Diana et al., 2019). Swine industry used a large number of unreasonable antibiotics to improve swine production performance and immune functions. Up to now, the swine industry has used antibiotics for more than 50 years, increasing bacterial resistance and decreasing quality of meat products. It has brought huge economic losses to swine industry (Xiao et al., 2015; Pérez-Duran et al., 2020).

Antimicrobial peptides come from a wide range of sources, from prokaryotes to humans. Antimicrobial peptides are considered to be ancient weapons against microbial infections. They are an important component of the innate immune system and play an important role in innate immunity. Antimicrobial peptides have become candidates for the design of new antimicrobial drugs due to their good antibacterial activity and not drug resistance.

This review focuses on the mechanism of action of antimicrobial peptides, especially improve performance, improve intestinal inflammation and nutrient digestibility, regulate the intestinal microbiota and enhance the immune function of swine. Overall, AMPs have great potential for application as an alternative to antibiotics in swine industry.

Key words: antimicrobial peptide, applications, swine.
destroying the cell membranes of bacterial, inhibiting DAN replication of bacterial and bacterial respiration and so on. It indicates that antimicrobial peptides have multiple mechanisms of action. Therefore, antimicrobial peptides are not susceptible to drug resistance (Cederlund et al., 2011; Xia et al., 2018). Compared with antibiotics, antimicrobial peptides have many advantages and become a research hotspot in recent years.

The mechanism of action of antimicrobial peptide. Due to the special properties of AMPs, such as amino acid composition variability, net charge, spatial conformation and structure, the mobility of amphiphilic modules and membranes is considered to have a special mechanism of action, and the antibacterial mechanism of several antimicrobial peptides is studied. The most scholars mainly agree that there are two kinds of mechanism of antimicrobial peptide, one is that the antimicrobial peptide destroy the cell membrane of the bacteria, forms a cavity, and causes the contents of the bacteria to leak out, leading to bacterial death. The other one is that antimicrobial peptide breaks the membrane after that enter into the bacteria body and combines with other substances in the cell, causing the bacteria die.

The membrane sterilization mechanism of antimicrobial peptide. At first, antimicrobial peptide aggregates on the surface of the bacterial cell membrane and inserted into the bilayer of the bacterial cell membrane phospholipid in a multimeric manner to form a channel. The external water molecules and ions use the channel to enter the interior of the bacteria, eventually leading to bacterial death (Jenssen et al., 2006). It can form a transmembrane potential after the antimicrobial peptide interacts with the bacterial cell membrane, thereby destroying the acid-base balance of the cell membrane, affecting the osmotic pressure of the bacteria, and inhibiting the bacterial respiration (Pandey et al., 2011). Su Jin Ko et al., 2017 used the circular dichroism to determine the structure of the antimicrobial peptide Macropin in the membrane simulation environment, and found that the antimicrobial peptide Macropin combined with LPS by destroying the bacterial cell membrane, resulting in bacterial death. Domenyuk V. et al., 2013 found that membrane labeled of antimicrobial peptides could against Staphylococcus aureus (UAB637) infection which mainly by destroying the bacterial cell membrane. It can be seen that the antimicrobial peptide exert bactericidal action by destroying the bacterial cell membrane.

Intracellular bactericidal mechanism of antibacterial peptides. In addition to destroying the cell membrane to produce bactericidal action, antimicrobial peptide can also enter the bacteria body and combine with other substances, and produce better bactericidal effect at a lower concentration (Wang et al., 2016). Dev K. Ranjit et al., 2010 used the hexamer peptide isolated from the laboratory to study its bactericidal mechanism and found that the antimicrobial peptide WRWYCR caused bacterial death by inhibiting bacterial DNA protein repair. Antibacterial peptides cause bacterial death by inhibiting the respiration of mycobacterium tuberculosis, depolarizing membranes and releasing bacterial ATP.

Scientific Messenger LNUVMB. Series: Veterinary sciences, 2020, vol. 22, no 98

75
Peptides have huge application potential in the swine industry. There are many scientific studies that proved the important role of antimicrobial peptides in increasing nutrient digestibility, improving swine intestinal morphology and improving health indicators, and significantly increase serum IgM, total serum complement (CH50) in the blood are mediated immune function (Levy, 2000). Weaned piglets were fed antimicrobial peptides with different concentrations (250 mg/kg; 500 mg/kg; 1000 mg/kg) for 28 days, and blood samples were collected at 32, 39, 46, and 53 days of age for testing. The levels of IgG, IgM, IgA, classical swine fever virus antibody (CSF-Ab) and total serum complement (CH50) in the blood are increasing with dose-dependent (Yuan et al., 2015). Adding antimicrobial peptides (10 mg/kg) to the diet of weaned piglets can reduce the diarrhea rate, and significantly increase IgG levels and peroxide dismutase activity, and reduce the total cholesterol concentration (Wang et al., 2011). Adding antimicrobial peptide HDP to the diet of weaned piglets can improve the digestibility of nutrients, intestinal morphology and growth performance of weaned piglets, and increase the concentration of serum IgA, IgG or IgM. PFM105 isolated from the rectum of healthy sows can improve growth performance of weaned piglets, reduce diarrhea rate, increase intestinal health indicators, and significantly increase serum IgM, IL-10, TGF-β and SCFAs levels in the colon (Wang et al., 2019). Porcine defensins are mixed with antimicrobial peptides derived from flies to the diet of weaned piglets, and found that the mixed antimicrobial peptides can increase the T cell population and significantly enhance the proliferation of T cells. It indicates that addition of antimicrobial peptides to the grain significantly enhances the immune function of swine (Ren et al., 2015). Artificially synthesized lactoferrin can enhance the proliferation of peripheral blood and splenic lymphocytes and increase the levels of IgG, IgA, IgM and IL-2 in the serum of weaned piglets (Shan et al., 2007). The piglets treatment with 100 mg/kg antimicrobial peptide cipB-LFC-LFA after enterotoxigenic E. coli infection can significantly increase the levels of IgA, IgG and IgM in serum (Tang et al., 2009). The above results indicate that antimicrobial peptides have important functions in enhancing the immune function of swine.

Conclusion

Antimicrobial peptides are expected to become one of the best alternatives to antibiotics with its unique antibacterial mechanism and immunomodulatory function. It has been proved that many antimicrobial peptides have huge application potential in the swine industry. Antimicrobial peptides provide the swine industry with functions such as improved growth performance and nutrient digestibility, improved intestinal health, regulation of intestinal microbiota and immune regulation. Antimicrobial peptides improve swine growth performance and are closely related to antibacterial and immunomodulatory functions. However, the studies on antimicrobial peptides exerting immunomodulatory functions and metabolism in vivo are relatively weak, and further studies are needed. With the continuous development of science and technology, the study on antimicrobial peptides will be more and more in-depth. Eventually, antimicrobial peptides become growth promoters and therapeutic agents of the swine industry by changing the effector molecules of the innate immune system medium immune function (Levy, 2000). Weaned piglets were fed antimicrobial peptides with different concentrations (250 mg/kg; 500 mg/kg; 1000 mg/kg) for 28 days, and blood samples were collected at 32, 39, 46, and 53 days of age for testing. The levels of IgG, IgM, IgA, classical swine fever virus antibody (CSF-Ab) and total serum complement (CH50) in the blood are increasing with dose-dependent (Yuan et al., 2015). Adding antimicrobial peptides (10 mg/kg) to the diet of weaned piglets can reduce the diarrhea rate, and significantly increase IgG levels and peroxide dismutase activity, and reduce the total cholesterol concentration (Wang et al., 2011). Adding antimicrobial peptide HDP to the diet of weaned piglets can improve the digestibility of nutrients, intestinal morphology and growth performance of weaned piglets, and increase the concentration of serum IgA, IgG or IgM. PFM105 isolated from the rectum of healthy sows can improve growth performance of weaned piglets, reduce diarrhea rate, increase intestinal health indicators, and significantly increase serum IgM, IL-10, TGF-β and SCFAs levels in the colon (Wang et al., 2019). Porcine defensins are mixed with antimicrobial peptides derived from flies to the diet of weaned piglets, and found that the mixed antimicrobial peptides can increase the T cell population and significantly enhance the proliferation of T cells. It indicates that addition of antimicrobial peptides to the grain significantly enhances the immune function of swine (Ren et al., 2015). Artificially synthesized lactoferrin can enhance the proliferation of peripheral blood and splenic lymphocytes and increase the levels of IgG, IgA, IgM and IL-2 in the serum of weaned piglets (Shan et al., 2007). The piglets treatment with 100 mg/kg antimicrobial peptide cipB-LFC-LFA after enterotoxigenic E. coli infection can significantly increase the levels of IgA, IgG and IgM in serum (Tang et al., 2009). The above results indicate that antimicrobial peptides have important functions in enhancing the immune function of swine.
virtue of their antibacterial and immunomodulatory effects.

**Author’s contributions**

All authors participated in this review design. Xueqin Zhao participated and performed writing and data collection. All authors read and approved the final manuscript. All authors contributed to the draft of the manuscript. All authors gave final approval for publication.

**Acknowledgements**

This work was supported by the National Natural Science Foundation of China (No. 31702259 and 31520103917), the Key Specialized Research and Development Breakthrough Program of Henan Province (182102110347). The authors would like to thank Hanna Fotina for language modification.

**Conflict of interest**

Author does not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

**References**

Burkey, T. E., Skjolaas, K. A., & Minton, J. E. (2009). Board-invited review: porcine mucosal immunity of the gastro intestinal tract. J Anim Sci., 87(4), 1493–1501. doi: 10.2527/jas.2008-1330.

Cederlund, A., Gudmundsson, G. H., & Agerberth, B. (2011). Antimicrobial peptides important in innate immunity, FEBS J., 278(20), 3942–3951. doi: 10.1111/j.1742-4658.2011.08302.x.

Choi, S. C., Ingle, S. L., Kim, J. S., Park, Y. K., Kwon, I. K., & Chae, B. J. (2013). An antimicrobial peptide-A3: effects on growth performance, nutrient retention, intestinal and faecal microflora and intestinal morphology of broilers. Br Poult Sci., 54(6), 738–746. doi: 10.1080/00071668.2013.838746.

Cutler, S. A., Lonergan, S. M., Cornick, N., Johnson, A. K., & Stahl, C. H. (2007). Dietary inclusion of colicin e1 is effective in preventing postweaning diarrhea caused by F18-positive Escherichia coli in pigs. Antimicrob Agents Chemother, 51(11), 3830–3835. doi: 10.1128/AAC.00360-07.

Diana, A., Boyle, L. A., Leonard, F. C., Carroll, C., Sheehan, E., Murphy, D., & Manzanilla, E. G. (2019). Removing prophylactic antibiotics from pig feed: does it affect their performance and health? BMC Vet Res., 15(1), 67. doi: 10.1186/s12917-019-1808-x.

Domenyuk, V., Loskovut, A., Johnston, S. A., & Diehnelt, C. W. (2013). A technology for developing synbodies with antibacterial activity. PLoS One, 8(1), e54162. doi: 10.1371%2Fjournal.pone.0054162.

Forkus, B., Ritter, S., Vlysidis, M., Geldart, K., & Kaznessis, Y. N. (2017). Antimicrobial Probiotics Reduce Salmonella enterica in Turkey Gastrointestinal Tracts. Sci Rep, 7, 40695. doi: 10.1038/srep40695.

Ramamorthy, G., Park, J., Seo, C., Vogel, H. J., & Park, Y. (2020). Antifungal and Antibiofilm Activities and the Mechanism of Action of Repeating Lysine-Tryptophan Peptides against. Microorganisms, 8, 758. doi: 10.3390/microorganisms8050758.

Hernandez-Gordillo, V., Geisler, I., & Chmielewski, J. (2014). Dimeric unnatural polyproline-rich peptides with enhanced antibacterial activity. Bioorg Med Chem Lett, 24(2), 556–559. doi: 10.1016/j.bmcl.2013.12.023.

Hurdle, J. G., O’Neill, A. J., Chopra, I., & Lee, R. E. (2011). Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. Nat Rev Microbiol, 9(1), 62–75. doi: 10.1038/nrmicro2474.

Choi, S. C., Ingle, S. L., Kim, J. S., Park, Y. K., Kwon, I. K., & Chae, B. J. (2013). Effects of dietary supplementation with an antimicrobial peptide-P5 on growth performance, nutrient retention, excreta and intestinal morphology of broilers. Animal Feedence & Technology, 185(1-2), 78–84. doi: 10.1016/j.anifeedsci.2013.07.005.

Jenssen, H., Hamill, P., & Hancock, R. E. (2006). Peptide antimicrobial agents. Clin Microbiol Rev, 19(3), 491–511. doi: 10.1128/cmrr.00056-05.

Jin, Z., Yang, Y. X., Choi, J. Y., Shinde, P. L., Yoon, S. Y., Hahn, T. W., Lim, H. T., Park, Y., Hahn, K. S., Joo, J. W., & Chae, B. J. (2008). Potato (Solanum tuberosum L. cv. Gogu valley) protein as a novel antimicrobial agent in weaning pigs. J Anim Sci., 86(7), 1562–1572. doi: 10.2527/jas.2007-0414.

Jindal, H. M., Zandi, K., Ong, K. C., Velayuthan, R. D., Rasid, S. M., Samudi, R. C., & Sekaran, S. D. (2017). Mechanisms of action and in vivo antibacterial efficacy assessment of five novel hybrid peptides derived from Indolicidin and Ranalexin against Streptococcus pneumoniae. PeerJ, 5, e3887. doi: 10.7717/peerj.3887.

Ko, S. J., Kim, M. K., Bang, J. K., Seo, C. H., Luchian, T., & Park, Y. (2017). Macropis fulvipes Venom component Macropin Exerts its Antibacterial and Anti-Biofilm Properties by Damaging the Plasma Membranes of Drug Resistant Bacteria. Sci Rep., 7(1), 16580. doi: 10.1038/s41598-017-16784-6.

Levy, O. (2000). Antimicrobial proteins and peptides of blood: templates for novel antimicrobial agents. Blood, 96(8), 2664. doi: 10.1182/blood.V96.8.2664.

Pérez-Duran, F., Acosta-Torres, L. S., Serrano-Díaz, P. N., Toscano-Torres, I. A., Olivo-Zepeda, I. B., García-Caxín, E., & Nuñez-Anita, R. E. (2020). Toxicity and anti-infective effect of silver nanoparticles in swine sperms. Systems biology in reproductive medicine, 1–9. doi: 10.1080/19396368.2020.1754962.

Pandey, B. K., Srivastava, S., Singh, M., & Ghosh, J. K. (2011). Inducing toxicity by introducing a leucine zipper-like motif in frog antimicrobial peptide, magainin 2. Biochem J., 436(3), 609–620. doi: 10.1042/bj20110056.

Ranjit, D. K., Rideout, M. C., Nefzi, A., Ostresh, J. M., Pinilla, C., & Segall, A. M. (2010). Small molecule
functional analogs of peptides that inhibit lambda site-specific recombination and bind Holliday junctions. Bioorg Med Chem Lett, 20(15), 4531–4534. doi: 10.1016/j.bmcl.2010.06.029.

Ren, Z. H., Yuan, W., Deng, H. D., Deng, J. L., Dan, Q. X., Jin, H. T., Tian, C. L., Peng, X., Liang, Z., Gao, S., Xu, S. H., Li, G., & Hu, Y. (2015). Effects of antibacterial peptide on cellular immunity in weaned piglets. J Anim Sci., 93(1), 127–134. doi: 10.2527/jas.2014-7933.

Shan, T., Wang, Y., Wang, Y., Liu, J., & Xu, Z. (2007). Effect of dietary lactoferrin on the immune functions and serum iron level of weaned piglets. J Anim Sci., 85(9), 2140–2146. doi: 10.2527/jas.2006-754.

Shi, J., Zhang, P., Xu, M. M., Fang, Z., Lin, Y., Che, L., Feng, B., Li, J., Li, G., Wu, D., & Xu, S. (2018). Effects of composite antimicrobial peptide on growth performance and health in weaned piglets. Anim Sci J., 89(2), 397–403. doi: 10.1111/asj.12933.

Tang, Z., Yin, Y., Zhang, Y., Huang, R., Sun, Z., Li, T., Chu, W., Kong, X., Li, L., Geng, M., & Tu, Q. (2009). Effects of dietary supplementation with an expressed fusion peptide bovine lactoferricin-lactoferrapin on performance, immune function and intestinal mucosal morphology in piglets weaned at age 21 d. Br J Nutr. 101(7), 998–1005. doi: 10.1017/s0007114508055633.

Veldhuizen, E. J., Schneider, V. A., Agustiandari, H., van Tang, Z., Yin, Y., Zhang, Y., Huang, R., Sun, Z., Li, T., Xin, J., & Hu, J. (2019). Antimicrobial and immunomodulatory activities of PR-39 derived peptides. PLoS One, 9(4), e95939. doi: 10.1371/journal.pone.0095939.

Wang, J. H., Wu, C. C., & Feng, J. (2011). Effect of dietary antibacterial peptide and zinc-methionine on performance and serum biochemical parameters in weanling piglets. Czech Journal of Animal Science, 56(1), 30–36. doi: 10.17221/341/2009-CJAS.

Wang, L., Zhao, X., Xia, X., Zhu, C., Zhang, H., Qin, W., Xu, Y., Hang, B., Sun, Y., Chen, S., Jiang, J., Zhang, G., & Hu, J. (2019). Inhibitory Effects of Antimicrobial Peptide JH-3 on Salmonella enterica Serovar Typhimurium Strain CVCC541 Infection-Induced Inflammatory Cytokine Release and Apoptosis in RAW264.7 Cells. Molecules, 24(3), 596. doi: 10.3390/molecules24030596.

Wang, S., Zeng, X., Yang, Q., & Qiao, S. (2016). Antimicrobial Peptides as Potential Alternatives to Antibiotics in Food Animal Industry. International Journal of Molecular Sciences, 17(5), 603. doi: 10.3390/ijms17050603.

Wang, T., Teng, K., Liu, Y., Shi, W., Zhang, J., Dong, E., Zhang, X., Tao, Y., & Zhong, J. (2019). Lactobacillus plantarum PFM 105 Promotes Intestinal Development Through Modulation of Gut Microbiota in Weaning Piglets. Front Microbiol, 10, 90. doi: 10.3389/fmicb.2019.00900.

Wang, Y. Z., Shan, T. Z., Xu, Z. R., Feng, J., & Wang, Z. Q. (2007). Effects of the lactoferrin (LF) on the growth performance, intestinal microflora and morphology of weaning pigs. Animal Feed Science & Technology, 135(3-4), 263–272. https://www.sciencedirect.com/science/article/pii/S0378401060030262?via%3Dihub.

Wu, S., Zhang, F., Huang, Z., Liu, H., Xie, C., Zhang, J., Thacker, P. A., & Qiao, S. (2012). Effects of the antimicrobial peptide cecropin AD on performance and intestinal health in weaned piglets challenged with Escherichia coli. Peptides. 35(2), 225–230. doi: 10.1016/j.peptides.2012.03.030.

Xia, X., Cheng, L., Zhang, S., Wang, L., & Hu, J. (2018). The role of natural antimicrobial peptides during infection and chronic inflammation. Anontie Van Leeuwenhoek, 111(1), 5–26. doi: 10.1007/s10482-017-0929-0.

Xiao, H., Shao, F., Wu, M., Ren, W., Xiong, X., Tan, B., & Yin, Y. (2015). The application of antimicrobial peptides as growth and health promoters for swine. J Anim Sci Biotechnol, 6(1), 19. doi: 10.1186/s40104-015-0018-z.

Yu, H., Zhang, L., Gan, Z., Xiong, H., Yu, C., Du, H., & Wang, Y. (2016). High therapeutic efficacy of Cathelicidin-WA against postweaning diarrhea via inhibiting inflammation and enhancing epithelial barrier in the intestine. Sci Rep., 6(1), 25679. doi: 10.1038/srep25679.

Yoon, J. H., Ingele, S. L., Kim, J. S., Kim, K. H., Lee, S. H., Park, Y. K., Lee, S. C., Kwon, I. K., & Chae, B. J. (2013). Effects of dietary supplementation of synthetic antimicrobial peptide-A3 and P5 on growth performance, apparent total tract digestibility of nutrients, fecal and intestinal microflora and intestinal morphology in weaning pigs. Journal of the Science of Food & Agriculture, 93(3), 587–592. doi: 10.1016/j.jfsa.2013.10.025.

Yu, H. T., Ding, X. L., Li, N., Zhang, X. Y., Zeng, X. F., Wang, S., Liu, H. B., Wang, Y. M., Jia, H. M., & Qiao, S. Y. (2016). Dietary supplemented antimicrobial peptide microcin J25 improves the growth performance, apparent total tract digestibility, fecal microbiota, and intestinal barrier function of weaned pigs. J Anim Sci., 95(11), 5064–5076. doi: 10.2527/jas2017.1494.

Yuan, W., Jin, H. T., Ren, Z. H., Deng, J. L., Zuo, Z. C., Wang, Y., Deng, H. D., & Deng, Y. T. (2015) Effects of antibacterial peptide on humoral immunity in weaned piglets. Food and Agricultural Immunology, 93(1), 127–134. doi: 10.1080/09540105.2015.1007448.

Zhang, C., & Yang, M. (2020). The Role and Potential Application of Antimicrobial Peptides in Autoimmune Diseases. Frontiers in immunology, 11, 859. doi: 10.3389/fimmu.2020.00859.

Zhai, Z., Ni, X., Jin, C., Ren, W., Li, J., Deng, J., Deng, B., & Yin, Y. (2018). Cecropin A Modulates Tight Junction-Related Protein Expression and Enhances the Barrier Function of Porcine Intestinal Epithelial Cells by Suppressing the MEK/ERK Pathway. Int J Mol Sci, 19(7), 1941. doi: 10.3390/ijms19071941.