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Antimicrobial Peptides Characteristics and their Application in Domestic Animals

Lu Lu, Xiu Huang, Long Zhang, Dan Lan, Di-yan Li, Yi-Ping Liu and Yao-Dong Hu
Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province,
Sichuan Agricultural University, Xinkang Road 46, 625014 Ya'an, P.R. China

Abstract: Antimicrobial Peptides (AMPs) are principal defense system for most organisms. Multiple AMPs have been characterized as widespread conserved component and showing a wide spectrum of effectiveness to the various microorganisms. Despite of the antibacterial activity, AMPs have also been found can serving as other immune effects including modulation of inflammatory responses, promotion of wound healing and chemotactic activity. AMP drugs have been actively developed and lightly applied in animal production. In this review, we will brief the activities and functional mechanisms of AMPs as well as a warning of the potential resistance to AMPs used directly. Moreover, we will focus on a potential application strategy, by regulating the expression of AMPs which may delay or avoid the resistance to AMPs. Finally, the status and obstacles of AMPs used in domestic animals will be described.

Key words: Antimicrobial peptides, application, domestic animals, regulation, AMPs

INTRODUCTION

Animal proteins are indispensable for human beings. The growing demand for animal proteins has given rise to the modern farming characterized by high density and large scale which increased the outbreak of disease. Antibiotics have been used in animal husbandry for disease preventing and growth promoting purposes. However, the problem of resistance is increasingly serious accompanied by the overuse of antibiotics (Neu, 1992; French, 2010). Antimicrobial Peptides (AMPs) comprised a large group of gene-encoded molecules have been proved wide existence ranging from prokaryotes to humans which prevent from infection with multitudinous microorganisms, playing a critical role in host defenseo (Brogden, 2005). Due to their broad spectrum of antimicrobial activity and the growing problem of resistance to conventional antibiotics, AMPs aroused great interest in developing new therapeutic for infections caused by multidrug-resistant bacteria and were recognized the ideal alternative for antibiotics.

MATERIALS AND METHODS

Activities and mechanisms of the action of AMPs: AMPs are appreciated of their broad-spectrum antimicrobial activity. The structure-function models of the Shai-Matsuzaki-Huang (SMH) provide a reasonable explanation for the antimicrobial activity for multiple bacteria through membrane permeation (Zasloff, 2002; Brogden, 2005). The antimicrobial activity of AMPs is closely related to their amphipathic fold, allowing the interaction with microorganisms (Yeaman and Yount, 2003). AMPs were attracted by electrostatic bonding between anionic or cationic peptides. After binding to the plasma membranes of cells, each antimicrobial peptide has two distinct physical states of binding to lipid bilayer (Huang, 2000). It is apparently reasonable that membrane permeation is a process of quantitative change to quality.

The susceptibility of a cell to an antimicrobial peptide depends on the value of P/L (Peptide/Lipid ratios) that is determined by the lipid composition of the cell and the transition from the safe state to the illegal state is specific to the peptide and is sigmoid related to the peptide concentration (Chen *et al*., 2003). Moreover, many recent studies suggested that antimicrobial peptides not only interact with biomembranes of specific composition and asymmetry but may also have effect on the properties within target cells. When antimicrobial peptide break through the cytoplasm, they lead to microbial killing indirectly in different ways such as altering cytoplasmic membrane, inhibiting cell-wall synthesis, binding nucleic acids, inhibiting nucleic-acid synthesis, protein synthesis and enzymatic activity (Brogden, 2005). Some AMPs adopt more than one strategy in killing or lysis for instance, melittin (Naito *et al*., 2000; Yang *et al*., 2001) causes "Toroidal" or "Carpet" membrane pore while pleurocidin (Patrzykat *et al*., 2001), dermaseptin (Patrzykat *et al*., 2001) and PR-39 (Boman *et al*., 1993) mediating a intracellular effect by inhibiting nucleic-acid synthesis or protein synthesis.

Corresponding Author: Yao-Dong Hu, Institute of Animal Genetics and Breeding, Sichuan Agricultural University, Chengdu Campus. Apt 211# Huimin Road, Wenjiang 611130 Sichuan, P.R. China

Despite the antimicrobial activity of AMP, AMPs can function as immune regulators, involved in the binding and neutralization of Lipopolysaccharide (LPS), chemotaxis of immune cells, the regulation of dendritic cell differentiation, induction of the angiogenesis and reepithelialization and modulation of cytokine and chemokine expression (Lai and Gallo, 2009; Semple and Dorin, 2011). The functions of these AMPs reach beyond mere inactivation of microorganisms and appear to play a role in connecting the innate and adaptive immune responses. Owing to their immunomodulatory effects, AMPs are being investigated as potential immunotherapeutic agents (Hamill *et al*., 2008). Innate Defense Regulators (IDRs) are synthetic peptides based on natural AMPs, for instance, IDR-1 can mediate protection against bacterial challenge without direct antimicrobial activity (Scott *et al*., 2007). Despite as anti-infective therapeutics, synthetic immunomodulatory IDR peptides have the potential to tailor the design for different purposes, such as enhance chemokine production and leukocyte recruitment or suppress excessive inflammation (Easton *et al*., 2009).

Several models has been proposed to clarify the antimicrobial mechanisms of AMPs, still there is an urgent to study AMPs more detailed, according to their structure, biological source and functions and the strategy of application.

Resistance to AMPs: Host immune system uses a variety of factors to destroy bacteria, including bacteriolytic enzymes and AMPs that disrupt the bacterial cytoplasmic membrane (Hancock and Chapple, 1999). Conversely, bacterial pathogens elaborate varieties of factors to colonize the hosts such as the display of adhesions on the bacterial surface to better attach to specific host cells, the secretion of tissue damage toxins and the possession of mechanisms that render them resistant to AMPs (Peschel, 2002). For instance, *Staphylococcus aureus* (Ernst *et al*., 2001; Jin *et al*., 2004), *Escherichia coli* (Winfield and Groisman, 2004) and the *Streptococcus pyogenes* (Frick *et al*., 2003) have evolved varieties of countermeasures to resist specific AMPs. The underlying mechanisms were well reviewed, including the repulsion of AMPs by decreasing the negative charge of the cell wall and bacterial membrane through covalent modification of the molecules (e.g., peptidoglycan, teichoic acids, phospholipids and lipid A); expelling AMPs through energy-dependent pumps; and inactivating AMPs by proteases and peptidases ; still *Shigella* species resist LL-37 and hBD1 relying on by inhibiting the production of these peptides (Peschel, 2002; Ganz, 2003).

Several genes have been proved conferring resistance to AMPs in response to environmental conditions. Disruption of the two-component transcriptional regulator PhoP/PhoQ increases the sensitivity of *S. typhimurium* to some AMPs (Murata *et al*., 2007). PhoP/PhoQ directly regulates the expression of many genes involved in the resistance to cationic peptides and also modulates a second two-component regulator PmrA/PmrB which is necessary for aminoarabinose transfer into lipid A (Gunn *et al*., 1998). The function of the downstream genes includes the covalent modification of LPS that decreases their affinity for AMPs and the expression of proteases that degrade cationic peptides (Guo *et al*., 1998; Guina *et al*., 2000). There is evidence that Dlt increased the sensitivity of the bacteria to defensins. The gene *Dlt* is required for the covalent modification of cell wall teichoic acid by alanine (Peschel *et al*., 1999; Dunman *et al*., 2001). The inactivation of regulatory gene *crgR* led to resistance to mouse cathelicidin (Nizet *et al*., 2001), yet the targets genes and signals controlling crgR remain to be identified. Homologues of these resistance genes have been identified in many bacterial species, indicating that these mechanisms might be widespread.

The existence of specific bacterial countermeasures argues that AMPs or other similar molecules have exerted significant influence on microbial evolution. The emergence and adaptation of microbial peptides-resistance mechanisms have affected the rapid evolution of AMPs, forcing the presence of multiple, divergent AMPs to better cope with different microbial challenges (Patil *et al*., 2004). Actually, the balance between host and pathogen is a result of dynamic co-evolutionary processes (Peschel and Sahl, 2006). Given the ongoing co-evolution between pathogen and host, it is surely a question not of 'if' but 'when' such adaptations will occur *in vivo*.

Regulation of AMPs: AMPs are antimicrobial genes act as final effectors and their expression pattern can be constitutive or inducible (Lehrer and Ganz, 1999). A variety of factors can modulate the expression of some AMPs (Table 1). However, the induction of AMP is still in the stage of infancy with the most of the work being conducted *in vitro* and very few animal studies have been documented. The sections below focus on Vitamin D (VD) and Free Fatty Acids (FFAs) as which can be added to dietary to enhance animals' immunity by up-regulating the expression of endogenous AMPs.

Vitamin D: VD was initially recognized as a hormone plays a crucial role in calcium and phosphorous

Table 1: The inducible expression pattern of AMPs

Regulators	AMPs	Cell or tissue	Genes or molecules related	References
VD	CAMP	Monocytes, macrophages	TLRs	Liu *et al.* (2006)
VD and LPS	CAMP, hBD-2	Human keratinocyes, monocytes and nutrophils	TLRs, NOD2, NF-KB, VDR, VDREs	Wang *et al.* (2004), Liu *et al.* (2007) and Wang *et al.* (2010)
FFAs	hBD-1,-2,-3 and LL-37	Human sebocytes, colonocytes	NFKB	Schauber *et al.* (2003) and Nakatsuji *et al.* (2010)
FFAs	AvBD9,14 and Cath B1	Chicken macrophages, primary monocytes	ND	Sunkara *et al.* (2011) and Sunkara *et al.* (2012)
LPS	hBD-2	Gingival keratinocyte, sebocytes	ND	Mathews *et al.* (1999) and Nagy *et al.* (2006)
Bacteria and LPS	Bovine â defensin	Tracheal epithelial cells	ND	Diamond *et al.*, (1996)
Salmonella enteritidis	AvBD 1, 9, 10, 12, 14	Epidigymis	ND	Anastasiadou *et al.* (2013)
IL-1α/β, -6,-17, -22,TNF-α	hBD-2	TBE cells, keratinocytes	NFKB, STAT3, JNK, PKC, PI3K,	Jang *et al.* (2004) and Kao *et al.* (2004), Wolk *et al.* (2004) and Wehkamp *et al.* (2006)
			p38MAPK, AP-1	Bando *et al.* (2007)
LCA	Human cathelicidin	NHEK	VDR, MEK-ERK	Peric *et al.* (2009)
Dermatitis, skin injury	CAMP, hBD-2, -3	Skin	ND	Aberg *et al.* (2008), Dressel *et al.* (2010) and Harder *et al.* (2010)
PBA, ST7	CAMP	VA10, HT-29, A498, U937	MEK 1/2, c-Jun N- terminal kinase	Steinmann *et al.* (2009)
Thapsigari, tunicamycin	CAMP, CRAMP	Epithelial cells	NFKB, C/EBPα	Park *et al.* (2011)

TLR, Toll-Like Receptor; hBD, human β-Defensin; NOD2, pattern recognition receptor; NFKB, Nuclear Factor; AvBD, Avianβ-Defensin; ND, Not Determined; IL, Interleukin; JNK, Jun N-terminal Kinase; PKC, Protein Kinase C; PI3K, Phosphatidylinositol-3-Kinase; P38MAPK, P38 Mitogen-Activated Protein Kinase; AP-1, Activator Protein-1; MEK 1/2, Mitogen-activated protein Kinase; NHEK, primary Human Keratinocytes; VDR, Vitamin D Receptor; LCA, Litocholic Acid; ST-7, α-metheylhydrocinnamate; VA10, a immortalized human bronchial epithelial cell line; HT-29, a human colonic adenocarrinoma cell line; A498, a human renal carcinoma cell line; U937, a human leukemic monocyte lymphoma cell line; PBA, 4-Phenylbutyrate; CRAMP, mouse cathelicidin antimicrobial peptide; C/EBP α, CCAAT/enhancer-binding protein α

homeostasis (Findling *et al.*, 1982), then was found the theoretical and preventive potential for cancer (Lappe *et al.*, 2007; Amir *et al.*, 2010), seasonal influenza A (Urashima *et al.*, 2010), multiple sclerosis (Burton *et al.*, 2010) and insulin resistance (Von Hurst *et al.*, 2010). $1,25(OH)_2D_3$ (the active form of VD) itself have no antimicrobial activity but numerous studies have proved that VD is a direct inducer of human cathelicidin (Liu *et al.*, 2007; Adams *et al.*, 2009) and β-defensin (Wang *et al.*, 2004; Liu *et al.*, 2006). The TLRs recognition of Pathogen-Associated Molecular Patterns (PAMPs) induced the expression of CYP27B1 which catalyzes the $25(OH)D_3$ to active form $1,25(OH)_2D_3$, then $1,25(OH)_2D_3$ signals through the VDR, a transcription factor that mediates the expression of human cathelicidin (Hewison, 2010). Once binding to VDR, they immediately arrive to the nucleus and bind Vitamin D Response Element (VDRE) located in the promoter of cathelicidin and then regulate human cathelicidin expression. But, the expression of three bovine *Cathelicidin* genes with potential VDRE are unresponsive to $1,25(OH)_2D_3$, as the VDRE is conserved in the CAMP promoter of primates (Gombart *et al.*, 2005; Nelson *et al.*, 2010). Cells have developed numerous repair or tolerance mechanisms to counteract the DNA damage caused by Ultraviolet-B (UV-B) (Sinha and Hader, 2002). Recently, VD was proved can protect human keratinocytes against UV-B-induced damage by up-regulating the expression of AMP (Tremezaygues *et al.*, 2009; Peric *et al.*, 2010). Meanwhile, UV-B was proved positive in regulating the expression of human β-defensin 2 in human keratinocytes *in vitro* and *in vivo* and the induction may represent part of a feedback loop to the suppressive effects of UVB on host immune (Glaser *et al.*, 2009). Thus, we infer that the UV-B-regulated expression of AMP may mediated by VD which derived from cholesterol following exposure to UV-B irradiation.

In addition inducing the expression of AMPs, vitamin D has many other effects on immune system. Both in human and bovine, $1,25(OH)_2D_3$ inhibits the proliferation of $CD4^+$ and γδ TCR $^+$T cells which are possibly responsible for the expression inhibition of IFN-γ and IL-17 (Nelson *et al.*, 2012).

Similarly, the decrease of Th1 and Th17 cells was observed in T cells treated with $1,25(OH)_2D_3$ which suggests that $1,25(OH)_2D_3$ can directly regulate T cell differentiation and prevent the autoimmune disease caused by Th1 and Th17 (Jeffery *et al.*, 2009; Tang *et al.*, 2009; Baeke *et al.*, 2010; Chang *et al.*, 2010a, b; Palmer *et al.*, 2011). As taken together, vitamin D-antimicrobial peptide pathway is an indispensable component of host immunity.

Free fatty acids: FFAs are important source of energy and components of cellular membranes. Recently, the FFAs were recently found functional in enhancing the immunity by inducing the expression of AMPs. The short-chain fatty acid butyrate was recognized to be capable of inducing AMPs expression in human (Schauber *et al.*, 2003, 2004), rabbits (Raqib *et al.*, 2006) and chicken (Sunkara *et al.*, 2011). Based on the number of carbon atoms in the aliphatic chain, FFAs are classified into short-chain fatty acids (<C5), medium-chain fatty acids

(C6-C11) and the long-chain fatty acids (≥C12) (Van Immerseel *et al*., 2006). It is intriguing that long-chain FFAs such as lauric acid, palmitic acid and oleic acid induced human β-defensin-2 (Nakatsuji *et al*., 2010) while short-chain fatty acids including butyrate and propionate induced LL-37 (Schauber *et al*., 2003). It has been proved that the induction of chicken AMP genes (*Cathelicidin B1* and *AvBD9*) is negative correlation with the chain length of FFAs and there is a synergetic effect in augmenting expression among three short-chain fatty acids (Sunkara *et al*., 2012). The mechanism of induction for AMPs expression by free fatty acids remains to be clarified but CD36 involved in regulating the uptake of FFAs and NF-KB mediated the transactivation pathway. Moreover, the feature that free fatty acids enhance human defensin gene expression without triggering proinflammatory makes FFAs to be potential antimicrobial therapeutic.

FFAs possess a direct antibacterial activity and the antibacterial activity is correlates with several characters of free fatty acids including the length of hydrocarbon chain, the saturability and the number of double bonds (Desbois and Smith, 2010). Unlike the induction activity, medium-chain fatty acids have the highest antibacterial activity and the activity decrease as the hydrocarbon chain gets longer or shorter. Notably, less is known about the consequences of the increased AMPs expression in the commensal intestinal flora which is critical for protection of the mucosa against enteropathogenic microbes.

RESULTS AND DISCUSSION

Application of the AMPs in domestic animals: The reduced effectiveness of some antibiotics and the wide spectrum bactericidal activity of AMPs enforce AMPs to be the ideal candidates for novel antimicrobial agents. Great progresses have been made in developing AMP drugs and peptides from different sources are at advanced stages of pharmaceutical development. For example, the porcine neutrophil-derived protegrins are the first of the mammalian cathelicidin peptides to reach clinical trial and phase 3 trials demonstrate the therapy ability of protegrin and its analogue in treating mucositis (Zaiou and Gallo, 2002). As polymyxins have been used for topical application, the cationic lantibiotic nisin and the cyclic cationic peptide gramicidin S have been applied in pharmacy and food industry respectively (Reddy *et al*., 2004; Falagas *et al*., 2006; Fjell *et al*., 2002). A series of references which focus on practical application of AMPs in livestock are listed in Table 2. Compared to the positive control with apramycin supplemented, the group of AMP supplemented behaved not so good but increasing levels of AMP-P3 linearly improved growth performance, coefficient of total tract apparent digestibility of nutrients and immune function (Yoon *et al*., 2012). The similar results were presented to cecropin AD in weaned piglets challenged with *Escherichia coli* (Wu *et al*., 2012). Although, there is little research on these compounds, the use of AMPs appears to have considerable potential as a replacement for antibiotics in rations fed. A commercial entity has started to market cecropin for use in swine rations in China (Thacker, 2013).

For use in pharmaceutical or biological applications, a reasonable amount of purified and active AMP molecules is needed. Even through different methods are available for producing AMPs such as the direct isolation from organism, chemical synthesis or recombinant expression via heterologous system (Desai *et al*., 2010; Parachin *et al*., 2012). The isolation of peptides from natural source is a strategy that is not economically sustainable for pharmaceutical companies, being restricted to universities and bio prospection labs. On the

Table 2: Efficacy of application of AMPs in domestic animals

Species	AMPs	Testing system	Effects	References
Pig	AMP-A3	*In vivo*	Significantly increased ADG, villus height, VH:CD and CTTAD of DM and CP; decreased crypt depth of the duodenum and jejunum	Yoon *et al*. (2012)
Pig	AMP-A5	*In vivo*	Dietary supplementation with 60 mg kg-1 AMP-P5 has the potential to improve the growth performance and apparent total tract digestibility of nutrients and reduce coliforms in weanling pigs	Yoon *et al*. (2013)
Pig	Lactoferrin	*In vivo*	Increased ADG and ADFI, decreased the F/G and the diarrhea. Reduced Escherichia coli and Salmonella in the small intestine	Wang *et al*. (2007)
Pig	Cecropin AD	*In vivo*	Decreased the incidence of diarrhea and reduced the negative effect (decreased weight gain, feed intake and feed efficiency) challenged by *E. coli*	Wu *et al*. (2012)
Pig	AP	*In vivo*	Synergistically worked with zinc methionine in improving growth performance, enhancing immune function, blood vessel function and antioxidant enzyme activity of piglets	Wang *et al*. (2011)
Chicken	AMP-P5	*In vivo*	Improved the growth performance, nutrient retention, intestinal morphology and reduce intestinal and excreta coliforms	Choi *et al*. (2013)
Chicken	BT	*In vivo*	Up-regulated chicken intestinal immune gene expression	Kogut *et al*. (2013)

ADG = Average Daily Gain; VH:CD = Villus Height to Crypt Depth; CTTAD = Coefficient of Total Tract Apparent Digestibility; DM = Dry Matter; CP = Crude Protein; AVD; ADFI = Average Daily Feed Intake; F/G = Feed efficiency

other hand, the problem that arises from the chemical synthesis of peptides is economic. This technique allows the large-scale production but is consequently limited to smaller AMPs lacking post-translational modifications (Li, 2011). Advances in recombinant DNA technology have enabled AMPs to be expressed in larger quantities for several applications (Parachin *et al*., 2012; Mulder *et al*., 2013). These techniques permit the cloning of genes encoding exogenous proteins in specific vectors for expression in prokaryotic and eukaryotic host cells (Park *et al*., 2008; Zelena *et al*., 2009), facilitating the production of these molecules on a large scale. Nevertheless, one enormous challenge in AMP production by heterologous system involves reducing intrinsic AMP toxicity to the vector.

CONCLUSION

The characterization of wide spectrum and excellent antibacterial effects make the AMPs an outstanding candidates for antibacterial therapeutics. When resistance to traditional antibiotics has been steadily increasing, there is an urgent need for novel, effective and safe antimicrobial therapeutics. Several attempts have been made over recent years to advance novel broad-spectrum AMPs into clinical use and have made big progress (Silva *et al*., 2013). There are two potential therapeutical options dealing with AMP, direct application of AMP or interference with AMP expression. While the balance between host and pathogen is a result of dynamic co-evolutionary processes, it is sooner or later that there will be AMP-resistance microorganism emerging directly using AMP agents just like antibiotics. The biggest difference between AMPs and antibiotics is that AMPs can be produced by the hosts themselves but antibiotics cannot. Thus, the strategy is that we can improve the defense of animals by up-regulating the expression of AMPs. The emergence of AMPs resistance may be delayed through an indirect application way, regulating the host its self's expression of AMPs to improve immunity. Different from human, it is more economical to take precautions against infection in breeding industry. All together, it's worth exploiting the induction mechanisms to control the infectious diseases in domestic animals.

LIMITATIONS

There are more limitations to be solved when AMPs were going to be applied in domestic animals besides the difficulty of production. One problem that bacteria may resist to AMPs (previously text for details) have been concerned us which may darken the future of applying AMPs in animal husbandry. Secondly, AMPs may be labile when incorporated in feeds, some harsh processing conditions, such as pelleting, dietary salt content and storage temperature may inactive AMPs. Moreover, the optimal dosage and treatment duration of different AMP to replace antibiotics in domestic animals remain to be elucidated. Lastly, we need to make sure the animal by-products are safe to consumers and environment. AMPs have arisen as a possible answer to antimicrobial resistance issues and these molecules can be used for human purposes is on the scientific agenda. And, it is important to emphasize that these clinical studies can be extended to agricultural purposes.

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REFERENCES

Aberg, K.M., M.Q. Man, R.L. Gallo, T. Ganz and D. Crumrine *et al*., 2008. Co-regulation and interdependence of the mammalian epidermal permeability and antimicrobial barriers. J. Invest. Dermatol., 128: 917-925.

Adams, J.S., S. Ren, P.T. Liu, R.F. Chun and L. Venu, 2009. Vitamin D-directed rheostatic regulation of monocyte antibacterial responses. J. Immunol., 182: 4289-4295.

Amir, E., C.E. Simmons, O.C. Freedman, G. Dranitsaris and D.E. Cole *et al*., 2010. A phase 2 trial exploring the effects of high-dose (10,000 IU/day) vitamin D(3) in breast cancer patients with bone metastases. Cancer, 116: 284-291.

Anastasiadou, M., M. Avdi and G. Michailidis, 2013. Expression of avian β-defensins and Toll-like receptor genes in the rooster epididymis during growth and *Salmonella* infection. Anim. Reprod. Sci., 140: 224-231.

Baeke, F., H. Korf, L. Overbergh, E. van Etten and A. Verstuyf *et al*., 2010. Human T lymphocytes are direct targets of 1,25-dihydroxyvitamin D_3 in the immune system. J. Steroid Biochem. Mol. Biol., 121: 221-227.

Bando, M., Y. Hiroshima, M. Kataoka, Y. Shinohara and M.C. Herzberg *et al*., 2007. Interleukin-1alpha regulates antimicrobial peptide expression in human keratinocytes. Immunol. Cell Biol., 85: 532-537.

Boman, H.G., B. Agerberth and A. Boman, 1993. Mechanisms of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. Infect Immun., 61: 2978-2984.

Brogden, A.K., 2005. Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? Nat. Rev. Microbiol., 3: 238-250.

Burton, J.M., S. Kimball, R. Vieth, A. Bar-Or and H.M. Dosch *et al*., 2010. A phase I/II dose-escalation trial of vitamin D_3 and calcium in multiple sclerosis. Neurology, 74: 1852-1859.

Chang, J.H., H.R. Cha, D.S. Lee, K.Y. Seo and M.N. Kweon, 2010a. 1,25-Dihydroxyvitamin D_3 inhibits the differentiation and migration of T_H17 cells to protect against experimental autoimmune enceph alomyelitis. PLoS One, Vol. 5. 10.1371/journal. pone. 0012925.

Chang, S.H., Y. Chung and C. Dong, 2010b. Vitamin D suppresses Th17 cytokine production by inducing C/EBP homologous protein (CHOP) expression. J. Biol. Chem., 285: 38751-38755.

Chen, F.Y., M.T. Lee and H.W. Huang, 2003. Evidence for membrane thinning effect as the mechanism for peptide-induced pore formation. Biophys. J., 84: 3751-3758.

Choi, S.C., S. Ingale, J.S. Kim, Y.K. Park, I.K. Kwon and B.J. Chae, 2013. Effects of dietary supplementation with an antimicrobial peptide-P5 on growth performance, nutrient retention, excreta and intestinal microflora and intestinal morphology of broilers. Anim. Feed Sci. Technol., 185: 78-84.

Desai, P.N., N. Shrivastava and H. Padh, 2010. Production of heterologous proteins in plants: Strategies for optimal expression. Biotechnol. Adv., 28: 427-435.

Desbois, A.P. and V.J. Smith, 2010. Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. Applied Microbiol. Biotechnol., 85: 1629-1642.

Diamond, G., J.P. Russell and C.L. Bevins, 1996. Inducible expression of an antibiotic peptide gene in lipopolysaccharide-challenged tracheal epithelial cells. Proc. Natl. Acad. Sci. USA., 93: 5156-5160.

Dressel, S., J. Harder, J. Cordes, M. Wittersheim, U. Meyer-Hoffert, C. Sunderkotter and R. Glaser, 2010. Differential expression of antimicrobial peptides in margins of chronic wounds. Exp. Dermatol., 19: 628-632.

Dunman, P.A., E. Murphy, S. Haney, D. Palacios and G. Tucker-Kellogg *et al*., 2001. Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the agrand/or sarA Loci. J. Bacteriol., 183: 7341-7353.

Easton, D.M., A. Nijnik, M.L. Mayer and R.E. Hancock, 2009. Potential of immunomodulatory host defense peptides as novel anti-infectives. Trends Biotechnol., 27: 582-590.

Ernst, R.K., T. Guina and S.I. Miller, 2001. *Salmonella typhimurium* outer membrane remodeling: Role in resistance to host innate immunity. Microbes Infect., 3: 1327-1334.

Falagas, M.E., S.K. Kasiakou, S. Tsiodras and A. Michalopoulos, 2006. The use of intravenous and aerosolized polymyxins for the treatment of infections in critically ill patients: A review of the recent literature. Clin. Med. Res., 4: 138-146.

Findling, J.W., N.D. Adams, J. Lemann Jr., R.W. Gray, C.J. Thomas and J.B. Tyrrell, 1982. Vitamin D metabolites and parathyroid hormone in Cushing's syndrome: Relationship to calcium and phosphorus homeostasis. J. Clin. Endocrinol. Metab., 54: 1039-1044.

Fjell, C.D., J.A. Hiss, R.E. Hancock and G. Schneider, 2002. Designing antimicrobial peptides: Form follows function. Nat. Rev. Drug Discov., 11: 37-51.

French, G.L., 2010. The continuing crisis in antibiotic resistance. Int. J. Antimicrob. Agents, 36: S3-S7.

Frick, I.M., P. Akesson, M. Rasmussen, A. Schmidtchen and L. Bjorck, 2003. SIC, a secreted protein of streptococcus pyogenesthat inactivates antibacterial peptides. J. Biol. Chem., 278: 16561-16566.

Ganz, T., 2003. Defensins: Antimicrobial peptides of innate immunity. Nat. Rev. Immunol., 3: 710-720.

Glaser, R., F. Navid, W. Schuller, C. Jantschitsch and J. Harder *et al*., 2009. UV-B radiation induces the expression of antimicrobial peptides in human keratinocytes *in vitro* and *in vivo*. J. Allergy Clin. Immunol., 123: 1117-1123.

Gombart, A.F., N. Borregaard and H.P. Koeffler, 2005. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D_3. FASEB J., 19: 1067-1077.

Guina, T., C.Y. Eugene, H. Wang, M. Hackett and S.I. Miller, 2000. A PhoP-regulated outer membrane protease of *Salmonella enteric* serovar *Typhimurium* promotes resistance to alpha-helical antimicrobial peptides. J. Bacteriol., 182: 4077-4086.

Gunn, J.S., K.B. Lim, J. Krueger, K. Kim, L. Guo, M. Hackett and S.I. Miller, 1998. PmrA-Pmr B-regulated genes necessary for 4-aminoarabinose lipid a modification and polymyxin resistance. Mol. Microbiol., 27: 1171-1182.

Guo, L., K.B. Lim, C.M. Poduje, M. Daniel, J.S. Gunn, M. Hackett and S.I. Miller, 1998. Lipid a acylation and bacterial resistance against vertebrate antimicrobial peptides. Cell, 95: 189-198.

Hamill, P., K. Brown, H. Jenssen and R.E.W. Hancock, 2008. Novel anti-infectives: Is host defence the answer? Curr. Opin. Biotechnol., 19: 628-636.

Hancock, R.E. and D.S. Chappel, 1999. Peptide antibiotics. Antimicrob. Agents Chemother., 43: 1317-1323.

Harder, J., S. Dressel, M. Wittersheim, J. Cordes and U. Meyer-Hoffert *et al*., 2010. Enhanced expression and secretion of antimicrobial peptides in atopic dermatitis and after superficial skin injury. J. Invest. Dermatol., 130: 1355-1364.

Hewison, M., 2010. Vitamin D and the intracrinology of innate immunity. Mol. Cell. Endocrinol., 321: 103-111.

Huang, H.W., 2000. Action of antimicrobial peptides two state model. Biochemistry, 39: 8347-8352.

Jang, B.C., K.J. Lim, J.H. Paik, Y.K. Kwon and S.W. Shin, 2004. Up-regulation of human β-defensin 2 by interleukin-1β in A549 cells: Involvement of PI3K, PKC, p38 MAPK, JNK and NF-KB. Biochem. Biophys. Res. Commun., 320: 1026-1033.

Jeffery, L.E., F. Burke, M. Mura, Y. Zheng and O.S. Qureshi *et al*., 2009. 1, 25-dihydroxyvitamin D_3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J. Immunol., 183: 5458-5467.

Jin, T., M. Bokarewa, T. Foster, J. Mitchell, J. Higgins and A. Tarkowski, 2004. *Staphylococcus aureus* resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. J. Immunol., 172: 1169-1176.

Kao, C.Y., Y. Chen, P. Thai, S. Wachi and F. Huang *et al*., 2004. IL-17 markedly up-regulates β-defensin-2 expression in human airway epithelium via JAK and NF-KB signaling pathways. J. Immunol., 173: 3482-3491.

Kogut, M.H., K.J. Genovese, H. He, C.L. Swaggerty and Y. Jiang, 2013. Modulation of chicken intestinal immune gene expression by small cationic peptides as feed additives during the first week posthatch. Clin. Vaccine Immunol., 20: 1440-1448.

Lai, Y. and R.L. Gallo, 2009. AMPed up immunity: How antimicrobial peptides have multiple roles in immune defense. Trends Immunol., 30: 131-141.

Lappe, J.M., D. Travers-Gustafson, K.M. Davies, R.R. Recker and R.P. Heaney, 2007. Vitamin D and calcium supplementation reduces cancer risk: Results of a randomized trial. Am. J. Clin. Nutr., 85: 1586-1591.

Lehrer, R.I. and T. Ganz, 1999. Antimicrobial peptides in mammalian and insect host defence. Curr. Opin. Immunol., 11: 23-27.

Li, Y.F., 2011. Recombinant production of antimicrobial peptides in *Escherichia coli*: A review. Protein Expression Purif., 80: 260-267.

Liu, P.T., S. Stenger, H. Li, L. Wenzel and B.H. Tan *et al*., 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science, 311: 1770-1773.

Liu, P.T., S. Stenger, D.H. Tang and R.L. Modlin, 2007. Cutting edge: Vitamin D-mediated human antimicrobial activity against *Mycobacterium tuberculosis* is dependent on the induction of cathelicidin. J. Immunol., 179: 2060-2063.

Mathews, M., H.P. Jia, J.M. Guthmiller, G. Losh and S. Graham *et al*., 1999. Production of β-defensin antimicrobial peptides by the oral mucosa and salivary glands. Infect. Immun., 67: 2740-2745.

Mulder, K.C., L.A. Lima, V.J. Miranda, S.C. Dias and O.L. Franco, 2013. Current scenario of peptide-based drugs: The key roles of cationic antitumor and antiviral peptides. Front Microbiol., 4: 321-321.

Murata, T., W. Tseng, T. Guina, S.I. Miller and H. Nikaido, 2007. PhoPQ-mediated regulation produces a more robust permeability barrier in the outer membrane of *Salmonella enteric* serovar typhimurium. J. Bacteriol., 189: 7213-7222.

Nagy, I., A. Pivarcsi, K. Kis, A. Koreck and L. Bodai *et al*., 2006. Propionibacterium acnes and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes. Microbes Infect., 8: 2195-2205.

Naito, A., T. Nagao, K. Norisada, T. Mizuno, S. Tuzi and H. Saito, 2000. Conformation and dynamics of melittin bound to magnetically oriented lipid bilayers by solid-state ^{31}P and ^{13}C NMR spectroscopy. Biophys. J., 78: 2405-2417.

Nakatsuji, T., M.C. Kao, L. Zhang, C.C. Zouboulis, R.L. Gallo and C.M. Huang, 2010. Sebum free fatty acids enhance the innate immune defense of human sebocytes by upregulating β-defensin-2 expression. J. Invest. Dermatol., 130: 985-994.

Nelson, C.D., T.A. Reinhardt, T.C. Thacker, D.C. Beitz and J.D. Lippolis, 2010. Modulation of the bovine innate immune response by production of 1α, 25-dihydroxyvitamin D_3 in bovine monocytes. J. Dairy Sci., 93: 1041-1049.

Nelson, C.D., T.A. Reinhardt, J.D. Lippolis, R.E. Sacco and B.J. Nonnecke, 2012. Vitamin D signaling in the bovine immune system: A model for understanding human vitamin D requirements. Nutrients, 4: 181-196.

Neu, H.C., 1992. The crisis in antibiotic resistance. Science, 257: 1064-1073.

Nizet, V., T. Ohtake, X. Lauth, J. Trowbridge and J. Rudisill *et al*., 2001. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature, 414: 454-457.

Palmer, M.T., Y.K. Lee, C.L. Maynard, J.R. Oliver, D.D. Bikle, A.M. Jetten and C.T. Weaver, 2011. Lineage-specific effects of 1, 25-dihydroxyvitamin D_3 on the development of effector CD4 T cells. J. Biol. Chem., 286: 997-1004.

Parachin, N.S., K.C. Mulder, A.A.B. Viana, S.C. Dias and O.L. Franco, 2012. Expression systems for heterologous production of antimicrobial peptides. Peptides, 38: 446-456.

Park, K., P.M. Elias, Y. Oda, D. Mackenzie, T. Mauro, W.M. Holleran and Y. Uchida, 2011. Regulation of cathelicidin antimicrobial peptide expression by an endoplasmic reticulum (ER) stress signaling, vitamin D receptor-independent pathway. J. Biol. Chem., 286: 34121-34130.

Park, J.H., M.C. Suh, T.H. Kim, M.C. Kim and S.H. Cho, 2008. Expression of glycine-rich protein genes, AtGRP5 and AtGRP23, induced by the cutin monomer 16-hydroxypalmitic acid in *Arabidopsis thaliana*. Plant Physiol. Biochem., 46: 1015-1018.

Patil, A., A.L. Hughes and G. Zhang, 2004. Rapid evolution and diversification of mammalian α-defensins as revealed by comparative analysis of rodent and primate genes. Physiol. Genomics, 20: 1-11.

Patrzykat, A., C.L. Friedrich, L. Zhang, V. Mendoza and R.E.W. Hancock, 2001. Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. Antimicrob. Agents Chemother., 46: 605-614.

Peric, M., S. Koglin, Y. Dombrowski, K. Grob, E. Bradac, T. Ruzicka and J. Schauber, 2009. VDR and MEK-ERK dependent induction of the antimicrobial peptide cathelicidin in keratinocytes by lithocholic acid. Mol. Immunol., 46: 3183-3187.

Peric, M., B. Lehmann, G. Vashina, Y. Dombrowski and S. Koglin *et al*., 2010. UV-B-triggered induction of vitamin D_3 metabolism differentially affects antimicrobial peptide expression in keratinocytes. J. Allergy Clin. Immunol., 125: 746-749.

Peschel, A. and H.G. Sahl, 2006. The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nat. Rev. Microbiol., 4: 529-536.

Peschel, A., 2002. How do bacteria resist human antimicrobial peptides? Trends Microbiol., 10: 179-186.

Peschel, A., M. Otto, R. Jack, H. Kalbacher, G. Jung and F. Gotz, 1999. Inactivation of the dlt Operon in Staphylococcus aureus confers sensitivity to defensins, protegrins and other antimicrobial peptides. J. Biol. Chem., 274: 8405-8410.

Raqib, R., P. Sarker, P. Bergman, G. Ara and M. Lindh *et al*., 2006. Improved outcome in shigellosis associated with butyrate induction of an endogenous peptide antibiotic. Proc. Nat. Acad. Sci. USA., 103: 9178-9183.

Reddy, K.V.R., R.M. Yedery and A. Clara, 2004. Antimicrobial peptides: Premises and promises. Int. J. Antimicrobial. Agents, 24: 536-547.

Schauber, J., C. Svanholm, S. Termen, K. Iffland and T. Menzel *et al*., 2003. Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: Relevance of signalling pathways. Gut, 52: 735-741.

Schauber, J., K. Iffland, S. Frisch, T. Kudlich and B. Schmausser *et al*., 2004. Histone-deacetylase inhibitors induce the cathelicidin LL-37 in gastrointestinal cells. Mol. Immunol., 41: 847-854.

Scott, M.G., E. Dullaghan, N. Mookherjee, N. Glavas and M. Waldbrook *et al*., 2007. An anti-infective peptide that selectively modulates the innate immune response. Nat. Biotechnol., 25: 465-472.

Semple, F. and J.R. Dorin, 2011. β-Defensins: Multifunctional modulators of infection, inflammation and more? J. Innate Immun., 4: 337-348.

Silva, N.C., B. Sarmento and M. Pintado, 2013. The importance of antimicrobial peptides and their potential for therapeutic use in ophthalmology. Int. J. Antimicrob. Agents, 41: 5-10.

Sinha, R.P. and D.P. Hader, 2002. UV-induced DNA damage and repair: A review. Photochem. Photobiol. Sci., 1: 225-236.

Steinmann, J., S. Halldorsson, B. Agerberth and G.H. Gudmundsson, 2009. Phenylbutyrate induces antimicrobial peptide expression. Antimicrobial Agents Chemotherapy, 53: 5127-5133.

Sunkara, L.T., M. Achanta, N.B. Schreiber, Y.R. Bommineni and G. Dai *et al*., 2011. Butyrate enhances disease resistance of chickens by inducing antimicrobial host defense peptide gene expression. PLoS One, Vol. 6. 10.1371/journal.pone.0027225.

Sunkara, L.T., W. Jiang and G. Zhang, 2012. Modulation of antimicrobial host defense peptide gene expression by free fatty acids. PLoS One, Vol. 7. 10.1371/journal.pone.0049558.

Tang, J., R. Zhou, D. Luger, W. Zhu and P.B. Silver *et al*., 2009. Calcitriol suppresses antiretinal autoimmunity through inhibitory effects on the Th17 effector response. J. Immunol., 182: 4624-4632.

Thacker, P.A., 2013. Alternatives to antibiotics as growth promoters for use in swine production: A review. J. Anim. Sci. Biotechnol., Vol. 4. 10.1186/2049-1891-4-35.

Tremezaygues, L., M. Seifert and W. Tilgen, 2009. 1,25-dihydroxyvitamin D(3) protects human keratinocytes against UV-B-induced damage: *In vitro* analysis of cell viability/proliferation, DNA-damage and-repair. Dermatoendocrinology, 1: 239-245.

Urashima, M., T. Segawa, M. Okazaki, M. Kurihara, Y. Wada and H. Ida, 2010. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am. J. Clin. Nutr., 91: 1255-1260.

Van Immerseel, F., J.B. Russell, M.D. Flythe, I. Gantois and L. Timbermont *et al*., 2006. The use of organic acids to combat Salmonella in poultry: A mechanistic explanation of the efficacy. Avian Pathol., 35: 182-188.

Von Hurst, P.R., W. Stonehouse and J. Coad, 2010. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient: A randomised, placebo-controlled trial. Br. J. Nutr., 103: 549-555.

Wang, T.T., F.P. Nestel, V. Bourdeau, Y. Nagai and Q. Wang *et al*., 2004. Cutting edge: 1,25-dihydroxyvitamin D_3 is a direct inducer of antimicrobial peptide gene expression. J. Immunol., 173: 2909-2912.

Wang, Y.Z., T.Z. Shan, Z.R. Xua, J. Feng and Z.Q. Wang, 2007. Effects of the lactoferrin (LF) on the growth performance, intestinal microflora and morphology of weanling pigs. Anim. Feed Sci. Tech., 135: 263-272.

Wang, T.T., B. Dabbas, D. Laperriere, A.J. Bitton and H. Soualhine *et al*., 2010. Direct and indirect induction by 1,25-dihydroxyVitamin D_3 of the NOD2/CARD15-defensin â2 innate immune pathway defective in Crohn disease. J. Biol. Chem., 285: 2227-2231.

Wang, J.H., C.C. Wu and J. Feng, 2011. Effect of dietary antibacterial peptide and zinc-methionine on performance and serum biochemical parameters in piglets. Czech J. Anim. Sci., 56: 30-36.

Wehkamp, K., L. Schwichtenberg, J.M.M. Schroder and J. Harder, 2006. Pseudomonas aeruginosa-and IL-1β-mediated induction of human β-defensin-2 in keratinocytes is controlled by NF-KB and AP-1. J. Invest. Dermatol., 126: 121-127.

Winfield, M.D. and E.A. Groisman, 2004. Phenotypic differences between Salmonella and Escherichia coli resulting from the disparate regulation of homologous genes. Proc. Nat. Acad. Sci. USA., 101: 17162-17167.

Wolk, K., S. Kunz, E. Witte, M. Friedrich, K. Asadullah and R. Sabat, 2004. IL-22 increases the innate immunity of tissues. Immunity, 21: 241-254.

Wu, S., F. Zhang, Z. Huang, H. Liu and C. Xie *et al*., 2012. Effects of the antimicrobial peptide cecropin AD on performance and intestinal health in weaned piglets challenged with *Escherichia coli*. Peptides, 35: 225-230.

Yang, L., T.A. Harroun, T.M. Weiss, L. Ding and H.W. Huang, 2001. Barrel-stave model or toroidal model? A case study on melittin pores. Biophys. J., 81: 1475-1485.

Yeaman, M.R. and N.Y. Yount, 2003. Mechanisms of antimicrobial peptide action and resistance. Pharmacol. Rev., 55: 27-55.

Yoon, J.H., S.L. Ingale, J.S. Kim, K.H. Kim and S.H. Lee *et al*., 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.

Yoon, J.H., S.L. Ingale, J.S. Kim, K.H. Kim and J. Lohakare *et al*., 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.

Zaiou, M. and R.L. Gallo, 2002. Cathelicidins, essential gene-encoded mammalian antibiotics. J. Mol. Med., 80: 549-561.

Zasloff, M., 2002. Antimicrobial peptides of multicellular organisms. Nature, 415: 389-395.

Zelena, K., S. Krugener, S. Lunkenbein, H. Zorn and R.G. Berger, 2009. Functional expression of the lipase gene Lip2 of *Pleurotus sapidus* in *Escherichia coli*. Biotechnol. Lett., 31: 395-401.