The Thai Journal of Veterinary Medicine

Volume 37
Issue 4 *December, 2007*

Article 10

12-1-2007

Review of the Efficacy and Safety of Poly (2-propenal, 2-propenoic acid): a Novel Antimicrobial Polymer

Alistair Iain Murdoch

Rosalie Dianne McCauley

David John Hampson

Review of the Efficacy and Safety of Poly (2-propenal, 2-propenoic acid): a Novel Antimicrobial Polymer

Alistair Iain Murdoch[1*] Rosalie Dianne McCauley[2] David John Hampson[3]

Abstract

The withdrawal of antibiotic growth promoters is likely to increase the incidence of enteric disease in pig and broiler farms; especially in poorly sanitised conditions. Therefore, replacements for the antibiotic growth promoters currently used in both pig and poultry production, which offer disease control and delay the further development of antibiotic resistance, will become increasingly important.

Poly (2-propenal, 2-propenoic acid) (pPPA) is a new chemical entity undergoing development by Chemeq Ltd, Australia. It was designed to contain aldehyde groups for antimicrobial activity, carboxylic acid groups to aid water solubility and conformationally is a long chain, high molecular weight polymer developed to minimise intestinal absorption. pPPA is postulated to kill bacteria by cross-linking bacterial lipoproteins and has *in vitro* bactericidal activity towards weaner pig and poultry pathogens. Results from *in vitro* and *in vivo* studies show weaner pigs treated with pPPA had significantly less intestinal colonisation with a challenge strain of β-haemolytic *Escherichia coli* and usually significantly less post-weaning diarrhoea than untreated control pigs. In broiler chickens, treatment with pPPA helped to maintain health and improved growth performance. Importantly, treatment of weaner pigs and broiler chickens ten times the recommended dose of 30 mg/kg of pig/day and 3 mg/kg chicken/day did not induce toxicity.

In summary, pPPA is an aldehydic antimicrobial compound that was developed to kill bacteria without significant intestinal absorption by treated animals. pPPA has bactericidal activity towards common pathogens of weaner pigs and broiler chickens and is proven to be safe for treated animals. The excellent combination of efficacy and safety mean pPPA has the potential to become an important new drug in the pig and poultry industry.

Keywords : Poly (2-propenal, 2-propenoic acid), weaner pig, broiler chicken, toxicity

[1]Spirogene Pty Ltd. Building 191, Murdoch University, Murdoch, WA, 6150 Australia.

[2]Chemeq Ltd, CornerPatterson and Alumina Roads, Rockingham, WA, 6168, Australia.

[3]Division of Veterinary and Biomedical Sciences, Murdoch University; Murdoch, WA, 6150 Australia.

[*]Corresponding author

Introduction

The withdrawal of antibiotic growth promoters is likely to increase the endemic nature of enteric disease in pig and broiler farms throughout the world; especially in poorly sanitised conditions. However, the well-sanitized management requires high cost investment and usually not economically practical in most poultry and livestock farms. The use of antibiotics at sub-therapeutic dose offers not only disease suppression but also helps to promote growth. It has long been considered as a cost-effective approach in terms of farm management. Resistance of bacterial pathogens to antibiotics is recognized as an increasing problem and is being widely studied (Van den Bogaard and Stobberingh, 1999; Lucy et al. 2002). As a result of increasing concerns over the transfer of resistant pathogens from animal origins to humans (Van den Bogaard and Stobberingh, 1999), the use of antibiotics particularly in food-producing animals has been under strict regulation (Tanner, 2000). Therefore, there is an urgent need for development of effective and safe replacements for the current antibiotic growth promoters used in both pig and poultry production.

Poly (2-propenal, 2-propenoic acid) (pPPA) is a new chemical entity undergoing drug development by Chemeq Ltd, Australia. CHEMEQ® polymeric antimicrobial is an aqueous formulation containing 5% w/w of pPPA and is advised for the control of post-weaning diarrhoea in weaner pigs and for the maintenance of intestinal health in broiler chickens. The aim of this review is to summarise the data from the past and on-going experiments carried out to assess the safety and antimicrobial efficacy of pPPA; and to promote further scientific discussion.

Chemistry

pPPA is a new class of therapeutic compound (ATCvet code: QA07AX90, 2006). It was designed to contain aldehyde groups for antimicrobial activity, carboxylic acid groups which aid in water solubility and conformationally is a long chain, high molecular weight polymer developed to minimise intestinal absorption. It is a polydisperse polymer with a number average molecular weight (Mn) of >1000 (aqueous Gel Permeation Chromatography). pPPA has limited solubility in water, but is soluble in aqueous buffers at pH 7, or higher, and is soluble in alcohols and ethers, including polyethylene glycol.

pPPA is produced by base catalysed polymerisation of 2-propenal (Figure 1). Polymerisation proceeds randomly via 2 modes of addition, resulting in a complex polymer with both aldehydes and vinyl groups within the polymer backbone. Secondary bonding gives rise to stable inter and intramolecular structures including tetrahydropyran rings (formed from adjacent aldehydes). Partial oxidation of this polymer results in about 10% of the polymer units converted to carboxylic acids (poly 2-propenoic acid). A partial structure of pPPA shows the polymer contains aldehyde groups and carboxylic acids group attached to a long polymer backbone (Figure 2).

Mode of action and antimicrobial resistance

pPPA is an aldehydic antimicrobial compound; therefore it has been postulated that it has a similar mode of action to the dialdehyde biocides such as gluteraldehyde and orthophthalaldehyde. Aldehyde groups react with amine groups within the lipoprotein molecules of the outer cell wall of micro-organisms leading to cross-linking. The cross-linked proteins are not able to carry out normal cellular activities and if enough proteins are cross-linked the organism will die without lysis (Mailland, 2002).

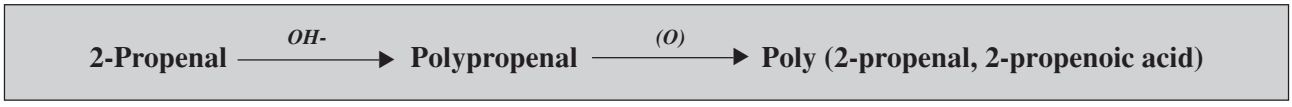

Figure 1 Summary of the formulation of Poly (2-propenal, 2-propenoic acid)

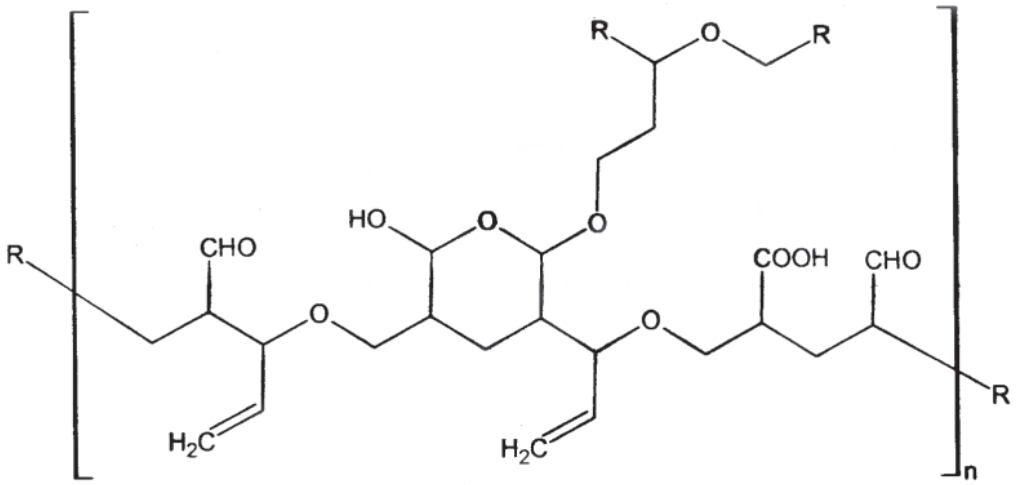

Figure 2 Partial structure of the Poly (2-propenal, 2-propenoic acid) polymer

Initial *in vitro* investigations showed bactericidal activity of pPPA is diminished by protein contained in standard microbiological media such as Mueller Hinton Broth. Therefore this characteristic of the active ingredient led to the development of minimum inhibitory concentration (MIC) and minimum kill concentration (MKC) tests using Minimal Glucose Media *(Glucose 5 g/L, Na_2HPO_4 6 g/L, KH_2PO_4 3 g/L, NH_4Cl 1 g/L, NaCl 0.5 g/L, $MgSO_4$ 0.12 g/L, $CaCl_2$ 0.01 g/L, pH 7.0.)* rather than Mueller Hinton Broth. Results of MKC testing using Minimal Glucose Media demonstrate that pPPA kills a wide range of pathogenic bacteria with MKC values in the range of 3 mg/L for *Clostridium perfringens* to 2000 mg/L for *Aspergillis niger* ATCC 16404 (Table 1)

Table 1 Minimum kill concentrations of Poly (2-propenal, 2-propenoic acid) towards selected microorganisms (V. Wycoco and F. Bahemia, unpublished observations).

Microorganism	Number Tested	MKC (ppm)	Comments
Aspergillis niger ATCC 16404	1	1000-2000	ATCC Reference microorganism
CANDIDA ALBICANS ATCC 10231	1	125	ATCC Reference microorganism
Clostridium perfringens	8	3-15	
Enterococcus faecium	4	125	Two strains with resistance to vancomycin
Escherichia coli ATCC 8739	1	15-31	ATCC Reference microorganism
Listeria monocytogenes	5	<15-31	
Pseudomonas aeruginosa ATCC 9027	1	125	ATCC Reference microorganism
Salmonella infantis	1	62.5	
Salmonella typhimurium	3	62.5	
Salmonella mbandaba	1	62.5	
Staphylococcus aureus ATCC 6538	1	<7-15	ATCC Reference microorganism

These *in vitro* studies have also provided information about the mode of action of pPPA. Phase contrast light microscopy showed 24 hours exposure of *Esherichia coli (E. coli)* ATCC 8739, *Proteus vulgaris* ATCC 4635, *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* var niger ATCC 9372 to 2000 mg/L of pPPA led to lysis of some of the bacteria (V. Wycoco, unpublished observations). However, results from examination of the mode of action of pPPA using the chromogenic plate assay of Mardones and Venegas (2000) suggest pPPA is not a bacteriolytic antimicrobial. This study used ampicillin, a known bacteriolytic antimicrobial, and tetracycline, an antimicrobial known not to be bacteriolytic as controls. The results confirmed ampicillin was bacteriolytic whilst tetracycline, pPPA and gluteraldehyde were not bacteriolytic. (R. McCauley and A. D'Hart, unpublished observations). A non-bacteriolytic mode of action for pPPA and gluteraldehyde is consistent with the cross-linking mode of action of aldehyde antimicrobials (Mailland, 2002).

Further to these studies a combined spectrophotometric repeat plating assay (Park et al., 1998) was undertaken (R. McCauley unpublished observations). Triplicate microplate wells containing β-haemolytic *E. coli* 0149 were exposed to 5 X MIC of pPPA or magainin II (a cationic peptide known to lyse bacteria) for one hour. Absorbance was monitored at 600 nm and samples were taken at 0, 10, 20, 30, 40, 50 and 60 minutes for serial dilution and plating to determine total viable count. As expected, both absorbance and total viable counts of the magainin II-treated wells decreased; however, only total viable count decreased in the wells treated with pPPA suggesting pPPA killed the *E. coli* 0149 without lysis.

An *in vitro* model has been used to assess the potential for pPPA to induce antimicrobial resistance. There was no change in MKC of pPPA to *E. coli* ATCC 8739 after 106 sequential exposures (K. Shaw-unpublished observations). In contrast, the MKC of apramycin sulphate to *E. coli* ATCC 8739 increased from 62 to 125 mg/L after 62 sequential exposures. These preliminary data suggest pPPA has a low propensity to induce antimicrobial resistance.

Results from a comparative efficacy study of pPPA, enrofloxacin, and timentin (ticarcillin plus clavulanic acid) highlight the biocidal nature of pPPA The MIC, MKC and minmum bacteriocidal concentrations (MBC) of pPPA enrofloxacin, and timentin to *E. coli* ATCC 25922, 35218 and 8739, and *Pseudomonas aeruginosa* ATCC 27853 were determined using Minimal Glucose Media, and Cation Adjusted Mueller Hinton Broth in Table 2; (D. Trott, J. Platell, E. Constantinou and S. Moss. unpublished observations). Comparison of the MIC, MBC and MKC values for each microorganism in minimal glucose media showed that the MKC, MIC and MBC for pPPA were consistent whilst the MKC values for enrofloxacin and timentin tended to be several dilutions higher than the MIC and MBC.

Efficacy against porcine and broiler chicken pathogens

In intensive pig production, Post Weaning Diarrhoea (PWD) remains one of the most problematic bacterial diseases (Hampson, 1994). Classical PWD is a common and severe diarrhoeal disease which occurs in the first 3 to 10 days after weaning. The diarrhoea results from the action of one or more serotypes of β-haemolytic enterotoxigenic *E. coli* which proliferate in the proximal small intestine during this post-weaning period (Bertschinger and Fairbrother, 1999). The *E. coli* adhere to villous enterocytes via specific pili, and release enterotoxins which are responsible for loss of fluid and electrolyte, and hence cause secretory diarrhoea (Morris and Sojka, 1985; Francis, 2002).

The use of oral systemic antibiotics , together with electrolyte replacement therapy is the most common method to treat PWD. Unfortunately, the strains of *E. coli* associated with PWD are becoming resistant to a range of antimicrobials (Barton, 1999; Amezcua et al., 2002). The results from laboratory and on-farm studies examining the effect of pPPA on PWD in weaner pigs has shown

Table 2 Minimum inhibitory concentrations, minimum bactericidal concentrations and minimum kill concentrations of Poly (2-propenal, 2-propenoic acid), enrofloxacin and timentin in two different media to *Escherichia coli* and *Pseudomonas aeruginosa* (D. Trott, J. Platell, E. Constantinou and S. Moss. unpublished observations).

Organism	Antimicrobial	Method [a,b]	MIC (ppm) or μg/mL	MBC (ppm) or μg/mL	MKC (ppm) or μg/mL
E. coli ATCC 25922	pPPPA[c]	MGM	7.1	31.2	31.2
	Enrofloxacin	CAMHB (0.008-0.03)[d]	0.008	0.015	0.015
		MGM	0.008	0.015-0.03	0.03-0.25
E. coli ATCC 35218	pPPPA[c]	MGM	15.1	31.25-62.5	62.5
	Timentin	CAMHB (4-16)[c]	32	32	32-256
		MGM	16	32	256
E. coli ATCC 8739	pPPPA	MGM	62.5	62.5	62.5
	Enrofloxacin	CAMHB	0.015	0.015	0.015
		MGM	0.008-0.015	0.015	0.015-0.25
	Timentin	CAMHB	2-4	2-4	4-8
		MGM	0.5-1	2-16	8-64
Ps. aeruginosa ATCC 27853	pPPPA	MGM	250-500	250-500	250-500
	Enrofloxacin	CAMHB (1-4)[c]	1-2	2-4	2-4
		MGM	2	4-8	4-16
	Timentin	CAMHB (8-32)[c]	32-64	32-128	128
		MGM	16	32-256	32-256

[a] MGM : Minimal Glucose Medium.

[b] CAMHG : Cation-Adjusted Mueller Hinton Broth).

[c] Poly (2-propenal, 2-propenoic acid).

[d] Values in parentheses represent the CLSI acceptable range MICs for each ATCC control strain.

that pPPA-treated pigs showed significantly reduced intestinal colonisation with the challenge strain of *E. coli*, less PWD, and required fewer treatments for PWD than pigs not treated with pPPA (Hampson et al., 2000; Hampson and Murdoch, 2003[a]; Van Barneveld et al., 2006).

Results from *in vitro* studies have shown pPPA kills multiple antibiotic resistant strains of bacteria isolated from animals in Australia and Brazil (Hampson et al., 2004; Vargas et al., 2006). The MKC of pPPA to seven strains of multiple antibiotic resistant strains of *E. coli* isolated in Australia was 62-125 mg/L (Table 3). More recently, (Vargas et al., 2006) reported the minimum kill concentration of pPPA to two multiple antibiotic resistant strains of *E. coli* isolated in Brazil was also 62.5 mg/L (Table 4).

Table 3 Antibiotic resistance profiles of australian isolates of enterotoxigenic *Escherichia coli*.

***E. coli* Serotype**	**O8**	**O101**	**O138**	**O141ab**	**O141ac**	**O149**	**O157:117**
pPPA[1]	Minimum kill concentration was 62-125 mg/L						
Spectinomycin	R*	R	R	R	R	R	R
Ampicillin	R	R	S**	R	R	S	R
Lincomycin	R	R	R	R	R	R	R
Bacitracin	R	R	R	R	R	R	R
Apramycin	S	S	S	R	R	S	R
Tetracycline	R	R	R	R	S	R	R
Gentamicin	S	S	S	R	R	S	R

l Poly (2-propenal, 2-propenoic acid) R.* : Resistant, no zone of clearing S.** : Sensitive, zone of clearing > 2 mm present

Table 4 Minimum kill concentrations for Poly (1-propenal, 2-propenoic acid) MKC towards *Escherichia coli* and *Salmonella typhimurium* strains from Brazilian animals. Data are mean ± SEM of at least two experiments.

Strain	**Adhesins and toxins****	**Antibiotic resistance***	**MKC (μg/mL)**
E. coli (control)	NT*	PEN	8735 ± 15.3
E. coli	F107, F4 STa, STb	NEO, AZI, PEN, SUT, EST, AMP	62.5 ± 0
E. coli	F4, F107, LT	PEN, AMP, SUT, EST	62.5 ± 0
S. typhimurium	NT	PEN, OXA, AZI, EST	156.2 ± 93.7
S. typhimurium	NT	PEN, OXA, AZI, EST	93.8 ± 31.2

Adhesins and Toxins** -heat labile (LT); heat stable (STa or STb); Shiga (STx); F;42 F;6; F4; F107; and F5. NT* -not tested Antibiotic Resistance - Penicillin (PEN); Trimethoprim sulfa (SUT); Neomycin (NEO); Azitromicin (AZI); Streptomycin (EST); Ampicllin (AMP); Gentamicin (GEN); Enrofloxacin (ENO); and Oxacillin (OXA) (Oxoid Ltd,. Hampshire, England).

Table 5 Minimum kill concentration values of Poly (2-propenal, 2-propenoic acid) towards bacteria isolated from broiler chickens.

Microorganisms	*Clostridium perfringens* (7 isolates)	*Escherichia coli* (6 isolates)	*Salmonella infantis*	*Salmonella typhimurium* (3 isolates)	*Salmonella mbandaba*	*Lesteria monocytogenes* (3 isolates)	*Lesteria monocytogenes* (2 isolates)
MKC (ppm)	3.5 - 15	62.5 - 125	62.5	62.5	62.5	31	< 15

University laboratory models have been used to evaluate the effect of pPPA on chickens. pPPA helped to maintain health and to improve growth performance of broiler chickens (Hampson and Murdoch, 2003). Furthermore, pPPA reduced the impact of *Eimeria tenella* challenge on weight gain and feed conversion ratio and reduced oocyst output from the experimentally infected birds (Molloy et al., 2007). As with pig pathogens, results of *in vitro* testing of pPPA towards poultry pathogens isolated from broiler chickens demonstrate that it has MKC values of between 3.5 to 125 mg/L towards these bacteria (Tables 4 and 5).

Safety studies in target species

Target species safety studies in pigs have been undertaken at Murdoch University, Australia (D. Hampson and A. Murdoch, unpublished observations). Thirty two weaner pgs were divided into four groups of eight pigs and treated with either 30, 150 or 300 mg/kg/day of pPPA for at least 14 days or untreated. Assessment of health status by daily clinical examinations, incidence of diarrhoea due to β-haemolytic *E. coli*, weight, and clinical signs of toxicity or drug intolerance by haematological, biochemical and pathological indices showed no evidence of toxicity. These data suggest treatment of weaner pigs for 14 days with 300 mg/kg/day of pPPA which is ten times the label dose of pPPA does not induced toxicity.

A similar study was undertaken in broiler chickens (D. Hampson and A. Murdoch, unpublished observations). In this study, 3 groups of 22 broiler chickens received either 3 or 30 mg/kg/day of pPPA for 42 days or no treatment. Assessment of health status by daily clinical examinations, feed conversion ratios, weight gains, and clinical signs of toxicity or drug intolerance by haematological, biochemical, and pathological indices showed no evidence of toxicity. The results of this experiment suggest treatment of chickens for 42 days with 30 mg/kg/day of pPPA which is ten times the label dose of pPPA does not induced toxicity.

Conclusion

In conclusion, based on the current efficacy and safety data, it is suggested that pPPA has a potential to become a valuable a new class of therapeutic compound, with antimicrobial activity, in pig and poultry industries.

References

Amezcua, R., Friendship, R.M., Dewey, C.E., Gyles, C.L. and Fairbrother, J.M. 2002. Presentation of postweaning *Escherichia coli* diarrhoea in southern Ontario, prevalence of haemolytic *E. coli* serogroups involved, and their antimicrobial resistance patterns. Can. J. Vet. Res. 66:73-78.

ATCvet code: QA07AX90. 2006. WHO Collaborating Centre for Drug Statistics Methodology, Oslo, Norway.

Barton, M.D. 1999. The down-side of antibiotic use in pig production: the effect on antibiotic resistance of enteric bacteria. In: Manipulating Pig Production VII. Cranwell P.D. (ed.). Werribee, Australia: Australasian Pig Science Association: 194-199.

Bertschinger, H.U., Fairbrother, J.M. 1999. Post weaning diarrhea and edema disease. In: Diseases of Swine 8th ed. B.E. Straw, S. D'Allaire, W.I. Mengeling, D.J. Taylor. (eds) Oxford, UK: Blackwell Science. 441-454.

Francis, D.H. 2002. Enterotoxigenic *Escherichia coli* infections in pigs and its diagnosis. J. Swine Health Prod. 10: 171-175.

Hampson, D.J. 1994. Postweaning *Escherichia coli* in pigs. In: *Escherichia coli* in Domestic Animals and Humans. 2nd ed. C.L. Gyles (ed). Wallingford, UK: CAB International. 171-191.

Hampson, D.J., Buddle, J.R. and Melrose, G.J.H. 2000. Evaluation of a novel antimicrobial polymer for the control of porcine postweaning colibacillosis. Aust. Vet. J. 78: 117-120.

Hampson. D.J. and Murdoch, A.I. 2003a. Futher evaluation of a novel polymer antimicrobial for the control of porcine postweaning colibacillosis. J. Swine Health Prod.: 11: 223-228.

Hampson, D.J. and Murdoch A.I. 2003b. Growth enhancement in broiler chickens receiving CHEMEQ® polymeric antimicrobial. an Pathology: 32: 605-611.

Hampson, D.J., Wycoco, V.L., Murdoch, A.I. and McCauley, R.D. 2004. CHEMEQ polymeric antimicrobial: efficacy against multiple drug resistant strains of beta haemolytic *Escherichia coli*. 18th International Pig Veterinary Society Congress. Vol 1: 284.

Lucy, B., Cryan, B., O'Halloran, F., Wall, P.G., Buckley, T. and Fanning, S. 2002. Trends in antimicrobial susceptibility among isolates of *Campylobacter* species in Ireland and the emergence of resistance to ciprofloxacin. Vet. Record 151: 317-320.

Maillard, J-Y. 2002. Bacterial sites for biocide action. J. Appl. Microbiol. Symp. suppl. 92: 16S-27S.

Mardones, G. and Venegas, A. 2000. Chromogenic plate assay distinguishing bacteriolytic from bacteriostatic activity of an antibiotic agent. J. Microbiol. Methods 40: 199-206.

Molloy, J., Jorgensen, W., Blight, G., Chaine, E. and McCauley, R. 2007. Polyaldehyde antimicrobial effective in a laboratory model of poultry coccidiosis. European Veterinary Summit 13-14 Feb 2007.

Morris, J.A. and Sojka, W.J. 1985. *Escherichia coli* as a pathogen in animals. In: The virulence of *Escherichia coli*. M. Sussman (ed). London, UK: Academic Press. 47-77.

Park, C., Kim, H. and Kim, S. 1998. Mechanism of action of the antimicrobial peptide Buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. Biochem. Biophys. Res. Commun. 244: 253-257.

Simons C., Walsh, S.E., Maillard, J-Y. and Russell, A.D. 1999. Studies on the mechanism of the antibacterial action of ortho-phthalaldehyde. J. Appl. Microbiol. 87: 702-710.

Simons C., Walsh, S.E., Maillard, J-Y, and Russell, A.D. 2000. A note: Ortho-phthalaldehyde: proposed mechanism of action of a new antimicrobial agent. Lett. Appl. Microbiol. 31: 299-302.

Tanner, A.C. 2000. Antimicrobial drug use in poultry. In: Therapy in Veterinary Medicine, 3rd ed. J.F. Prescott, J.D. Baggot and R.D. Walker (Eds). Ames: Iowa State University Press. 637-655.

Vargas, A.C. de., Monego, F., Potter, M.K., McCauley, R.D. and Murdoch, A.I. 2006. Prelinimary results from minimum kill concentrations testing of CHEMEQ® polymeric antimicrobial towards multi-drug resistant Brazilian bacteria. 19th International Pig Veterinary Society Congress. Copenhagen. Denmark. P.31(20): 453.

Van Barnveld R.J., Welch, B., McCauley, R.D. and Murdoch A.I.M. 2006. Commercial application of Chemeq polymeric antimicrobial for the control of post-weaning diarrhoea. 19th International Pig Veterinary Society Congress. Copenhagen. Denmark. P.22(05): 337.

Van den Bogaard, A.E. and Stobberingh, E.E. 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. Drug 58: 589-607.