Chances for Phytogenic Feed Additives in Antibiotic-free Animal Production

PEER REVIEWED

The discovery of antibiotics is one of the most important achievements in modern medicine. Currently, about 30 different classes of antibiotics exist. Most antibiotics used for animals are also used in human medicine; only nine are exclusively used in animals.

In modern agriculture, antibiotics have been routinely used as growth promoters (long-term prophylactic application of antibiotics in sub-therapeutic doses) in animal feeding. Consequently, the widespread application of antibiotic growth promoters has strongly contributed to the development of resistant bacteria (Laxminarayan *et al*. 2015).

This basic situation encounters two completely different global conditions: On the one hand, in industrial nations the demand for healthy food increases. Moreover, in Western societies life expectancy increases and concomitantly the number of elderly, immunocompromised and hospitalised people. These people are more susceptible to chronic infections, many of which are caused by *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

On the other hand, in developing countries, the demand for animal-derived food increases exponentially, reflecting the upgraded lifestyle and upcoming wealth. Nevertheless, in both parts of the world, the containment of the further development of multi-resistant bacteria and with it the protection of consumer's health represents a major health issue (Givskov 2012).

Currently, a complete ban on antibiotics as growth promoters exists only in the European Union and in South Korea. In Australia, New Zealand and Mexico, the feeding of some, but by no means all, antibiotics is prohibited. The USA and Canada plan the withdrawal of antibiotics with relevance for the curation of human bacterial diseases at the beginning of 2017. However, in numerous other countries in the world, a tendency to exacerbate regulations regarding the use of feed antibiotics can also be observed (Laxminarayan *et al*. 2015).

The post-antibiotic era in animal feeding is associated with two major risks:

- An imminent loss of productivity
- A so far unpredictable increase in intestinal and systemic bacterial infections

The ban on feed antibiotics as growth promoters has expedited the research in alternative substances. Phytogenic substances, such as ground herbs and spices, or preparations, like essential oils, extracts or oleoresins, contain myriad highly active secondary plant metabolites, unfolding a broad range of therapeutic effects, including antibacterial effects.

This article briefly reviews the mode of action of antibiotics and evaluates the potential of phytogenic substances in their treatment in antibiotic-free livestock production.

Anti-bacterial Modes of Action of Antibiotics and of Phytogenic Substances

The main advantage of antibiotics is that they kill sensitive bacteria by specific mechanisms, summarised in Figure 1. One way by which antibiotics can kill bacteria consists in the inhibition of peptidoglycan synthesis of the bacterial cell wall. Another important class of antibiotics, namely tetracyclines and macrolides, inhibit bacterial growth via the inhibition of bacterial protein biosynthesis at the 30S-ribosomal or the 50S-ribosomal subunit, respectively. Two further important modes of action are inhibition of DNA topo-isomerase or RNA polymerase, inhibiting DNA or RNA synthesis. And last but not least, inhibition of folic acid synthesis will reduce bacterial enumeration as folic acid is essential for bacterial growth. Bacteria can develop resistance mechanisms against all antibiotic target sites, which risk is increased in said use as antimicrobial growth promotion (Apotheken Umschau 2013; Blair *et al*. 2015). With regard to bactericidal effects of phytogenic substances, it has been frequently postulated that essential oils can penetrate or damage the bacterial cell wall and cell membrane. Once inside the bacterium, essential oils are assumed to trigger the coagulation of cytosolic proteins and the efflux of essential intracellular compounds, and with it the destruction of bacteria (see Figure 2).

By definition, the terminus 'minimum inhibitory concentration' (MIC) represents the lowest concentration of a compound capable of inhibiting bacterial growth by more than 90 % (Mann & Markham 1997). The 'minimum bactericidal concentration' (MBC) even means a reduction of bacterial viability by more than 99 %. Table 1 gives an overview of the MIC concentrations of selected essential oils and essential oil compounds towards several microorganisms (prepared from information from Burt 2004). Considering an additional dilution effect of the feed in the intestine, the MIC concentrations give evidence that genuine bactericidal effects of phytogenic compounds in the animal cannot be obtained with phytogenic additives, unless at very high concentrations. Thus, it becomes evident that the in-feed application of phytogenic substances in amounts unrolling direct antibacterial or even bacteriostatic effects is neither economically feasible, nor they would be sensorially accepted by the animals.

Nevertheless, phytogenic substances have been shown to clearly reduce the pathogenicity of bacteria in the intestinal tract.

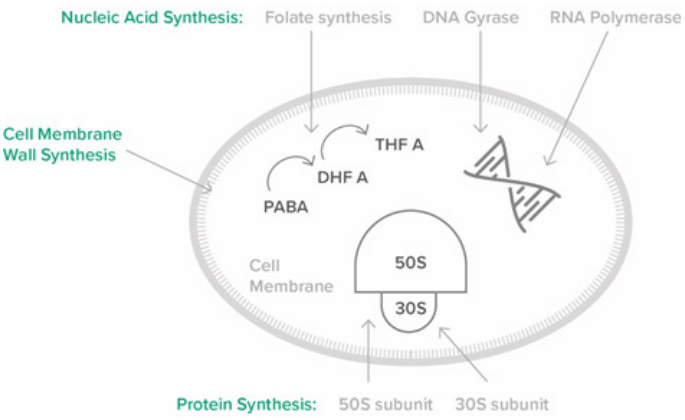

Figure 1. Damaging mechanisms of different antibiotic classes towards bacteria

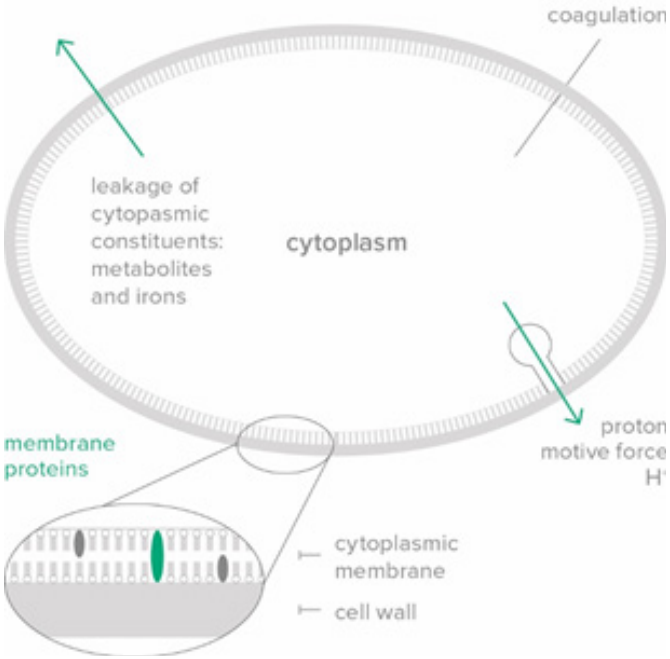

Figure 2. Assumed antibacterial mechanism of essential oils

Bacterial Quorum Sensing and Anti-quorum Sensing Effects of Phytogenic Additives

As shown above, the majority of antimicrobials target a limited number of basal life processes in bacteria, such as DNA and RNA replication, protein biosynthesis and cell wall synthesis. These were the 'low-hanging fruits' for traditional methods of antibiotic discovery. The prospect of a future post-antibiotic era calls for novel targets and innovative approaches to the control and cure of infectious diseases. An emerging topic in this regard is the bacterial regulation systems, especially the quorum sensing (QS). In many bacteria, the expression of virulence-associated genes and shielding against (or resistance to) the phagocytic cells of the innate immune system in already-formed biofilms is controlled by quorum sensing. QS signals are mediated by chemical signalling

Plant origin of essential oil	Bacteria species	MIC (estimated range) µl/ml ~ mg/ml ~ g/l ~ kg/t
Rosemary		
	Escherichia coli	4.5 - 10.0
	Salmonella typhimurium	> 20
	Bacillus aureus	0.20
	Staphylococcus aureus	0.4 - 10
Oregano		
	Escherichia coli	0.5 - 1.2
	Salmonella typhimurium	1.2
	Staphylococcus aureus	0.50 - 1.2
	Listeria monocytogenes	0.20
Thyme		
	Escherichia coli	0.45 - 1.25
	Salmonella typhimurium	0.45 - 20.0
	Staphylococcus aureus	0.20 - 2.5
	Listeria monocytogenes	0.15 - 0.45
Sage		
	Escherichia coli	3.5 - 5.0
	Salmonella typhimurium	10.0 - 20.0
	Staphylococcus aureus	0.75 - 10.0
	Listeria monocytogenes	0.20
Clove		
	Escherichia coli	0.40 - 2.5
	Salmonella typhimurium	> 20.0
	Staphylococcus aureus	0.40 - 2.5

Table 1. Minimum inhibitory concentrations of various essential oils towards different bacteria (after Burt 2004)

molecules referred to as autoinducers (AI). Both the type of AI and the intrabacterial receptors vary between bacteria. Whereas gram-negative bacteria produce acyl-homoserinelactones as AI, gram-positive bacteria mainly use short peptides for QS regulation (Figures 3 and 4). Accordingly, in gram-negative bacteria LUXR/I-type receptors mediate acyl-homoserinelactone-induced changes in gene expression, whereas gram-positive bacteria have more complex kinase-dependent signalling systems to regulate their QS-related gene expression. The luxS/AI-2 system is used for interspecies communication and the AI3/epinephrine/norepinephrine system, triggered by stress in the hosts organism, mediates inter-kingdom communication (Reading & Sperandio 2005).

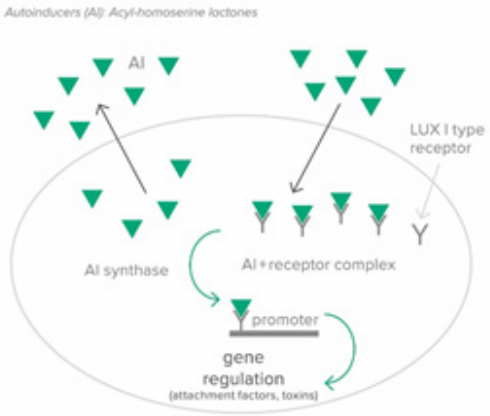

Figure 3. Quorum sensing in gram-negative bacteria using N-Acyl-Homoserine-Lactone derivatives as autoinducer molecules (AI)

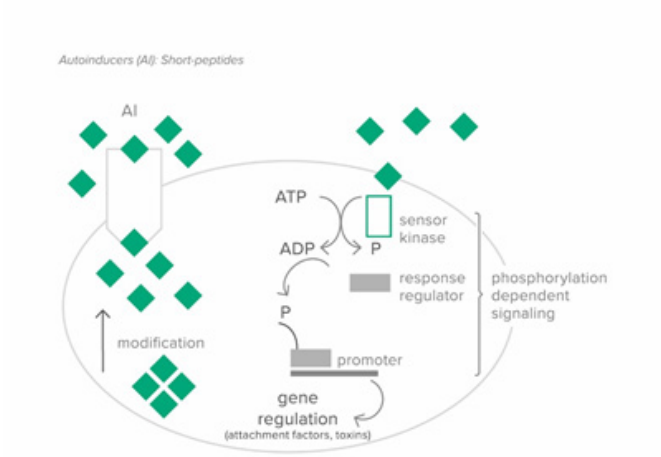

Figure 4. Quorum sensing in gram-positive bacteria using Short-Peptide-Molecules as autoinducer molecules (AI)

Regarding relevant pathogenic bacteria in animal husbandry, QS systems are well described for numerous *E. coli* strains, *Salmonella* and *Clostridia*. For enterohemorrhagic *Escherichia coli* (EHEC), the production of shigatoxins and formation of intestinal lesions are virulence mechanisms controlled by QS. The bacterial QS in the mentioned bacteria is additionally triggered by stress hormones released by the host due to the bacterial infection.

One important aspect studied in many publications on phytogenics and QS is their effect on biofilm formation. The development of a stable biofilm represents the first QS-dependent process within an infection in most pathogenic bacteria. Phytogenic substances, such as essential oils, have the power to counteract biofilm formation by QS inhibition. Thus, these phytogenic compounds are effective in disturbing the adhesion of pathogens in their potential hosts, even at concentrations at which neither the bacterial growth is inhibited nor an anti-bactericidal effect can occur. Girennavar *et al.* (2008), for example, demonstrated the inhibiting effects of grapefruit juice and isolated grapefruit furocoumarins on biofilm formation in *E. coli* and in *Salmonella typhimurium*. In a similar way, ursolic acid purified from plants showed strong inhibition on biofilm formation of an *E. coli* strain (Ren *et al.* 2005), which is controlled by AI-2 in that bacterium (Gonzáles Barrios *et al.* 2006).

A further example for the interference of phytogenics with virulence factor production without affecting bacterial growth was given by Lee *et al.* (2014). In this study, the effects of different coumarins on MIC, biofilm formation and virulence-related genes of *E. coli* O157:H7 were determined. The MIC values of coumarin, umbelliferone and esculetin against this EHEC strain were higher than 200 µg/ml. Incubation of the *E. coli* with 50 µg/ml of the above-mentioned substances reduced the biofilm biomass after 24 hours by 90 %, 80 % and 35 %, respectively. Interestingly, esculetin showed a downregulation of Shigatoxin like gene stx2. Despite having the higher impact on biofilm formation compared to esculetin, coumarin and umbelliferone did not downregulate stx2. The effect of plant extracts or their active compounds on virulence-associated genes or toxin production was determined also in several other studies (see Table 2).

Substance	Concentration [µg/ml]	Effect	Reference
Allspice (Ethanolic extract)	200	Decreased Shigatoxin 1 and 2 production	Takemasa et al. (2009)
Clove (Ethanolic extract)	200	Decreased Shigatoxin 1 and 2 production	
Cumin (Ethanolic extract)	200	Decreased Shigatoxin 1 and 2 production	
Eugenol	50	Decreased Shigatoxin 1 and 2 production	
Oregano oil	50	Downregulation of intrabacterial AI-receptor, intestinal attachment factor, Shigatoxin 2, and fimbriae synthesis	Mith et al. (2015)
Carvacrol	50	Downregulation of of intrabacterial AI-receptor, intestinal attachment factor, Shigatoxin 2, and fimbriae synthesis	
Quercetin	50	Downregulation of *AI-2 synthesis, flagella regulation and type III regulation*	Lee et al. (2011)
Kaempferol	50	Downregulation of *AI-2 synthesis, flagella regulation and type III regulation*	
Myricetin	50	Downregulation of *AI-2 synthesis, flagella regulation and type III regulation*	

Table 2: Effect of phytogenics on *E. coli* O157:H7 virulence-associated genes and toxin production

The *in vivo* importance of QS inhibition can be demonstrated by use of *Caenorhabditis elegans* as model organism. It was shown by Lee *et al.* (2014) that addition of 50 µg/ml or 100 µg/ml esculetin to culture medium with *E. coli* O157:H7 infected *C. elegans* reduces virulence of the pathogen and increases the lifespan of *C. elegans*. Similar observations were also made in *E. coli* O157:H7 infected *C. elegans* grown in a culture medium, containing either no or 0.5 % broccoli extract (Lee *et al.* 2011). The addition of the broccoli extract increased the survival rate of *C. elegans* to 28.5 % compared to the untreated control.

In summary, a wide range of studies demonstrates the effect of phytogenic substances against QS regulated virulence factors of pathogenic bacteria at concentrations below their MIC values. Substances interfering with QS may have a longer economic lifetime compared to antibiotics. In the post-antibiotics era, the manipulation of the 'small molecule controlled QS signaling pathways' using plant compounds is therefore an emerging topic with regard to the maintenance and improvement of animal health. However, literature data on the impact of phytogenics on the QS systems is focused mainly on model organisms such as *Vibrio harveyi* or *Chromobacterium violaceum*, or bacteria with clinical importance such as multi-drug resistant Pseudomonas aeruginosa. Among the important pathogens for livestock species, considerable research was performed with *E. coli* O157:H7 strains, whereas information on other pathogens is scarce. Further research is necessary to unravel QS-inhibition in additional pathogens. The immense variety of phytogenics provides doubtlessly the

promising basis to investigate the reduction of virulence in many bacterial species by QS inhibition.

Conclusion

Whereas the use of in-feed antibiotics globally is pushed back by law and customer pressure for health reasons, the use of phytogenic feed additives has become an increasingly important and accepted alternative strategy to enhance animal performance. Since phytogenic substances have been shown to prevent the colonialisation and virulence of pathogenic in concentrations even below their MIC, by interference and disturbance of the major bacterial regulation system 'quorum sensing'. In conclusion, phytogenic substances represent one important pillar in fighting bacterial disorders in farm animals in the post-antibiotics era.

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