SMALL MOLECULES OF NATURAL ORIGIN AS A SOURCE OF ANTIMICROBIAL AGENTS IN PIG BREEDING - REVIEW

***Rozkot M.* [1], *Frydrychová S.* [1], *Benešová N.* [2], *Opletal L.* [2]**

[1] *Institute of Animal Science, Prague, Czech Republic*

[2] *Charles University in Prague, Czech Republic*

Abstract

The use of antibiotics is currently limited within the framework of the prevention of the infectious disease in farm animals in the EU. Formerly used antibiotic growth stimulators markedly enhanced the quality of animal breeding and decreased the incidence of infectious diseases. After their ban, the new substances, which do not cause a possible resistance to pathogenic agents, are searched. One option is to use the small molecules of natural origin, particularly the primary and secondary plant metabolites. It is possible to consider the use of a new generation of cationic peptides isolated from plants or animal and bacteriocins. The use of these substances in the pig breeding is directed into the feeding stuff in a form of additive compounds, whose aim is to prevent the development of microbial infection in GIT and the area of abdomen, increasing the immunity of animals and decreasing the inflammatory processes in boar genitals. The second area is the application of these substances in the cryopreservation of boar semen and into the extenders for the short-term storage of semen prepared for AI. According to the EU recommendation, a substantially higher attention must be paid to the research of natural substances in agricultural sphere.

Key Words: Pig breeding, Natural antimicrobial substances, Feeding stuff, Preservation of boar semen

A widespread application of antibiotics, for the prevention and therapy of infections, leads to the occurrence of the antibiotic-resistant bacterial strains in human and veterinary medicine. The farm animals (particularly bovines) may act as potential gene resources of this resistance to anti-invasive drugs and that is the cause of complications in the public health field. From the point of view of the farm animals, the situation has not been understood fully yet. At the foreground, an economic interest is more important than a later hazard which may arise from an extensive application of the antibiotics. There are views that methicillin-resistant strains of *Staphylococcus aureus* and multi-resistant gram-negative bacteria should be thoroughly examined, because they are broadly involved in the human field as well as in the animal production [Garcia-Alvarez et al., 2012]. An antimicrobial multi-drug resistance [Nikaido, 2009] is quite often developed, so it makes impossible to succeed in the therapy.

There have been methods by which a possible development of undesirable resistance is ascertained, but they have limited validity. The fact that the substance does not build up the resistance cannot be recognized sooner than after 10 years of circulation in normal natural biological cycle at least. It cannot be questioned that the antibiotic resistance has a genetic origin [Votava, 2001]. In principal, it is possible to state that certain bacterial species are primarily resistant: e.g., gram-negative enteric bacilli are resistant to penicillin, macrolides and lincosamides, *Pseudomonas aeruginosa* is resistant to clotrimoxazol, members of *Klebsiella* genus to ampicillin, *Streptococcus* genus to aminoglycoside antibiotics and *Enterococcus* genus to cephalosporins. Currently, there is a worldwide problem with an acquired bacterial resistance. The origin of the resistance could be possibly obtained by one of these ways: gene mutation of a chromosome, transfer of a plasmid containing a resistant gene during bacteria conjugation and transfer of plasmid by bacteriophage.

There is no doubt, that a cross-resistance forms to chemically similar antibiotics (it means to penicillins and cephalosporins, tetracycline and macrolide groups, etc.). Therefore, if the bacterium is resistant to G-penicillin after the transfer of plasmid, it will be certainly resistant to V-penicillin as well. It is known that the members of *Staphylococcus* genus, who are resistant as methicillin and resistant to penicillin. However, there is a great variability of bacteria. A single principle is not valid here. From the point of view of bacteria's sensitivity to antibiotics, a „specific“ microorganism identification is necessary to carry out by methods of DNA sequence analyses, but even this may not be able to discover interactions of genes. An informative value has the ratio of guanine and cytosine bases and adenine and thymine bases, use of gene probes and determination of metabolites under strictly controlled conditions by the GC/ MS (gas chromatography – mass spectrometry) methods. From the phylogenetic point of view, these microorganisms keep a number of primary adaptive phenomena. They are ancient and simple. So, it is hard to predicate the clarity of their metabolic behaviour within the change of conditions, therefore the whole matter of antibiotics' usage, whether additive or therapeutic, is very risky. Every possible hazard must be considered very carefully.

In some fields, if we want to be informed about the resistance, respectively cross-resistance of low-molecular substances used as a replacement of antibiotics, we will find a relative lack of information. It is mentioned that the resistance is developed through a different mechanism to these substances than to antibiotics (a transfer of plasmid is probably not applied here). A significant fact is that it is not supposed to be permanent.

With all the considerations on applicability of antimicrobial agents, if it is related to the antibiotics, it is vital to take into account that the essential negative role, for human, plays subliminal doses of these substances [DuPont et al., 1987]. Therefore the efforts, in using other efficient substances, are very stimulating.

Alternatives to Antibiotic Treatment

Of course, quitting the use of antibiotics in therapy (sometimes even in the prevention) is virtually impossible for three reasons. The first is seriousness of the disease (elimination of resistant strains in actual stable infections is virtually impossible without the use of antibiotics), the second main reason is the tradition and the certainty that the antibiotic is the most efficient remedy for coping with an infection (and sometimes also the price). The third reason is a limited knowledge of the effect of natural substances in the field of agricultural research sector. These substances can be an alternative in numerous infections, especially of preventive character [Opletal, 2003].

The demandingness, which the eradication of serious gram-positive infections in human requires, is great [Metzger et al., 2009]; it is without any doubts that the same problem occurs in the animal production field as well. We have to admit that human treatment seems to be simpler, taking into account every social and therapeutic background. It is because, it covers just single animal species which is, to a considerable measure, consistent to its physiology and needs. But in the case of the farm animals, the situation is markedly divergent: there are sea animals, terrestrial animals (ruminants, non-ruminants, birds) and social insect. Each of these groups has its own physiological requirements and mainly a specific strictly defined reservoir of pathogens, which are characteristic just for this group. The prevention and treatment of microbial infection, by means of low-molecular natural substances, is reluctant to a certain extent. Even if there have been overview studies on the use of natural substances for the treatment of a microbial infection in aquatic animals [Makkar et al., 2007], ruminants [Benchaar et al., 2007], cattle [Makkar et al., 2007] and broilers [Huyghebaert et al., 2001], resp. poultry [Applegate et al., 2010], they cannot give any definite answer. This interest is based on the political and social pressure of the EU, resp. the efforts in minimising pollution and damage to the environment as a result of intensive livestock production. From the point of view of the European prospective, there is observed, for example, the influence of essential oils and aromatic plants in the animal nutrition [Franz et al., 2010], use of plant extracts and other products for influencing veterinary infections [Eloff et al., 2009] and others.

Natural Substances Usable in Prevention and Therapy

Although, low-molecular non-peptide plant, yeasts and fungi metabolites are meant under the term „antimicrobial substances of non-antibiotic character", recently some statements appear on the antimicrobial peptides as an alternative to the antibiotics. This group, of low-molecular substances of a new generation of a cationic peptide nature, is isolated from plants and animals. These substances are more specific to the microbial agents and could be produced by the genetically modified organism [Marhall et al., 2003]. A similar role could play the bacteriocins – peptides which are synthesized in ribosomes [Naidu et al., 2006] – produced by lactic acid bacteria [Galvez et al., 2007]. Substances with usable antimicrobial action have been isolated also from marine algae [McDougald et al., 2006] and cyanophytes (phycobiliproteins) [Rodriguez et al., 2005].

The major part of substances with antimicrobial effect belongs to the group of low-molecular metabolites which may be added in the feedstuffs in a very simple form of dry extracts from different plant parts. Finding these substances in medicinal plants represents a great area of natural resource phytochemistry [Rios et al., 2005]. It has been observed, e.g. in the case of *Vibrio cholera*, that the extracts from neem (*Azadirachta indica*), Guazuma (*Guazuma ulmifolia*), apple (*Malus sylvestris*), hop (*Humulus lupulus*), green tea (*Camellia sinensis*) decrease the secretion of the cholera toxin [Yamasaki et al., 2011].

The phenolic compounds of various structures (tannins, flavonoids, anthocyanins, simple phenolic glycosides, etc.) show a significant antimicrobial effect. They are contained in fruits, vegetables, green tea, wine and propolis. Except an anti-invasive activity on pathogenic bacteria, they have also an antioxidant, anti-allergic and anti-inflammatory effects and can also operate as chelators of certain metals [Balouche et al., 2009]. Berry plants (cranberry, cloudberry, raspberry, strawberry and bilberry) are very significant sources of these substances. In particular, they contain ellagitannins and substances which counteract against *Salmonella* and *Staphyllococcus* genera in digestive organs [Puupponen-Pimiae et al., 2005]. Catechin derivatives (epigallokatechin-3 -gallate) and some phenolic acids (caffeic, chlorogenic, gallic and quinic) are significantly applied in this area [Chirumbolo, 2011]. The immunomodulatory activity has been found in certain substances of tannin nature [Kolodziej et al., 1999].

However, the usable antimicrobial effects are not peculiar just for phenolic substances of flavan and organic acid type. But they could be caused by the biologically active glycosides of stilbenoid, phenylethanoid, phenylpropanoid types and cyclic aromatic structures as well [Dembitsky et al., 2005a]. From this point of view, the assessing of secondary plant's and fungus' metabolites must consider hemi- and monoterpene glycosides of acyclic, monocyclic and bicyclic type, including iridoids [Dembitsky, 2006], carotenoid glycosides [Dembitsky et al., 2005b] and a number of further compounds, which can be classified in various chemical structure types, as usable for their antimicrobial effect. The dipicolinic acid (natto), sarcocodins (*Sarcodon scabrosus*), hinokitiol (*Juniperus* sp.), chitosan (shrimps), pectin-degrading product from Howthorn (*Crataegus* sp.), Yucca saponins (*Yucca shidigera*) and capsaicin (*Capsicum annuum*) are also among them [Sakai, 2000]. For the food protection are also designed some nontoxic substances of primary metabolite character which originate from natural models, e.g., 1,5-anhydro-D-fructose [Lundt et al., 2010], e-polylysine, pectin degradation products and glycine [Inatsu et al., 2007]. We cannot omit the products of yeast's nature [Cerqueira et al., 2011], especially those of galactomannan type, which can be used for encapsuling of particles in the feedstuff and may act as protective agent, and substances of animal origin – milk protein [Inatsu et al., 2007], lysozyme and lactoferrin [Davidson et al., 2007], which currently play an important role antimicrobial protection.

Essential ingredients from members of Brassicaceae family (thiosulfinates) and allicin from garlic (*Allium sativum*), including its degradation products [Sakai, 2000], are also taken into account. Certainly favourable is the fact that these substances have not only antimicrobial action, they even show the anti-oxidative, anti-inflammatory, antimutagenic properties and, in some cases, they also operate as surfactants.

From the literature, it is apparent that the essential oils are viewed as a raw material with the best availability of antimicrobial action. More than 18 overview studies and 470 papers dealing with original studies, patents and clinical evaluation of essential oils were published in the literature with impact factor in the last 10 years. The essential oils are represented by a complicated mixture of terpenic compounds of a various chemical structure (particularly monoterpene hydrocarbons, alcohols, ketones and some other derivates). They are thoroughly studied, even if they are examined through historical experience [Rios et al., 2005]. In term of their use, a particular attention shall be given mainly to pathogenic strains of bacteria (*Salmonella* sp., *Esche-richia coli* O157:H7, *Listeria* sp., *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Clostridium* sp., *Campylobacter* sp.) and to microscopic fungi (*Aspergillus* sp., *Cladosporium* sp.) and yeasts (*Candida* sp.) influencing full production animal health. Single essential oils or some of their compounds are efficient not only against gram-negative and gram-positive bacteria but also against some yeasts and further organisms. Plant taxons, which produce these metabolites with anti-infection action, are numerous. However, it has been applied only in a small fraction of them in practise yet.

An attention shall be paid to the effect of essential oils from various species of *Citrus* [Padilla-de La Rosa et al., 2011], *Alpinia zerumbet* [Correa et al., 2010], *Mentha* [Deans et al., 2007], *Lavadnula* [Deans et al., 2002a], *Eucalyptus* [Deans 2002b], representatives of essential species from *Asteraceae* [Martinez et al., 2008] and *Cistaceae* [Bedoya et al., 2009] families, from endemic flora of the Mediterranean area [Kintziou et al., 2001] and to many others. Their antimicrobial effect is permanently monitored and assessed [Skrinjar et al., 2009]. The essential oils are also observed in the term of influencing the bacterial flora in rumen (*Ruminobacter amylophilus*) [Wallace et al., 2004], respectively an effect on ruminal metabolism with other substances – saponins and tannins [Benchaar et al., 2007]. Their use in the food protection is under discussions [Tajkarimi et al., 2010]. These studies are supposed to have the same validity for the use within the feeding stuff production.

Practically usable resources originate from a small number of taxa located in several families, see Blaschek [Blaschek et al., 2011].

A benefit of the application of natural substance into the feeding stuff has a sense only when the substances are stable and significant losses do not occur. Essential oils, the most frequently named and used sources, create only one group of substances. In plant sources are also other secondary metabolites, which by means of their action synergise the effect of essential oils. As an example, we can mention plant species *Zingiber officinale* and members of *Curcuma* genus which are popularly used in India [Meena et al., 2010]. Rhizomes of these plants contain the essential oils as well as a group of other secondary metabolites. These metabolites have a certain antimicrobial effect, but their further biological action (anti-inflammatory, cholagogic, hepatoprotective effect, antioxidant activity, etc.) is supported by the effect of the essential compound. This phenomenon of supportive effect of metabolites is mentioned in a number of studies [Singh, 2012].

Applicability of Natural Antimicrobial Substances in Pigs

On this subject is only a small group of studies in the literature which does not provide options for creating a deeper opinion in what way the application of natural sources of antimicrobial substances shall proceed in pig breeding in several years. This is a paradoxical position to a certain extent: pig is, due to its metabolism, very close to *Homo sapiens* species and so there exists an idea that the research should provide a wider basis for the application of appropriate knowledge in this area. The reality does not correspond to it: the research results are more numerous in the field of ruminant and poultry.

A presumption, that among secondary metabolites of algae, fungi and plants shall be discovered substances of the type of small molecules, which are of the same efficiency as antibiotics, is creating an incorrect conceptions. However, the accessibility, not very significant financial demandingness, the complexity of their effect and the fact, that they do not generate the resistance on them, are a great benefit of the natural substances. Naturally, a condition for filling these factors is a systematic and consequent research in the field of phytogenic additives.

Infection Prevention

The best way to eliminate a possible development of infectious diseases in pigs is an administration of natural substance mixture which shall not have only anti-invasive (antimicrobial, antifungal and anticandidose effects), but enhance the immunity and provide an anti-inflammatory effect, as well. Some of the authors mention it very instructively [Gallois et al., 2009].

An immune response by a *per os* administration of antigens is managed very well due to the function of local intestinal mucosa system. It must respond to the potential pathogens in the case of various antigens from feeding stuff or it may lead to allergic or inflammatory conditions in consequence of the commensal bacteria's metabolism. These processes are managed by means of oral tolerance in adult animals, but the immune system of piglets is not developed enough and its modulation may play a decisive role in a final response to these antigens [60]. In this respect, it is appropriate to use a complex mixture of substances given below and take into account the animal ontogenesis.

Yeast polysaccharides

Some mannoproteins, b-D-glucans and a-D-mannans modulate the immune response in the mammals through some specific interactions with a various immunocompetent cells [Kogan et al., 2007]. For the b-D-glucans, a direct interaction, between macrophages and polymorphonuclear cells, occurs [Tzianabos, 2000]. The mannans are changed by immune reaction as a result of specific bonds on mannose receptors [Tzianabos, 2000]. For these purposes, the preparations from yeasts (*Saccharomyces cerevisiae*) cell walls are used, which are commonly applied in pig feeding stuffs.

Plant metabolites (plant extracts)

The above mentioned polysaccharides cannot be expected to possess other than immunostimulatory effects. But the experience shows that plant extracts may offer advantages not only in the immune system boosting and so prevent the diseases of farm animals [Wenk, 2003]; this fact has been reflected in the interest to apply them into feeding stuffs [Windisch et al., 2008]. The biological active compounds are various. There are not the sufficient numbers of studies, which are completed with a statistical evaluation, related to the influence of immunity *in vivo*. A mixture of compounds is always used, often unstandardized, which keeps us from getting an unambiguous assessment. Historically proven medicinal plants are used in human therapy to cure gastrointestinal tract disorders [Blaschek et al., 2011]. Some of these plants have an antimicrobial activity due to their content of essential oils [Mimica-Dukic et al., 2007], e.g. Peppermint (*Mentha ´ piperita*), or Chamomile (*Matricaria recutita*), others have only an immunostimulatory effect, e.g. Cat´s Claw (*Uncaria tomentosa*), Echinacea (*Echinacea pallida*), Astragalus (*Astragalus membranaceus*), but some of them show both types of the action, e.g., Aloe (*Aloe vera*), Angelica (*Angelica* sp.), Reishi Mushroom (*Ganoderma lucidum*), Baikal skullcap (*Scutellaria baicalensis*), Ginger (*Zingiber officinale*) [Tan et al., 2004] and Garlic (*Allium sativum*). Their use can be particularly beneficial from the point of view of antimicrobial and immunomodulation effects [Pendbhaje et al., 2011, Chaturvedi et al., 2011].

Plants of Lamiaceae family (Basil, Dill, Fennel, Marjoram, Mint, Rosemary, Oregano, Sage and Thyme) [Craig, 1999] represent a significant reservoir of essential oils. From the point of antimicrobial view, they seem to be promising, especially, those essential oils with a higher content of thymol and carvacrol (oregano, thyme, savory) [Burt, 2004], because they have an antimicrobial [Baydar et al., 2004] and, potentially, an immunomodulation effects [Woollard et al., 2007]. In a number of studies has been described an influence of essential oils, with a content of both mentioned terpenes, on a pig health and development [Walter et al., 2004, Burt, 2004]. The cinnamaldehyde (*Cinnamomum* sp.) and the capsaicinoids from oleoresins of Cayenne Pepper (*Capsicum frutescens*) are the other components found in studied mixtures.

Within the study of antimicrobial and immunostimulatory activities, the plants from field of the Traditional Chinese Medicine (*Angelica sinensis, Atractylodes ovata, Codonopsis pilosula, Glycyrhizza uralensis, Ligusticum chuanxiong, Paeonia albiflora, Rehmannia glutinosa*, etc.) also begin to find their use. These plant sources have been solved in a large study [Opletal et al., 2009]. The extracts from these plants show an applicable immunostimulatory and antimicrobial effects.

The immunostimulatory effect (and even antimicrobial, possibly antiviral) can be also applied from other plants, especially from the alkamides from Echinacea (*Echinacea* sp.) [Nasir et al., 2009]. The extracts of these plants (*Echinacea angustifolia, E. pallida, E. purpurea*) are commonly and successfully used in human therapy. But in the case of application in pigs, it will be necessary to carry out a number of following studies [Gallois et al., 2009], because the results are not unambiguous.

The extract (saponin fraction) from the wood of Quillaja (*Quillaja saponaria*) [Milgate et al., 1995] is already successfully used in order to enhance intestinal immunity [Francis et al., 2002]. But in the case of *Salmonella enterica*, serovar *Typhimurium*, the extract itself has not shown any significant immunostimulatory efficiency [Turner et al., 2002]. Apparently, it is a result of a poor purity of used saponin preparation [Ilsley et al., 2005]. Most likely, there has been applied a content of tannins, which have some antinutritional properties [Singh et al., 2003].

The Use for Sperm Protection

A microbial decontamination of controlled sampled boar sperm is currently problematic. A sampled sperm is frozen until its use and subsequently diluted by means of an appropriate extender (with an addition of antibiotics). By the current knowledge, this is a process related to negative factors in term of a later vitality of spermatozoa and capability to fertilise the egg.

It is evident that the addition of antibiotic growth stimulators may improve the reproductive capabilities of boars and sows. For example, there has been observed that grisein, added into the feeding stuff, increases the fertilizing capacity of spermatozoa, fertility of sows and decreases a number of stillborn piglets [Sergeev, 1969]. The addition of cormogrisin or bacitracin, into the feeding stuff, improved the ovarian development in sows, increased the fertility and decreased the embryonic mortality and improved the quantity and quality of sperm, as well [Sergeev et al., 1974]. But this way is currently completely closed.

At the past, it was possible to use the substances such as sulfonamides and antibiotics for preserving, e.g. sodium salts of sulfamerazine, sulfamethazine, sulfisomidine, penicillin, streptomycin [Mizuho et al., 1963], and also the salts of carbenicillin, gentamycin and sulphonamide of streptocid [Prokoptsev et al., 1992]. According to the sensitivity of bacterial strains isolated from the ejaculate, the antibiotics have been selected, with the respect to the standard 72 hours of storing periods, after the dilution [Mazurova et al., 1991]. A spectrum of antibiotics has been selected according to the predominant presence of microorganisms (*Staphylococcus* sp., *Escherichia coli*). Especially, there has been applied neomycin, streptomycin, monomycin, tetracycline, biomycin, levomycetin, penicillin and erythromycin [Charenko et al., 1975]. Similarly, linkomycin and colistin have been recommended in the diluents together with erythromycin. The decreased cell motility occurred only in ~2% sperm samples [Hovorka, 1983]. Within the study of the effect of linkomycin and spectinomycin (Lincospectin), there has not been found any effect on sperm mobility and acrosome integrity in a dose of 4.5 mg/ml of the diluents. Similar results have been proven for the combination of Lincospectin with penicillin, streptomycin or gentamycin where has not been observed any negative effects after the routine stable insemination [Waberski et al., 1990].

Generally, the result of an artificial insemination, which carrying out from a frozen boar sperm, is the decreased fertility and reduced number of the young in farrow. After the dilution, the unfrozen sperm can be stored, without the occurrence of the decreased capacity of fertility, for 72 hours at maximum. Microbial contamination, bacteria effect on sperm surviving, effect of bacterially contaminated boar sperm on reproductive efficiency and search of the prospect antibiotics which will be effective to a bacterial growth during storing and decreasing effect of undesirable substances present in sperm have been observed. The result is a finding that a storage life at 15°C, which is suitable for the improvement of becoming pregnant, can be extended for 7-8 days assuming that a bacterial growth has stopped. In particular, the aminoglycosides (dibecacin, amicacin, gentamycin), polymyxin B or colistin are effective against the growth of enteric bacteria (*E. coli*) [Sone, 1991]. Also, it has been shown that polymyxin B neutralizes bacterial endotoxins and enhances the quality after the dilution within cryopreservation [Okazaki et al., 2010]. The SLC (Single Layer Centrifugation) method, designed for the bacteria elimination from ejaculate, may also decrease the application of antibiotics within the sperm preservation [Morrell et al., 2011]. Currently, it is not yet known to what measure the method can be practically exploitable in terrain.

If the antibiotics are administered systemically (phenoxymethylpenicillin, oxytetracycline), it may occur adverse changes in boar testes related to the content of ribonucleoproteins, sulfhydryl groups of proteins, cytoplasmic glycoproteins and hydrolase activity [Sazonov, 1974]. To what measure may the antibiotics, formerly used for the sperm preservation, affect the acrosome structure has not been determined absolutely yet. There is a report, that calcimycin (A23187) induces morphological changes in human sperm cells [Russell et al., 1979], but the transfer of these results on pig cells is difficult due to a different structure of membrane microfilaments and tubular microelements.

After assessing a current knowledge is evident, that the knowledge, regarding possibilities of the use of natural substances of a small molecules type for the decontamination of boar sperm, is low. The sperm can be contaminated by 25 invasive agents of a various structure at minimum [Althouse et al., 2008], and therefore the action against them is problematic. Bielanski summarizes that the application of germicide procedures and substances for the regulation of microorganism presence in sperm and embryos of human and farm animals in his very instructive study [Bielanski, 2007]. Besides the antibiotics application, he introduces a sperm acidification, enzyme (trypsin) use, immunostimulants (immunoextenders) (colostrum, lactoferrin), photosensitive substances and pigments (hematoporphyrin, thiopyronin). A subsequent sperm fertilisation capacity is not known in a number of these procedures and substances.

A recent single study shows an inhibition effect of (*E*)-cinnamaldehyde on *Campylobacter* in poultry sperm. The substance does not affect the sperm viability and may be used during *in vitro* storing of poultry sperm [Liu et al., 2012]. This message creates a precondition that not only aromatic aldehyde, present in essential oil from Cinnamon (*Cinnamomum verum*), but a number of other monoterpene substances, introduced in this overview, could have similar antimicrobial effects. Taking into account, that aldehydes together with ketones are relatively reactive substances (and so potentially toxic), then an idea emerges that a number of essential oils could be effective even in sperm. But these studies with essential oils and further low-molecular natural substances were carried out in a low measure until now [Lustyková et al., 2012, Kukla et al., 2012].

Conclusion

It is possible to assume that a quality of boar sperm is not dependent just on a microbial spectrum, which occurs in it. But the whole functional state – immunity, inflammation and microbial dissemination in abdomen and boar reproductive organs is also very important [Opletal et al., 2010a, Opletal et al., 2010b]. Therefore, it is desirable, for the application of natural substances, to use not only compounds which favourably influence the immunity and has an antimicrobial effect but also to solve a rational application of prebiotics and probiotics. These compounds may very favourably influence the bacterial abdominal macrophage activity and secure a decreased measure of invasive agents' infiltration into boar reproduction organs, softening possible inflammatory processes and decreasing undesirable "waste" materials in ejaculate.

Though, it has not been proven that terpenic compounds in essential oils had some distinctively toxic effects yet. It could be the silent toxicity here. It concerns the spermicidal effect, which has a number of antimicrobial substances. This has been proven, e.g., after the application of essential oil from *Trachyspermum ammi* [Kang et al., 2012] in human spermatozoa. This type of action is well detectable, but if it is light, it may not signalize the toxicity of the substance in the first phase. But the affection of the cell membrane stands at the beginning of sperm cell damage, particularly, if we add the effect of the ROS (Reactive Oxygen Species) established during the manipulation and sperm storing. The ROS do not affect only the acrosome and flagellum but intervene into the mitochondrial system with a subsequent influence on respiration. The essential oil from Garlic (*Allium sativum*), which were applied on mouse hepatic microsomes, has shown that the diallyldisulfid damages the mitochondrial functions directly. It is a result of membrane lipids oxidation initiated due to glutathione- and Fe-dependent formation of the ROS [Caro et al., 2012]. For these reasons, it is necessary to apply appropriate antioxidants in ejaculate before the cryopreservation.

Prospective area, which should be developed, is the determination of antimicrobial proteins (lantibiotics, etc.) effect. These substances will be of a great significance in near future. Their use can be considered in the sperm purity protection, but not all of them will have favourable effects, e.g., antimicrobial peptide, bacteriocin subtilosin A (*Bacillus subtilis*), are effective against *Gardnerella vaginalis* and does not affect physiological vaginal flora. A member of *Lactobacillus* genus has a marked spermicidal effect [Silkin et al., 2008].

From the above mentioned reasons, for essential oils and other natural substances, which have been indicated as promising for the use in practise will be necessary to solve their technological implementation into the feeding stuffs. The essential oils are relatively volatiles and their release from the compounds of the feeding stuffs, oxidative changes during feeding stuff production and organoleptic properties create a certain barrier for their use. But this barrier can be overcome, because there are known methods of their protection from the other branches of the industrial use of these substances which can be analogically used even in the feed industry.

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Corresponding Address:
Lubomír Opletal
Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Pharmaceutical Botany and Ecology, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
E-mail: opletal@faf.cuni.cz

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