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SUBSTITUTION OF ANTIBIOTIC GROWTH PROMOTERS IN ANIMAL NUTRITION BY BIOACTIVE PLANT-DERIVED PRODUCTS

A REVIEW OF THE LITERATURE

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Abstract

Based on the analysis of scientific literature, four plant species with the most intensive biological effects beneficial for growth promotion (namely antimicrobial, antioxidative, immunomodulatory and anti-inflammatory effects) were identified as a result of this thesis. From the total number of 16 plants, *Melissa officinalis, Ocimum sanctum, Thymbra capitata*, and *Zingiber officinale* were found to be the most prospective species. Their antimicrobial effect was proved *in vitro* by low values of MIC ranging from 167 to 300 μg/ml for *M. officinalis, O. sanctum* and *Z. officinalis*; for *T. capitata* MIC was 0.05 μl/ml. The antioxidative effect was evaluated *in vitro* by DPPH assay, when the IC_{50} ranged from 8 to 88 μg/ml. The immunomodulatory effect was observed *in vivo* to be significant for *O. sanctum* and *Z. officinale* at the doses of 0.2 mg/kg and approximately 20 mg/kg respectively. Anti-inflammatory effect was proved for *T. capitata* by *in vitro* 5-lypoxygenase assay, when IC_{50} was 93 μg/ml. *M. officinalis*, *O. sanctum* and *Z. officinale* were tested *in vitro* showing significant effects at doses ranging from 50 mg/kg to 400 mg/kg.

The immunomodulatory activity still has to be tested in *M. officinalis* and *T. capitata* prior to their further investigation on the growth promoting effect. As a result of this thesis, *M. officinalis, O. sanctum, T. capitata*, and *Z. officinale* are suggested for further research focused on the development of possible alternatives to antibiotic growth promoters in livestock animals.

Key words: antibiotic, growth promoter, plant, extract, antimicrobial, antioxidative, anti-inflammatory, immunomodulatory.

Abstrakt

Na základě analýzy odborné literatury byly jako výsledek této práce identifikovány čtyři rostlinné druhy vykazující nejintenzivnější biologické účinky (konkrétně antimikrobiální, antioxidační, imunostimulační a protizánětlivé účinky) příznivé pro podporu růstu hospodářských zvířat. Z celkového počtu šestnácti rostlin byly nejperspektivnějšími druhy shledány *Melissa officinalis*, *Ocimum sanctum*, *Thymbra capitata* a *Zingiber officinale*. U *M. officinalis*, *O sanctum* a *Z. officinalis* byl antimikrobiální účinek prokázán nízkými hodnotami minimálních inhibičních koncentrací v rozmezí od 167 do 300 µg/ml. U *T. capitata* byla minimální inhibiční koncentrace 0,05 µl/ml. Antioxidační účinky byly hodnoceny v *in vitro* DPPH testech s hodnotami IC_{50} v rozmezí od 8 do 88 µg/ml. Imunomodulační aktivita byla výrazná u *O. sanctum* a *Z. officinale* v *in vivo* testech při dávkách 0,2 mg/kg a přibližně 20 mg/kg. Protizánětlivý účinek byl prokázán u *T. capitata in vitro*, kdy byla inhibována 5-lypoxygenáza při IC_{50} 93 µg/ml. Protizánětlivá aktivita *M. officinalis*, *O. sanctum* a *Z. officinale* byla testována *in vivo* a výrazný efekt byl zjištěn při dávkách od 50 mg/kg do 400 mg/kg. Jako výsledek této práce jsou rostliny *M. officinalis*, *O. sanctum*, *T. capitata* a *Z. officinale* doporučeny k dalšímu výzkumu zaměřenému na vývoj možných alternativ antibiotických promotorů růstu ve výživě hospodářských zvířat. Nicméně u *M. officinalis* a *T. capitata* je nutné nejdříve doložit jejich imunomodulační aktivitu.

Klíčová slova: antibiotikum, promotor růstu, rostlina, extrakt, antimikrobiální, antioxidativní, protizánětlivý, imunomodulační.

Certification

I declare that I have worked on this thesis independently, using only the sources listed in the bibliography.

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Amálie Balaštíková

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Foreword

The discovery of antibiotics caused a revolution in the treatment of both humans and animals. In livestock, however, the use of antibiotics has not been limited solely to medical treatment and they soon started to be used also as growth promoters. Along with the intensification of animal production and the health problems of animals, which this trend brings, the use of antibiotics as growth promoters rose steadily in the world. Unfortunately, inappropriate use of antibiotic growth promoters and the excessive use of antibiotics in human and veterinary medicine caused the development of resistance among bacteria. Therefore, because of problems connected to the use of antibiotics as growth promoters and their banned use in the European Union, it is necessary to find for them efficient substitutes.

Several alternatives to antibiotic growth promoters are already in use in animal production. Their effect may not be so pronounced but they do not bring such risks as antibiotic growth promoters do. One option of substituting antibiotic growth promoters are plant species and their biologically active constituents, since they exhibit several biological activities beneficial for animal nutrition and growth promotion. Nowadays, essential oils are popular and commercially available. The future use of plants and their products in animal production will depend on further research, on the development of animal production and on the demands of the market.

I have chosen this topic for several reasons. I consider the excessive use of antibiotics to be a great problem and I am also not satisfied with the situation in current conventional animal production. I believe a more nature-oriented approach should be applied both in animal nutrition and in the whole system of conventional animal husbandry. While working on this thesis and getting to know the problems of animal production in more detail, I started to ask myself questions about to what extent should we, humans, use animals for our needs and if we have the right to do so. Although these questions exceed the scope of this thesis, I consider it important to mention them.

1. Introduction

1.1. Antibiotics in animal production

Ever since the accidental discovery of penicillin by Sir Alexander Fleming in 1928, huge amounts of antibiotics have appeared on the market and are now available not only to cure diseases in humans and animals but also serve as growth promoters in livestock husbandry in some countries (Sarmah et al., 2006). The first use of antibiotics in animal production was therapeutic, namely, to treat infectious (streptococcal) mastitis in dairy cattle (Woolcock, 1991). The growth promoting effect of antibiotics was discovered in the 1940s (Castanon, 2007), when Stoksad et al. (1949) fed chickens residues from fermentative production of chlortetracycline produced by *Streptomyces aureofaciens* in order to provide them with a cheap source of vitamin B_{12}. This growth-promoting effect was quickly confirmed in chickens and pigs and was also produced by the addition of other antibiotics to the feed of young animals. Antibiotics were increasingly used as feed additive not only due to their growth-promoting effect but also for their profitability, since only very small amounts are required (Woolcock, 1991).

In 1951 the United States Food and Drug Administration approved the use of antibiotics as animal feed additives without veterinary prescription (Jones and Ricke, 2003). Thus, the amount of antibiotics produced in the United States grew rapidly. In 1957 these antibiotics constituted 450 tons of all antibiotics produced there (Hejzlar et al., 1980). In 2004 antibiotic production was 9,900 tons, of which 60–80% was used for non-therapeutic purposes (Arikan et al., 2008). Nowadays, the use of antibiotics as growth promoters is under scrutiny in the United States (Dibner and Richards, 2005). Recent data from 2005 reveal that arsenicals, bacitracin, carbadox, chlortetracycline, monensin, penicillin, tylosin and virginiamycin are used as approved antibiotic growth promoters (Giguere et al. 2006). However, consumers are pressuring companies to remove antibiotic growth promoters from animal feed altogether. For example, both the McDonald's Corporation and the KFC claim that they do not accept chicken meat grown using antibiotic growth promoters (Dibner and Richards, 2005).

In the 1950s and 1960s, each European country approved its own national regulations concerning the use of antibiotics in animal feed (Castanon, 2007). Following the ban of all growth-promoting antibiotics in Sweden in 1986, and the ban on avoparcin and virginiamycin in Denmark in 1995 and 1998, the European Union banned the use of

avoparcin in 1997 and bacitracin, spiramycin, tylosin and virginiamycin in 1999 (Casewell et al., 2003). According to Opletal and Skřivanová (2010), non-antibiotic growth promoters carbadox and olachindox were also banned in the same year and since 1. January 2006 a ban applies to all antibiotics used as growth promoters, although antibiotics still can be used for therapeutic purposes. Producers that seek to export to markets within the European Union are thus forced to stop using antibiotic growth promoters altogether (Dibner and Richards, 2005). In Denmark, Norway and Sweden a study focused on the amount of therapeutic antibiotics used after the ban on antibiotic growth promoters was carried out. It revealed that in Sweden the use of therapeutic antimicrobials increased only temporarily. In Norway the use of therapeutic antimicrobials in animals declined markedly after the withdrawal of antibiotic growth promoters mainly because of a campaign supporting the avoidance of the use of antibiotics. The exception were weaning pigs in Denmark, where therapeutic use of antibiotics did not decline to previous levels after the increase in relation with the ban on antibiotic growth promoters (Grave et al., 2006). As could have been expected, performance did decrease, as observed in weaning pigs (Millet and Maertens, 2011). As far as economic impact is concerned, by withdrawing antibiotic growth promoters in Denmark, the cost per pig produced increased by 1.03 Euro (Barug et al., 2006). The ban also led to lower resistance of the enterococci to various antibiotics, including tylosin and virginiamycin (Aarestrup et al., 2001; Boerlin et al., 2001).

As far as Australia is concerned, before the year 2000 a number of antimicrobials, including arsenicals, avoparcin (a glycopeptide antibiotic), ionophores, macrolides, polypeptides, quinoxalines, virginiamycin (a streptogramin antibiotic) and others were registered as growth promoters. In June 2000 avoparcin was withdrawn. Because of its carcinogenicity carbadox was also prohibited. However, there are no available data on the quantities of various growth promoters used. Considering other countries of the eastern hemisphere, the use of antibiotics as growth promoters in food-producing animals is prohibited in Japan, however, antibiotics are permitted as a component of feed additives following Ministerial approval. In China regulation of the use of antibiotics in animal feed has been introduced in 1989 and since then only non-medicated antibiotics are permitted as feed additives (Sarmah et al., 2006). Also, raw mycelia can be used as animal growth promoters. Unlike in Japan and China, in Southeast Asia the use of antibiotics in shrimp farming is unregulated (Witte, 1998).

Witte's data from 1998 reveal that in the countries of the developing world, which are responsible for about 25% of world meat production, policies regulating veterinary use of antibiotics are poorly developed or absent. The study of Sarmah et al. (2006) reveals that in many developing countries, such as India, Thailand and Indonesia there is a lack of control concerning antibiotic use in animals. Therefore, there is no data available on the types of veterinary antibiotics and amounts used. Considering Africa, the data on the consumption of antibiotics by food-producing animals is lacking. However, in a study of Mitema et al. (2001) focused on antibiotics in food producing animals in Kenya, it was confirmed that hardly any antibiotics were used as growth promoters. It is still possible, however, that some soluble tetracyclines and sulfonamides in the form of soluble powders or solutions were used. According to the data of Mitema's study, between the years 1995-1999, almost 15 tons of active antimicrobials were used in animal food production. Of these, tetracyclines and sulfonamides and trimethoprim combination account for nearly 78% (56% and 22% respectively). In other African countries, such as the United Republic of Tanzania and Uganda, veterinary antimicrobials are easily accessible and under low control by the government (Sarmah et al., 2006).

1.1.1. Veterinary use of antibiotics

Modern agriculture is characterized by mechanization and intensification. When livestock is concentrated on too small a space and when poor husbandry is applied, the probability of disease increases (Parker, 1980). Another sign of modern agriculture practices is the increasing immaturity of livestock animals. By improving performance and due to breeding, animals are reaching market weight much earlier than before. Thus, there are more young animals in the livestock units with still undeveloped immune systems which are, therefore, much more prone to disease infection. The most widespread infections are respiratory diseases, which are caused by a large number of different pathogens and may not respond to available medicines. Another group of important infections are enteric infections, which are also caused by different groups of pathogens and are, therefore, difficult to cure. This group of infections is becoming more widespread not only for the reasons mentioned above but also because of the modern trend of eliminating the bedding of animals. When bedding is lacking, it is more difficult to

separate animals from their excrements. Other infections increasing in modern livestock husbandry are caused by bacteria, such as *Escherichia coli*, *Clostridia* spp., *Pasteurella* spp. and *Salmonella* spp. Many of these pathogens are common inhabitants of the animal intestine but with poor livestock husbandry a disease can break out (Sainsbury, 1986).

Many infections can be treated with antibiotics. Unlike in human medicine, however, in animals the antimicrobial drugs are used not only for therapeutic purposes but also for metaphylaxis, prophylaxis and growth promotion. The therapeutic use is similar to the use in human medicine, when animals are examined and treated individually. This approach is applied mainly in dairy cows and calves and antibiotics are applied by injection or orally. Metaphylaxis means that a whole group of animals, such as poultry or pigs, is medicated when a single animal starts to express signs of illness. This treatment is commonly administered with feed or water. On the other hand, prophylactic use is a more preventive approach when treatment can be applied both to groups and individual animals. It is usually used after a surgical operation or in cows at the end of lactation to prevent mastitis (intramammary administered therapeutic levels). Prophylaxis can also be applied at key times, such as weaning, or when mixing animals from different herds together, since in such cases it is highly probable that some disease will occur. Both prophylactic use and the use of antimicrobials for growth promotion (which will be discussed in further chapters) are criticized for building resistance among bacteria (Schwarz, 2001).

In contrast with conventional animal husbandry is organic animal husbandry, which takes a different approach to animals and the use of medicaments. In organic farming, preventive use of any chemical or synthetic remedy or antibiotic is prohibited, as is the use of antibiotics, coccidiostatics and other synthetic substances to promote growth. The treatment of an illness or injury must be as natural as possible and the main principle of fighting a disease is prevention. Animals must have an access to fresh air, to pastures and free paddocks, all of which strengthen their immune systems. The number of animals in the herd is also limited. Furthermore, straw or other natural materials are usually used for bedding and it is optimal to keep traditional animal breeds, since they are well adapted to local conditions. It is possible to use phytotherapeutics and homeopathics for treating a disease but the use of antibiotics is prohibited. They can be used only in cases when other permitted remedies are ineffective and the treatment of the animal is urgent (Moudrý et al., 2007).

1.1.2 Antibiotics in growth promotion

In this chapter I would first like to focus on the mode of action of antibiotic growth promoters and then describe selected antibiotic growth promoters in more detail. Orally administered antibiotics promote growth and feed efficiency in livestock and poultry (Dibner and Richards, 2005), while injected antibiotics have no or little growth promoting effect. Furthermore, the stimulation of growth is greater in broad-spectrum antibiotics, in cases with poor animal zoohygiene on farms, and it is more evident in young animals than in older ones (Opletal and Skřivanová, 2010). When explaining the mode of action of antibiotics, we can consider autochthonous microorganisms in the animal gut, which protect the gut from colonization by pathogenic and non-autochthonous microbes. They produce some nutrients beneficial for the animal, such as vitamin B and K. But there are also some drawbacks to these actions. The bacteria compete with the host for nutrients and amino acids. Bacteria fermenting these amino acids can produce toxins, such as amines, ammonia, indoles and phenols, decreasing animal growth. Some bacteria also decrease the digestibility of fat by degrading bile acids and their salts resulting in toxic products (Dibner and Richards, 2005). Antibiotics reduce the number of microorganisms, and thus protect the nutrients from competing microorganisms in the digestive system. Furthermore, antibiotics inhibit bacteria producing toxins (Feighner and Dashkevicz, 1987; Opletal and Skřivanová, 2010).

Many bacteria also digest the protective mucus layer of the intestine, resulting in increased secretion of the mucus, costing the host energy (Dibner and Richards, 2005). In a study of Costa et al. (2011) with chlortetracycline and mice infected with *Citrobacter rodentium*, it was concluded that the growth promoting effect of antibiotics is more due to their ability of modulating intestinal immune response than due to antibiotic action. While chlortetracycline had no effect on the density of *C. rodentium* in feces and did not impact colonic microbial flora, it did lessen pathologic changes in the distal colon and regulated transcription levels of inflammatory cytokines Th1 and Th17 temporarily. Similar conclusion was made years before in a study of Roura et al. (1992) in an experiment with chickens, where results indicated that feeding them antibiotics may promote growth by preventing immunologic stress. On the contrary, in the study of Collier et al. (2003) concerning pigs fed tylosin or administered an antibiotic rotation sequence, it was observed that the total amount of bacteria was significantly decreased. This theory is supported by

the observation in an experiment of Coates et al. (1963) in chickens, which shows that antibiotics do not promote the growth of germ-free animals. When administered doses of 45.5 mg/kg of penicillin, the weight gain was improved only in conventional chickens but not in germ-free ones, since their intestinal epithelium is thinner and, therefore, nutrients are more easily absorbed (Feighner and Dashkevicz, 1987; Opletal and Skřivanová, 2010). The germ-free chickens gained more weight than control groups receiving or not receiving penicillin (Coates et al., 1963). In spite of these experiments and theories, the scientific world is still not sure about the exact mechanisms by which antibiotics promote growth (Dibner and Richards, 2005).

Chlortetracycline

Chlortetracycline belongs to tetracyclines, which are broad-spectrum bacteriostatic antibiotics with a hydronaphthacene nucleus containing four fused rings (Murray et al., 1999). This antibiotic is produced by *Streptomyces aureofaciens* (Hejzlar et al., 1980) and it was first recognized as a growth promoter in the feed of chickens around 1950 (Jukes and Williams, 1953). All tetracyclines have similar antimicrobial activity spectra ranging from Gram-positive and Gram-negative bacteria, mycoplasmas, chlamydiae, rickettsiae to some types of protozoa (Murray et al., 1999). Hejzlar (1980) states that chlortetracycline is administered to treat particularly Gram-negative and mixed bacterial infections and that it is often prescribed as a basic antibiotic for treating infections where therapy should be initiated before the result of microbiological investigation is obtained. According to Murray et al. (1999), tetracyclines are the drug of choice for treating acute and uncomplicated urinary infections caused by *Escherichia coli*. Many pathogenic spirochetes including *Treponema pallidum* and *Borrelia burgdorferi* or protozoans such as *Plasmodium falciparum* are also inhibited by chlortetracycline. It is also used in cases of resistance or hypersensitivity to penicillin and where the clinical picture is not sufficiently clear. The most common complications accompanying the use of chlortetracycline are the irritating effect on mucous membranes of the digestive tract (Hejzlar et al., 1980) and prolonged use can thus result in diarrhea and pseudomembranous colitis (Murray et al., 1999). Also, bacterial and candidal superinfections and metabolic disorders caused by deficiency of vitamins B and K due to suppression of useful intestinal microflora can be observed. This can be avoided by simultaneous supply of some *Lactobacillus* species (*L. acidophilus* and

L. bulgaricus) and higher doses of vitamins B and K (Hejzlar et al., 1980).

According to Mackie et al. (2006) tetracyclines are commonly used as antibiotic growth promoters in pig and poultry industry in the United States. Mackie mentions that in the 1990s chlortetracycline along with oxytetracycline made 48% of all antibiotics fed to pigs. In 2004 the amount of chlortetracycline in animal feed for non-therapeutic purposes used in the United States was 1,800-2,400 tons (Arikan et al., 2008). The effective doses for starter pigs improving the average daily gain and the feed/gain ratio were observed by Zimmerman (1986) to be 11, 22 and 110 mg/kg. Considering chickens, Proudfoot et al. (1988) found out in their experiment with male broiler chickens that low levels of chlortetracycline (5.5 mg/kg), which were permitted at that time in Canada, had no significant effect on mortality and neither weight gain nor feed conversion ratios were affected. The results of this experiment were in sharp contrast with some previous works, where both weight gain and feed efficiency were significantly improved. But in those studies higher amounts of chlortetracycline were used (10-25 mg/kg and 25-55 mg/kg respectively). Chlortetracycline works well in the rearing of calves where the antibiotics are used more for their prophylactic or therapeutic effect on disease rather than for their specific effect on growth promotion. Chlortetracycline also prevents methemoglobinemia in cattle when 0.5-10 ‰ is added to the feed. Considering lambs, chlortetracycline gave higher growth rates than in control groups at 12 to 17 mg/kg. As a growth promoter chlortetracycline is particularly suitable in cases where housing conditions are not appropriate and lead to chronic diseases (Hejzlar et al., 1980; Woolcock, 1991).

Bacitracin

Bacitracin is a cyclic peptide antibiotic made of ten amino acids joined in a ring (Finch et al., 2012). It is produced by *Bacillus subtilis* (now recognized as *B. licheniformis*) and it was first isolated in 1943 from an infected wound of a girl called Tracy (which then became the basis for its name) (Block, 2001; Phillips, 1999). The main component is bacitracin A which, in its pure form, is also the most active (Hejzlar et al., 1980). Bacitracin disrupts bacterial cytoplasmic membrane and inhibits dephosphorylation of bacterial cell wall synthesis. Antimicrobial spectrum of bacitracin covers mainly Gram-positive bacteria, particularly the streptococci. *Neisseria* spp. are also susceptible (Murray et al., 1999) but there is little activity against Gram-negative bacteria (Block, 2001),

however, bacitracin shows an effect on some strains of *Haemophilus influenzae* and *Haemophilus ducreyi* (Hejzlar et al., 1980). Bacitracin is often combined with neomycin, polymyxin B, or both, since alone it has a narrow antimicrobial spectrum (Murray et al., 1999). Its antimicrobial activity is also increased by cadmium, manganese and zinc (Hejzlar et al., 1980). Initially, bacitracin was introduced to treat severe staphylococcal infections, yet its use in treatment is now mainly topical because of its systemic toxicity. Still, administered orally it is effective in treating antibiotic associated *Clostridium difficile* colitis (Murray et al., 1999).

Bacitracin has growth-promoting qualities and can be added to animal feed. One of its advantages is its selectivity, since bacitracin does not affect all microorganisms, but only some groups. As mentioned previously, bacitracin inhibits mainly the development of Gram-positive bacteria (clostridia, micrococci, pneumococci, staphylococci and streptococci), which means it does not destroy useful Gram-negative bacteria in the digestive tract. Without Gram-positive competitors, Gram-negative bacteria can afterwards increase the production of vitamins (Hejzlar et al., 1980). Butaye et al. (2003) states that bacitracin is more stable as a zinc salt. Both bacitracin and Zn-bacitracin are used as growth promoters and in some topical preparations in human and veterinary medicine. Another advantage is that all types of bacitracin are absorbed very little or not at all in the intestines (shown in chickens, pigs and rats). Therefore, no residues can be found in meat when the product is administered orally (Butaye et al., 2003; Froyshov et al, 1986) Bacitracin favorably affects body weight gains of calves, chickens and pigs (Hejzlar et al., 1980) and in the United States it is a common antibiotic used in pig and poultry industry (Arikan et al., 2008). According to Opletal and Skřivanová (2010), before the ban of antibiotics as growth promoters in the European Union, Zn-bacitracin was used at the doses of 5 to 20 mg/kg in calves to the maximum age of 6 months, in pigs it was also used until the age of 6 months and in poultry the maximum age was 16 weeks. In chickens it was observed that with the administration of bacitracin at the doses of 55 to 110 ppm to the feed, necrotic enteritis caused by *Clostridium perfringens* was prevented (Butaye et al., 2003).

Virginiamycin

Virginiamycin is produced by *Streptomyces virginiae* and was first isolated in 1955

by De Somer. It is a depsipeptide antibiotic composed of two components A and B (Hejzlar et al., 1980). When used individually, bacteriostatic qualities can be observed, but in combination the components show marked synergism and are bactericidal against Gram-positive bacteria. The antibiotics are inhibitors of protein synthesis and act on the ribosomes (Petroski and McCormick, 1992). In medicine virginiamycin is applied only locally in the form of creams, drops, ointments or powders. As possible side effects one can consider contact allergy and photosensibilization. When administrated orally, diarrhea, nausea and stomach pain can appear (Hejzlar et al., 1980). The drug is not absorbed after oral administration (Giguere et al., 2006), therefore, no residues are found in muscles and organs, as was seen in chickens (Butaye et al., 2003).

Besides the use of virginiamycin in topical preparations for human and veterinary medicine, it is also used at levels around 20 ppm to promote growth in animals in many countries (Butaye et al., 2003; Giguere et al., 2006). For example in the United States it is commonly served to promote growth in both chickens and pigs (Mackie et al., 2006). According to Opletal and Skřivanová (2010), before the ban in the European Union, virginiamycin was used at the doses of 5 to 20 mg/kg in calves until the age of 16 weeks, in pigs with the maximum age of 6 months and in poultry with no limitation on the maximum age. As for pigs, virginiamycin also affects litter performance when applied to sows during pregnancy and lactation. Considering poultry, in an experiment of Cervantes et al. (2011) on turkey hens it was demonstrated that birds fed diets supplemented with 22 ppm of virginiamycin were significantly heavier than those from the control group. Furthermore, they also had significantly better feed conversion ratio. Johnson et al. (1998) found out in his study that nontherapeutic levels of virginiamycin reduce fermentative acidosis in the hindgut and thus lessen some behavioral problems associated with management of stabled horses and the intake of grain.

Flavomycin

This growth promoting antibiotic was invented in 1962-1969 by the Farbwerke Hoechst AG Company (Hejzlar et al., 1980). Flavomycin (also known as: bambermycin, flavophospholipol and moenomycin) is a glycolipid antibiotic produced by *Streptomyces bambergiensis*, *S. ghanaensis*, *S. geysirensis*, and *S. ederensis* (Butaye et al., 2003; Huber and Nesemann, 1968). The product is manufactured as a complex of very similar

components, of which moenomycin A, a phosphorus-containing glycolipid, is the main component (Butaye et al., 2003). Flavomycin is active primarily against Gram-positive organisms but it also inhibits certain Gram-negative bacteria, such as *Pasteurella*, *Brucella* (Huber and Nesemann, 1968) and *Listeria* (Hejzlar et al., 1980). Its spectrum of activity also covers staphylococci and streptococci (Butaye et al., 2003)

Flavomycin is not used for medical treatment. It is only applied as a growth-promoting antibacterial in animal feeds, commonly in cattle, chickens, pigs and turkeys (Butaye et al., 2003; Pfaller, 2006). For this purpose it is produced as a mixture of mycelia from producing strains and antibiotic at the concentration of 5g of active substance on 1kg. Pure product is used just for experiments, reasons for this are mainly economical. As a growth promoter, flavomycin has many favorable attributes, for example it is not absorbed (only 0.02% is absorbed) so there are no residues in animal tissues. Flavomycin does not have any side effects and does not cause allergic reactions (Hejzlar et al., 1980). According to Pfaller (2006), it also has the following advantages: flavomycin does not kill beneficial bacteria in the gut, such as *Lactobacillus* spp. and *Bifidobacterium* spp., but is known to suppress certain microorganisms, for example *Staphylococcus* spp. and *Enterococcus faecalis*. Therefore, this growth promoter contributes to the improvement of gut microflora, providing a barrier to colonization by pathogenic bacteria and resulting in improved weight gain and feed conversion. Before the withdrawal of antibiotic growth promoters in the European Union, flavomycin had been used in calves of up to six months of age at the dose of 6 to 16 mg/kg, in pigs and poultry at the dose of 1 to 20 mg/kg for six months and sixteen weeks respectively. It was also used in cattle at the dose of 2 to 10 mg/kg and in rabbits at the dose of 2 to 4 mg/kg (Opletal and Skřivanová, 2010). In broilers flavomycin reduces the incidence of the animal pathogens *Salmonella* and *Clostridium* (Bolder et al., 1999) and in ruminants it modulates the gut microflora. The dose of 20 mg per day results in decreased ruminal ammonia and total volatile fatty acid concentrations (Edwards et al., 2005). Flavomycin is also used as a growth promoter in aquacultures in China, since it also has an effect on the autochthonous intestinal microflora in some fish (He et al., 2010).

1.2. Problems in the use of antibiotics in animal production

1.2.1. Antibiotic resistance

According to Drlica and Perlin (2011), resistance is a condition when an antibiotic fails to harm the pathogen enough to cure a disease. Emergence of resistance often begins with a large pathogen population in which a tiny fraction is naturally resistant to the antibiotic, either through spontaneous changes or through the acquisition of resistance genes from other microbes. Antibiotic treatment kills or halts the growth of the major, susceptible portion of the microbial population. That, unfortunately, favors the growth of the pathogen population composed of resistant cells. Subsequent treatment with the same antibiotic does little good. If the resistant organisms spread to other people, the resulting infection is resistant even before treatment and the control of such an infection requires a different antibiotic. The development of resistance is accelerated by mutagenic action of some antibiotics, by the movement of resistance genes from one microbial species to another, and by excessive and inappropriate use of antibiotics. Considering genetic transferability of resistance, mobile elements (such as plasmids, transposons and other genetic material) play a key role in the horizontal spread of resistance genes among bacteria. Plasmids contain resistance genes and can replicate independently of the host chromosome. Transposons can exist on plasmids or integrate into other transposons or the host's chromosome (Devirgiliis et al. 2011; Alekshun and Levi 2007).

As is mentioned above, resistance may also be caused by inappropriate and excessive use of antibiotics both in human medicine and in animal husbandry (Mateus et al., 2011). In humans, the most serious problems are caused by methicillin resistant *Staphylococcus aureus* (MRSA), extended spectrum β-lactamases (ESBLs) produced by Gram-negative bacteria, penicillin-resistant pneumococci and multi-resistant *Mycobacterium tuberculosis*. None of these have any connection to the use of antibiotics in agriculture (Barton and Hart, 2001). When resistance appeared, pharmacological industry started to develop new compounds to deal with this problem. In recent years the invention of new agents slowed down, which had a negative impact on the ability to treat serious infections (Phillips et al., 2004). In animal husbandry antibiotics are used for prophylaxis, chemotherapy and growth promotion. Routine use of antibiotics for growth promotion in food-animals constitutes a serious public health problem, especially in cases where the same classes of antibiotics are used in humans. In 1969, the Swann Committee of the

United Kingdom concluded that antibiotics used in human medicine or those that promote cross resistance should not be used as growth promoters in animals. Since then, there has been continuous debate about the extent to which the use of antibiotics in food animals contributes to resistance in bacteria that infect humans. The resistant bacteria can spread to humans by food or by animal contact. Improved analytical techniques have provided evidence that resistance is increased by antibiotics in animal feed. The ban on antibiotics as growth promoters in the European Union has led to reductions in the prevalence of resistant bacteria in food and food animals, as well as in humans (Wegener 2003, Witte 1998). Still, opinions differ. Whereas some are convinced about the dangers of unregulated and unnecessary use of antibiotics, especially of growth promoters in animal husbandry (Singer et al., 2003), others, such as Phillips (2007) disagree with the ban on antibiotics just on the basis of the Precautionary Principle and argue that there has been little opportunity for discussion on the withdrawal of antibiotic growth promoters in a purely scientific forum.

1.2.2. Antibiotic Residues

Antibiotic residues can be found both in animal products and in the environment. In the first case antibiotic residues can appear in animal tissues and in milk when sufficient withdrawal periods between treatment and slaughter or milking are not followed (O'Keeffe and Kennedy, 1998). The withdrawal period is the time needed for the active substance to decrease to the maximum residue level. The maximum residue level has been established by the Commission of the European Union and is set individually for each food product and species. It is based not only on the level of the active substance which remains in the animal tissue at the end of medical treatment but also on the amount of this particular food product consumed by the population on daily basis (Serratosa et al., 2006). Examples of selected maximum residue levels in the European Union can be seen in Table 1. One of the drawbacks of the definition of maximum residue levels is that there are individuals who do not consume the average diet and also those who can be supplied with foods from only one particular source (O'Keeffe and Kennedy, 1998). The negative effect of exposure to antibiotic residue can be allergic reactions, especially to neomycin, penicillin and sulfonamides. This type of allergic reaction is not dose dependent, therefore, even very small amounts can have an intense effect (Woolcock, 1991). Furthermore, other

Table 1: Maximum residue levels of selected antibiotics (Regulation EC/37/2010)

Antibiotic	**Animal species**	**Target tissue**	**Maximum residue level**
Ampicillin	all food producing animals	fat, kidney, milk, muscle, liver	4-50 µg/kg
Bacitracin	bovine, rabbit	fat, kidney, milk, muscle, liver	100-150 µg/kg
Chlortetracycline	all food producing animals	eggs, kidney, milk, muscle, liver	100-600 µg/kg
Monensin	bovine	fat, kidney, milk, muscle, liver	2-30 µg/kg
Neomycin	all food producing animals	eggs, kidney, milk, muscle, liver	500-5,000 µg/kg
Oxytetracycline	all food producing animals	eggs, kidney, milk, muscle, liver	100-600 µg/kg
Sulfonamides	all food producing animals	eggs, kidney, milk, muscle, liver	100 µg/kg
Tylosin	all food producing animals	eggs, kidney, milk, muscle, liver	50-200 µg/kg

pathological effects can occur, such as bone marrow toxicity (chloramphenicol), carcinogenicity (oxytetracycline), hepatotoxicity, mutagenicity, nephropathy (gentamicin), reproductive disorders and other effects (Nisha, 2008). When residues of antimicrobial agents surpass the agreed maximum residue levels, they can contribute to the development of resistance in bacteria in humans (Al-Dobaib and Mousa, 2009).

As far as antibiotic residues in the environment are concerned, many antibiotics are poorly absorbed in the gut and subsequently both the compound itself and its breakdown products (Mackie et al., 2006) are largely excreted in the feces and urine of animals and humans (Khan et al., 2008). The amount of antibiotics excreted can reach up to 75 % (Arikan et al., 2009; Mackie et al., 2006) and even up to 100 % for streptomycin and lincomycin (Woolcock, 1991). Together with antibiotic residues, resistant microbial

population can also be excreted with feces (Mackie et al., 2006) as was shown in a research of Haack and Andrews (2000), where 71 % of isolates of *Enterococcus faecalis* obtained from a swine farrowing house were resistant to tetracycline.

Since animal excrements and urine are usually used for land application in the way of manure, they can act as the source of contamination of soil, surface and groundwater (Mackie et al., 2006). Interestingly, in a study of Schlusener et al. (2006) focused on antibiotics in liquid manure tanks before their application on fields, it was shown that for some antibiotics the storage time enhanced degradation. The resulting half-lives were 41 days for erythromycin, 130 days for roxithromycin and 6 days for salinomycin. Only tiamulin remained unchanged for the whole of the 180 day long experiment. In several studies of Arikan et al. (2007, 2009) aiming at the behaviour of chlortetracycline and oxytetracycline in the composting process, it was proved that composting lowers the levels of extractable chlortetracycline and oxytetracycline in manure. Furthermore, the impact of composting on the number of chlortetracycline-resistant organisms was also investigated. It was shown that their number was greatly lowered. When we consider antibiotic residues after manure application on the field, resulting residual concentrations can vary from a few μg up to g/kg. The mobility and transport of antibiotics in the soil is greatly influenced by the soil pH and many antibiotics can be photoderaded. However, this process is not very effective in the soil. On the other hand, biotransformation by microbial processes works better for antibiotic degradation and inactivation. The higher the number of microorganisms and the more aerobic the setting, the more effective the degradation is. Still, some antibiotics can persist in the soil and their metabolites can preserve their antibiotic effect, which influences microbes in the soil (Thiele-Bruhn, 2003) and plants, as was demonstrated in an experiment by Migliore et al. (1996) with *Hordeum distichum* treated with 300 mg of sulfadimethyoxine per liter and grown on synthetic medium and soil. This experiment showed that bioaccumulation of the drug was higher in the synthetic medium, while in the soil it was lower but still quite high. Therefore, there is a potential risk for human health. Some antibiotic residues can also be found in groundwater or surface water due to soil leaching or rain (Khan et al., 2008), as was shown in the study of Campagnolo and his colleagues (2002) of antimicrobial compounds contaminating surface and groundwater near swine and poultry farms.

1.3. Alternatives to antibiotic growth promoters

New alternatives to antibiotic growth promoters, which are currently in research or are already in practical use, do not have such a pronounced impact as early antibiotic growth promoters. Unlike antibiotic growth promoters, whose effect was basically antibacterial and antiprotozoal and which were affecting muscle growth or egg production, newer agents influence more areas of animal physiology, in milder and more complex way, without reducing the safety of feed and food chain (Opletal and Skřivanová, 2010).

Still, in the search for new methods of how to improve animal health in modern animal production systems, we should consider not only factors like nutrient supply or feeding strategy but also other important features like stocking density, environmental temperature and hygienic level (Thomke and Elwinger, 1998). In the following chapter I would like to describe several non-antibiotic growth promoters and their use in animal husbandry.

1.3.1. Non-antibiotic growth promoters

Probiotics

Probiotics are live microbial feed supplements beneficial for the host by improving its intestinal microbial balance, since they are able to prevent the colonization of the intestinal tract by potentially pathogenic microorganisms. While a wild animal acquires gut microflora from its mother and from the environment contaminated with its mother's bacteria, in modern methods of rearing animals we have to deal with the problem of restricted access to the mother and, therefore, with the lack of microflora which would otherwise protect the new-born animal. In poultry industry, the egg is usually taken from the hen and hatching happens in a clean incubator. Thus, probiotics work well in poultry (Fuller, 1989; Verstegen and Williams, 2002), as was shown in the experiment of Shivaramaiah et al. (2011) when chickens and poults were administered *Bacillus* spp., resulting in reduced *Salmonella typhimurium* incidence and in increased body weight gain in both chicks and poults. In a different study of Zulkifli et al. (2000) it was shown that chickens which were administered *Lactobacillus* cultures had greater body weight and better food efficiency than the control group. Body weight and weight gain was similar to chickens with oxytetracycline diet, while food efficiency was even better. In a review of Mantere-Alhonen (1995) beneficial results in calves and piglets were also proved. Strains

of bacteria commonly used as probiotics are as follows: *Bacillus* spp., *Bifidobacterium* spp., *Enterococcus faecium*, avirulent *Escherichia coli*, *Lactobacillus* spp., *Lactococcus lactis*, *Pediococcus pentosaceus*, *Saccharomyces cerevisidae* and *Streptococcus thermophilus* (Vondruskova et al., 2010). Probiotics are commercially available, as an example can serve Cernivet®, which contains *Enterococcus faecium* strain and is designed for calves, chickens and pigs (Anonymous, 2009b). Other probiotic products are manufactured by Vit-E-Men Company for cattle, pigs, poultry and sheep (Anonymous, 2010e).

Prebiotics

Prebiotics are non-digestible food ingredients that improve the host's health by stimulating the growth and/or activity of one or a limited number of bacteria in the colon, namely, the lactobacilli and the bifidobacteria (Gibson and Roberfroid, 1995). Their main components are oligosaccharides and their most common natural source is grain legumes. Some oligosaccharides can also be derived from fruits and wheat (Huyghebaert et al., 2011). Oligosaccharides used as prebiotics are as follows: fructooligosaccharides, galacto-oligosaccharides, inulin, isomaltooligosaccharides, lactulose, mannanoligosaccharides, soybeanoligosaccharides and xylooligosaccharides (Vondruskova et al., 2010). In an experiment of Belle et al (2009) on growing and finishing pigs the use of prebiotics (fructooligosaccharide and mannanoligosaccharide) showed similar results on performance as apramycin treatment. In chickens the beneficial effect on body weight gain and feed conversion ratio due to supplementation with prebiotics, probiotics and their combination was also proved (Nyamagonda et al., 2011). An example of commercially available prebiotic is Celmanax®, which contains mannanoligosaccharide, d-mannose, galactosamine, glucomannans and glucosamine and is designed for aquacultures, cattle, horses and poultry (Anonymous, 2009c). Another example is the Vitalan® prebiotic feed additive, which is designed especially for cows (Anonymous, 2006).

Synbiotics

Synbiotics are defined as a mixture of probiotics and prebiotics that beneficially affects the host. They improve the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by stimulating the growth and/or by activating the

metabolism of one or a limited number of health-promoting bacteria (Gibson and Roberfroid, 1995). Nemcova et al. (1999) confirmed the synergistic effect of *Lactobacillus paracasei* and fructooligosaccharide combination on faecal microflora of weaned pigs. It was observed that lactobacilli and bifidobacteria counts were increased while clostridia, enterobacteriacea and *Escherichia coli* counts were lowered. Also, in calves a beneficial effect of synbiotics was proved in the study of Jatkauskas and Vrotniakiene (2009) when the occurrence of post weaning diarrhea and its severity was lowered by 40% and the average daily gain and feed conversion rate were improved by 15.3% and 12.8% respectively. Example of commercially available synbiotic is Proflora™ (Anonymous, 2012a).

Enzymes

Cereal animal feeds contain non-starch polysaccharides (such as arabinoxylans, β-glucans, celluloses, hemicelluloses and pectins). Non-starch polysaccharides can have a negative effect on growth and performance (Huyghebaert et al., 2011), since they increase gut viscosity as was seen in birds. This reduces the speed of passage and diffusion of digestive enzymes and it also contributes to the loss of endogenous enzymes and stimulates bacterial proliferation. Supplementation with enzymes reverses this effect and leads to increased performance (Choct et al., 1996; Verstegen and Williams, 2002). It is now common that poultry feeds contain enzymes such as xylanases and β-glucanases, which break down non-starch polysaccharides (Huyghebaert et al., 2011). An example of a commercially available product is Profytase 5000, which contains phytase enzyme and is designed for cattle, chickens and pigs (Anonymous, 1999). Other examples of enzyme feed additives are Natuphos® (containing enzyme phytase) and Natugrain® TS (containing glucanase and xylanase) designed especially for pigs and poultry (Anonymous, 2012b).

Organic acids

Organic acids are widely distributed in nature as common constituents of plants or animal tissues (Huyghebaert et al., 2011). They are also formed in the ceca of poultry, where they contribute to the reduction of *Enterobacteriaceae* (van der Wielen et al., 2000). Beneficial effects of organic acids are probably not only due to their energy contribution but also due to improved protein digestion, decreased gastric pH and decreased bacterial

growth (Partanen and Mroz, 1999; Verstegen and Williams, 2002). An effect on pigs was observed by Partanen et al. (2007), when a mixture of formic acid, propionic acid and potassium sorbate decreased feed conversion ratio, lessened the severity of post-weaning diarrhea and also enhanced weight gain during finishing period and total fattening. In a study of Haque et al. (2010), which compared the effect of citric acid and flavomycin in broilers, it was concluded that citric acid increases weight gain, feed intake, tibia ash deposition and non-specific immunity, as well as feed efficiency and carcass yield and that citric acid is a potential alternative to the antibiotic growth promoter flavomycin. An example of commercially available organic acid feed additive is Selacid-Green, which contains short chain fatty acids and medium chain fatty acids and is designed for aquacultures, pigs and poultry (Anonymous, 2011c).

1.3.2. Biologically active constituents of plants

In this chapter I describe the beneficial effects of herbs and botanicals. I focus mainly on essential oils, since they are the most commonly used compounds of phytogenic feed additives in animal husbandry, as can be seen in Table 2. According to Huyghebaert et al., (2011) many plants have beneficial multifunctional properties derived from their specific bio-active components. Biologically active constituents of plants are mostly secondary metabolites, such as terpenoids (mono- and sesquiterpenes, steroids, etc.), phenolics (tannins), glycosides and alkaloids (present as alcohols, aldehydes, ketones, lactones, esters, ethers, etc.). The use of constituents derived from plants is problematic because there is a lot of variation in composition, mainly due to biological factors, such as plant species, growing location and harvest conditions. Variation is further influenced by the techniques of extraction, distillation and stabilization and also by storage conditions, which are affected by light, temperature and time. Herbs and botanicals can have various beneficial effects on farm animals. They may for example reduce the negative effects of stress factors in the animal, act preventively against pathogens of microbial and protozoal character, improve immunity, have anti-inflammatory effect, stimulate microflora of the digestive tract, increase the bioavailability of nutrients, have an antioxidative effect, act as promoters of growth, increase performance and fertility, influence the appearance and quality of animal products and improve the welfare of farm animals (Barug et al., 2006; Opletal and Skřivanová, 2010).

Table 2: Commercially available phytogenic feed additives

Product	Company	Applied animal	Active ingredient	Effect	Dosage	Source
AROMEX® ME Plus FRESTA® F Plus FRESTA® F Wean FRESTA® F Profertil FRESTA® Gel BIOSTRONG® 510 BIOSTRONG® 505 BIOSTRONG® 505 Plus PERFORMIZER® RUMEX® RUMEX SC®	DELACON	dairy cows, pigs, poultry	basic constituents are for example essential oils, saponins, bitter substances and flavonoids	Improvement of the palatability of feed and for reduction of ammonia, stabilisation of digestive tract, prevention against weaning problems, etc.		(Anonymous, 2011a) (Anonymous, 2011b)
Biomin® P.E.P	Biomin®	aquatic animals, poultry, swine, young ruminants	essential oils from oregano, anise and citrus	Supports digestion and feed utilization.		(Anonymous, 2010a) (Steiner, N/A)
CRINA® Finishing Pigs and Sows CRINA® Poultry CRINA® Ruminants	DSM	cattle, pigs, poultry	blend of essential oil compounds	Increase live weight gain, improve feed efficiency, etc.	cattle: 300-1,200 mg / head /day, rest: 50-200 mg/kg of feed	(Anonymous, 2009a)
CRINA® Poultry Plus	DSM	broiler	benzoic acid and blend of essential oil componds (including thymol, eugenol, piperine)	Reduces the populations of potentially pathogenic bacteria, does not affect the beneficial Lactobacilli, increases daily weight gain, improves feed conversion ratio.	300 mg/kg	(Anonymous, 2011c)

Table 2: Commercially available phytogenic feed additives (Continued)

Product	Company	Applied animal	Active ingredient	Effect	Dosage	Source
DOSTO® Powder DOSTO® Liquid DOSTO® Green DOSTO® Emulsion	DOSTOFARM®	calves, lambs, pigs, poultry	origanum oil	Stimulates appetite, improves feed consumption, etc.	powders: 0.25-2,000 g/ton of feed, liquid: 0.25-1,000 g / 1,000 l, piglets 2-3 ml/head	(Anonymous, 2008)
ENVIRO Plus	DELACON	shrimps	active ingredients are based on a standardized complex of triterpenoid saponins	Optimizes weight gain, feed conversion and survivability.		(Anonymous, 2011a)
Extra-Health (sow lactogenic powder)	Guangzhou Tanke Industry Co., Ltd.	pigs	angelica, hemlock parsley, dangshen, wangbuliuxing, etc.	Resolves the sows constipation, improves feed intake, increases production, improves the survival rate and birth weight of piglets, enhances sow lactation and anti-stress, reduces the boar's cleft feet and other hoof disease, etc.	2-3 kg/ton of feed	(Anonymous, 2010b)
Orego-Stim® Fin Fish Orego-Stim® Poultry Orego-Stim® Ruminant Orego-Stim® Shrimp Orego-Stim® Swine	Meriden Animal Health Ltd.	aquatic animals, cattle, pigs, poultry	oregano essential oil	Utilising feed more efficiently, boosting immunity against pathogens, increasing weight gain, increasing survivability, improving feed conversion ratio, maximising intestinal health, etc.	aq. animals: 125-1,000 ppm, rest: 125 ml – 500 ml/1,000 l of water	(Anonymous, 2010c), email communication

Table 2: Commercially available phytogenic feed additives (Continued)

Product	Company	Applied animal	Active ingredient	Effect	Dosage	Source
Saccharicterpenin	Hangzhou Tangtian Technology Co., Ltd.	aquatic animals, cattle, pigs, poultry	standardized complex of triterpenoid saponin and saccharide (*Camellia* L.)	Regulate immune function, lower mortality and morbidity, improve average daily gain and feed conversion rate, etc.	aq. animals: 100-2,000 g/ton of feed, cattle: 8-10 g /day /head, rest: 100-500g/ ton of feed	(Anonymous, 2010d)

Table 2 shows phytogenic feed additives, which are currently in commercial use, their effect, target animal species and recommended dosage. In the table one can see that essential oils are of main interest within the commercial sphere. In their review Franz et al. (2010) recommend for essential oils to be microencapsulated when they are administered to animals, since then the losses and reaction with air can be avoided. The benefits of essential oils are their antimicrobial effect and also slight irritation of intestine tissue, which causes a pronounced production of mucus and thus prevents the adhesion of pathogenic microorganisms. Another benefit of the use of essential oils and plants providing these essential oils (aromatic plants) is their antioxidative effect, which causes an improved oxidation stability of the carcass, egg yolk and fat. However, the drawback of the use of essential oils is that they can influence the flavor of animal products. Special care should be taken especially while using certain species, such as parsley and caraway. The growth promoting effect of essential oil mixture from anise, citrus and oregano (Biomin® P.E.P. 125 poultry) was proved in an experiment by Hong et al., 2012 with broilers. The group supplemented with essential oil had improved feed/gain ratio in the course of the whole experiment (42 days), while the group supplemented with oxytetracycline had improved feed/gain ratio during the first 21 days. Compared to control group, both oxytetracycline and essential oil increased the survival rate by 10% and decreased ileum ammonia concentration. Other beneficial effect of supplementing the feed with essential oil was that breast muscles were more tender and tight muscles were juicier, compared to both control group and oxytetracycline supplemented group. Interestingly, the broiler supplemented with essential oil had longer duodenum villi. In a study of Mathlouthi et al. (2012) dealing with the effectiveness of essential oils (namely oregano, rosemary, mixture of oregano and rosemary and commercial blend), it was observed that the supplementation of broilers' diet with essential oils significantly increased feed efficiency compared to the control group. Moreover, the body weight and body weight gain was greater in groups receiving essential oil than in the control group. There was no difference observed in feed efficiency and growth performance among broilers supplemented with essential oil or with avilamycin.

When searching for new medical materials on promoting health and well-being of animals, ethnoveterinary medicine can be a useful approach (Wynn and Fougère, 2007). Stein et al. (2005) in their ethnoveterinary study on antifungal drugs claim that only a very

small part of the known plant species has been evaluated for antifungal properties. Due to the rapid speed of plant species extinction it is necessary to collect and screen plants in order to avoid the loss of important sources. Interesting information can be found in a review of Viegi et al. (2004), which states that in Italy more than 280 plants have been used in folk veterinary medicine. Plants, such as *Quercus cerris* bark, *Smilax apera* shoots, *Quercus pubescens* bark, *Tamus communis* berries were used for treating the inflammation caused by yoke or saddle. For the treatment of mastitis nine plants were used, including *Brassica oleracea*, *Avena sativa*, *Anagallis arvensis*, *Linum usitatissimum* and *Scrophularia canina*. Interestingly, to prevent mastitis *Buxus sempervirens* was placed in the litter. Twenty-three plants were found to treat diarrhea and twenty to treat respiratory difficulties. The family with the most medicinal plants was found to be Asteraceae, followed by Lamiaceae, Ranunculaceae, Fabaceae, Apiaceae, Rosaceae, Liliaceae, Poaceae and Euphorbiaceae. In British Columbia several plants such as *Chenopodium ambrosioides*, *Ch. album* and *Artemisia vulgaris* were used to treat internal parasites in poultry. Leaves of *Nicotiana rustica* were applied for the treatment of *Heterakis gallinarum* and *Histomonas meleagridis* (Lans and Turner, 2011).

Based on the above mentioned data, plants seem to be promising materials for the development of products with properties, such as antimicrobial, antioxidative, anti-inflammatory and immunomodulatory effects, suitable for substitution of antibiotic growth promoters. However, only a limited number of studies or reviews is focused on plant-derived products with the whole complex of desired biological action. Thus, I presume that detailed systematic analysis of literature data could lead to identification of plant species promising for further evaluation in *in vitro* as well as *in vivo* tests focused on the development of growth promoting agents.

2. Objectives

The aim of this thesis is the identification of plant species with complex antimicrobial, antioxidative, anti-inflammatory and immunomodulatory effects as potential substituents of antibiotic growth promoters in animal nutrition. The additional objective is summarization of data on introduction.

3. Materials and methods

Data on biological activity (antimicrobial, antioxidative, anti-inflammatory and immunomodulatory) of the individual plants were collected from scientific databases (Web of Knowledge, PubMed and Google Scholar) using the following keywords: plant*, extract*, antimicrobial*, antioxidative*, immunomodulatory* and anti-inflammatory*. Plant names were verified using on-line databases, namely the International Plant Name Index (IPNI) and the Multilingual Multiscript Plant Name Database (MMPLD). Information on introduction was retrieved from the scientific databases (Web of Knowledge, PubMed and Google Scholar) and from other scientific literature.

4. Results and discussion

As a result of literature analysis, I have identified five plants (*Balanites aegyptiacus, Echinacea purpurea, Ocimum sanctum, Phyllanthus emblica* and *Zingiber officinale*) in which the complex antibacterial, antioxidative, anti-inflammatory, and immunomodulatory properties have previously been reported in various studies. I have also identified eleven plants (*Acacia nilotica, Baccharis dracunculifolia, Capparis spinosa, Dicranopteris linearis, Dittrichia viscosa, Laurus nobilis, Melissa officinalis, Nigella sativa, Prunella vulgaris, Thymbra capitata,* and *Vitis vinifera*) with three out of four of the above-mentioned effects. The detailed data on scientific name, common synonyms, family, part tested, extract solvent or fraction and biological properties (antimicrobial, antioxidative, immunomodulatory and anti-inflammatory) is shown in Table 3 (containing plants with all the required biological effects) and in Table 4 (containing plants with three out of the four required biological effects). To demonstrate biological properties of an individual plant, the best value of a pharmacological effect was always chosen and the particular target of the assay used was noted. As far as the extracts are concerned, the search was focused on polar solvents, such as ethanol, methanol and water, due to the presence of polar substances, which should guarantee good solubility and accessibility for a living organism. Considering biological properties in Table 4, the form of essential oil was also searched due to the lack of data on polar solvents.

Considering antimicrobial action, *B. aegyptiacus*, *O. sanctum* and *Z. officinale* previously exhibited significant *in vitro* growth-inhibitory effect against various species of pathogenic microorganisms (*Candida albicans, Helicobacter pylori* and *Trichophyton mentagrophytes*) with lowest MICs ranging from 125 to 300 μg/ml (Balakumar et al. 2011; Maregesi et al. 2008; Nanjundaiah et al. 2011), which corresponds with parameters proposed by Rios and Recio (2005) for antimicrobially effective plant-derived products (MIC < 1 mg/ml for extracts). Moreover, the leaf aqueous extract of *O. sanctum* has been reported to possess antimicrobial activity also in *in vivo* treatment of bovine sub clinical mastitis, when total bacterial count was significantly lowered (Mukherjee et al. 2005). The proved *in vivo* effectiveness together with relatively strong *in vitro* action observed by Balakumar et al. (2011) against broad spectrum of microorganisms (*Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum gypseum, Microsporum nanum* and *Epidermophyton flocossum*) suggest *O. sanctum* as a prospective antimicrobial agent.

Table 3: Plant species possessing complex biological effects

Scientific name/Common synonyms/Family	Part tested	Extract/ Fraction	Biological effect			
			Antimicrobial Method/Concentration/ Microorganism/Reference	Antioxidative Method/Concentration/ Effect/Reference	Immunomodulatory Method/Concentration/ Effect/Reference	Anti-inflammatory Method/Concentration/ Effect/Reference
Balanites aegyptiacus (L.) Delile/*Balanites aegyptiaca* (L) Delile/Balanitaceae	stem barks	ethanol or methanol extract	*In vitro* (broth dilution method) MIC = 125 µg/ml *Candida albicans* (Maregesi et al., 2008)	*In vitro* (DPPH assay) IC_{50} = 53 µg/ml (Karou et al., 2011)	*In vitro* [cell oxidative burst response (blood)] C = 100 µg/mL Inhibition: 76% (Koko et al., 2008)	*In vitro* (reduction of paw oedema in rat) Dose = 200 mg/kg: (in 2.5 h) Reduction: 28% (Speroni et al., 2005)
Echinacea purpurea (L.) Moench/*Rudbeckia purpurea* L., *Brauneria purpurea* (L.) Britton/Asteraceae	aerial parts (stems, leaves, and flowers)	ethanol or methanol extract	*In vitro* (agar well diffusion method) C = 20,000 µg/ml *Saccharomyces cerevisiae* Zone of inhibition = 26 mm (Stanisavljevic et al., 2009)	*In vitro* (DPPH assay) C = 2000 µg/ml Inhibition: 61% (Orhan et al., 2009)	*In vitro* (Flow cytometry) C = 50 µg/ml Murine bone marrow-derived dendritic cells phenotypically examined: Increased frequency of CD11c+ cells by 19%. (Benson et al., 2010)	*In vitro* (COX-2 assay) C = 2 µg/ml Reduction: 28% (Benson et al., 2010)
Ocimum sanctum L. /*Geniosporum tenuiflorum* (L.) Merr., *O. album* Blanco, *O. brachiatum* Hassk., non Blume, *O. flexuosum* Blanco, *O. frutescens* sensu Burm. f., non L, *O. gratissimum*sensu Lour., non L., *O. hirsutum* Benth., *O. inodorum* Burm. f., *O. monachorum* L., *O.*	leaf	aqueous or ethanol or methanol extract	*In vitro* (broth dilution method) MIC = 167 µg/ml *Trichophyton mentagrophytes* (Balakumar et al., 2011) *In vivo* (treatment in bovine sub clinical mastitis, total bacterial count) Dose = 100 mg/day (day 30): total bacterial count lowered by 85% (Mukherjee et al., 2005)	*In vitro* (DPPH assay) IC_{50} = 88 µg/ml (Dutta and Maharia, 2012)	*In vivo* (milk differential leukocyte count) Dose = 0.2 mg/kg Neutrophyl: day 3: increase by 12% *In vivo* (phagocytic activity) Dose = 0.2 mg/kg day 3: increase by 58% (Mukherjee et al., 2005)	*In vivo* (excision wound model): Dose = 400 mg/kg Wound contraction (in 14 days): 100% (Shetty et al., 2008)

Table 3: Plant species possessing complex biological effects (Continued)

Scientific name/Common synonyms/Family	Part tested	Extract/ Fraction	Biological effect			
			Antimicrobial Method/Concentration/ Microorganism/Reference	Antioxidative Method/Concentration/ Effect/Reference	Immunomodulatory Method/Concentration/ Effect/Reference	Anti-inflammatory Method/Concentration/ Effect/Reference
sanctum L. var. *hirsuta* (Benth.) Hook. f., *O. tomentosum* Lam., *O. villosum* Roxb., *O. virgatum* Blanco, non Thunb., *Plectranthus monachorum* (L.) Spreng., *P. striatus*sensu Muschler & Hosseus, non Benth./Lamiaceae						
Phyllanthus emblica L./*Emblica officinalis* Gaertn., *Cicca emblica* Kurz, *Mirobalanus embilica* Burm., *P. mairei* Lév. /Euphorbiaceae	fruit or fruit pulp	aqueous extract or methanol or supernatant (*in vivo*)	*In vitro* (agar dilution method) MIC = 7,000 µg/ml *Candida albicans* (Mehmood et al., 1999)	*In vitro* (DPPH assay) IC_{50} = 4 µg/ml (Nampoothiri et al., 2011)	*In vivo* (mice sensitized with sheep red blood cells, white blood cell count) Dose = 100 mg/kg day 19: increase by 57% (Suja et al., 2009)	*In vivo* (adjuvant induced arthritic rat model, animals injected with complete Freund's adjuvant, paw oedema measurement) Dose = 25 mg/kg day 5: inhibition: 58% (Ganju et al., 2003)

Table 3: Plant species possessing complex biological effects (Continued)

Scientific name/Common synonyms/Family	Part tested	Extract/ Fraction	Biological effect			
			Antimicrobial Method/Concentration/ Microorganism/Reference	Antioxidative Method/Concentration/ Effect/Reference	Immunomodulatory Method/Concentration/ Effect/Reference	Anti-inflammatory Method/Concentration/ Effect/Reference
Zingiber officinale Roscoe/*Amomum angustifolium* Salisb., *A. zingiber* L./Zingiberaceae	rhizome	aqueous or methanol extract or dried and ground (fed *in vivo*)	*In vitro* (broth dilution method) MIC = 300 µg/ml *Helicobacter pylori* (Nanjundaiah et al., 2011)	*In vitro* (DPPH assay) IC_{50} = 24 µg/ml (Saputri and Jantan 2011)	*In vivo* (phagocytic activity) Dose = 10 or 17 mg/kg Phagocytic activity (day 14): increase by 25% *In vivo* (white blood cells count) Dose = 20 or 34 mg/kg Increase by 15% (Nya and Austin 2009)	*In vivo* (adjuvant-induced arthritis rat model, inhibition of paw oedema) Dose = 200 mg/kg day 29: inhibition: 76% (Ramadan et al., 2011)

The data was rounded up to whole numbers and standard deviation was not considered.

Table 4: Plant species possessing three out of four required biological effects

Scientific name/Common synonyms/Family	Part tested	Extract/ Fraction	Biological effect			
			Antimicrobial Method/Concentration/ Microorganism/Reference	Antioxidative Method/Concentration/ Effect/Reference	Immunomodulatory Method/Concentration/ Effect/Reference	Anti-inflammatory Method/Concentration/ Effect/Reference
Acacia nilotica (L.) Delile/*Mimosa nilotica* L., *A. arabica* (Lam.) Willd., *A. scorpioides* W. Wight, *A. vera* Willd., *M. arabica* Lam., *M. scorpioides* L./Mimosaceae	stem bark	ethanol extract	*In vitro* (broth dilution method) MIC = 1,560 µg/ml *Mycobacterium aurum* (Eldeen and Van Staden 2006)	*In vitro* (DPPH assay) C = 4 µg/ml Inhibition: 87% (Sultana et al., 2007)	*In vitro* (luminol/lucigenin-based chemiluminescence assay, cell oxidative burst response of isolated polymorphonuclear and mononuclear cells) C = 6 µg/mL Inhibition 67% (Koko et al., 2008)	
Baccharis dracunculifolia DC./ Asteraceae	leaves	ethanol extract or dichlormethane extract	*In vitro* (broth dilution method) MIC = 40 µg/ml *Cryptococcus neoformans* (Filho et al., 2008)		*In vitro* (H_2O_2 production by peritoneal macrophages) C = 25 µg/ml H_2O_2 production: Increas by 160% (Missima et al., 2007)	*In vivo* (carrageenan-induced paw oedema) Dose = 300 mg/kg Inhibition (3rd hour): 39% (dos Santos et al., 2010)
Capparis spinosa L./*C. ovata* Desf., *C. rupestris* Sm., *C. spinosa* L. subsp. *rupestris* (Sm.) Nyman/Capparaceae	aerial parts, flower buds	ethanol or methanol extract	*In vitro* (agar diffusion method) C = 2,000 µg/disc *Bacillus cereus* Zone of inhibition: 17 mm (Mahasneh 2002)	*In vitro* (DPPH assay) EC_{50} = 178 µg/mL (Germano et al., 2002)		*In vitro* (NO assay, Griess reaction) C= 10 µg/mL Inhibition: 57% (Panico et al., 2005)
Dicranopteris linearis (Burm.f.) Underw./*Polypodium lineare* Burm.f./ Gleicheniaceae	leaves	aqueous or methanol extract	*In vitro* (broth dilution method) MIC = 1,250 µg/ml *Staphylococcus aureus* (Zakaria et al., 2010)	*In vitro* (DPPH assay) C = 100 µg/ml Inhibition: 85% (Zakaria et al., 2011)		*In vivo* (carrageenan-induced paw oedema test) Dose = 66 mg/kg Thickness of paw oedema: hour 3: inhibition: 17% (Zakaria et al., 2008)

Table 4: Plant species possessing three out of four required biological effects (Continued)

Scientific name/Common synonyms/Family	Part tested	Extract/ Fraction	Biological effect			
			Antimicrobial Method/Concentration/ Microorganism/Reference	Antioxidative Method/Concentration/ Effect/Reference	Immunomodulatory Method/Concentration/ Effect/Reference	Anti-inflammatory Method/Concentration/ Effect/Reference
Dittrichia viscosa (L.) Greuter/ *Erigeron viscosus* L./*Inula viscosa* (L.) Aiton/Asteraceae	aerial parts	essential oil	*In vitro* (agar dilution method) MIC = 1 µl/ml *Staphylococcus epidermidis, Streptococcus faecalis, Proteus vulgaris* (Blanc et al., 2006)	*In vitro* (DPPH assay) IC_{50} = 1011 µg/mL (Albano et al., 2012)		*In vitro* (5-Lypoxigenase assay) IC_{50} = 291µg/mL (Albano et al., 2012)
Laurus nobilis L./ Lauraceae	leaves	essential oil	*In vitro* (broth dilution method) MIC = 100 µg/ml *Candida albicans* (Ozcan et al., 2010)	*In vitro* (DPPH assay) IC_{50} = 94,655 µg/ml (Ozcan et al., 2010)		*In vivo*: (formalin induced paw edema) Dose = 0.1 ml/kg Inhibition (3 hours) 35% (Sayyah et al., 2003)
Melissa officinalis L./*M. citriodorata* hort., *M. cordifolia* Pers., *M. hirsuta* (Pers.) Hornem./Lamiaceae	aerial parts	aqueous extract	*In vitro* (agar diffusion method) MIC and MFC = 200 µg/ml *Dekkera anomala, Torulaspora delbrueckii* (Araujo et al., 2003)	*In vitro* (DPPH assay) IC_{50} = 8 µg/ml (Mimica-Dukic et al., 2004)		*In vivo* (histamine-induced paw oedema) Dose = 50 mg/kg Inhibition (after 5 hours): 48% (Birdane et al., 2007)
Nigella sativa L./*N. indica* Roxb. ex Flem., *N. truncata* Viv./Ranunculaceae	seeds	essential oil	*In vitro* (broth dilution method) MIC = 4 µg/ml *Bacillus cereus, Staphylococcus aureus, S. epidermidis* (Kokoska et al., 2008)	*In vitro* (DPPH assay) IC_{50} = 460 µg/ml (Burits and Bucar, 2000)		*In vitro* (NO assay) C = 4 µg/ml Inhibition of NO: 95% (Bourgou et al., 2010) *In vivo* (croton oil induced ear oedema in mice) Dose = 10 µl/kg Reduction by: 39% (Hajhashemi et al., 2004)

Table 4: Plant species possessing three out of four required biological effects (Continued)

Scientific name/Common synonyms/Family	Part tested	Extract/ Fraction	Biological effect			
			Antimicrobial Method/Concentration/ Microorganism/Reference	Antioxidative Method/Concentration/ Effect/Reference	Immunomodulatory Method/Concentration/ Effect/Reference	Anti-inflammatory Method/Concentration/ Effect/Reference
Prunella vulgaris L./*Brunella vulgaris*, Benth./Lamiaceae	aerial parts, herb	aqueous extract		*In vitro* (DPPH assay) C = 7 µg/ml Inhibition: 46% (Kyung-A et al., 2011)	*In vitro* (mitogenic activity, flow cytometric analysis) C = 400 µg/mL Lymphocyte proliferation: Increase by 5% (Harput et al., 2006)	*In vitro* (NO production, Griess method) C = 400 µg/ml NO inhibition: 82% (Harput et al., 2006)
Thymbra capitata (L.) Cav/*Satureja capitata* L./Lamiaceae	flowering aerial parts	essential oil	*In vitro* (broth dilution method) MIC = 0.05 µl/ml *Listeria monocytogenes* (Faleiro et al., 2005)	*In vitro* (DPPH assay) IC_{50} = 61 µg/ml (Albano et al., 2012)		*In vitro* (5-Lypoxygenase assay) IC_{50} = 93 µg/ml (Albano et al., 2012)
*Vitis vinifera L./Vitis laciniosa L./*Vitaceae	leaves	aqueous extract	*In vitro* (agar diffusion method) MIC = 1,250 µg/mL *Candida albicans* (Yigit et al., 2009)	*In vitro* (DPPH assay) IC_{50} = 300 µg/mL (Kosar et al., 2007)		*In vivo* (carrageenan-induced paw oedema in mice) Dose = 100 mg/kg Inhibition (in 360min): 27% (Kosar et al., 2007)

The data was rounded up to whole numbers and standard deviation was not considered.

A moderate antiplasmodial activity against *Plasmodium falciparum* (IC_{50} = 25 μg/mL) was recorded for *B. aegyptiacus* (Karou et al. 2011), which supports the prospective anti-infective potential of this species.

Considering the antioxidative effect, the extracts of *B. aegyptiacus, O. sanctum, P. emblica* and *Z. officinale* previously exhibited marked *in vitro* antioxidative properties in DPPH assays, with lowest IC_{50} ranging from 4 to 88 μg/ml (Dutta and Maharia, 2012; Karou et al. 2011; Nampoothiri et al. 2011; Saputri and Jantan 2011). *P. emblica* possesses the lowest IC_{50} value of 4 μg/ml (Nampoothiri et al. 2011), which is a value comparable with the effectiveness of a standard antioxidant ascorbic acid.

Although *B. aegyptiacus* and *E. purpurea* exhibited significant *in vitro* effect in initial screening tests, further investigation did not confirm their immunomodulatory properties (Koko et al. 2008; Benson et al., 2010). Since *in vivo* experiments are more relevant models for evaluation of biological effects, *O. sanctum, P. emblica* and *Z. officinale* exhibiting potent immunostimulatory action in tests with animal models (Mukherjee et al. 2005; Nya and Austin 2009; Suja et al. 2009) can be considered as more prospective than *B. aegyptiacus* and *E. purpurea*. However, it is difficult to compare these results between each other, because different animal species (cows, mice and trout), different way of administration (oral and intramammary) and different experiment designs (e.g. observation times) were used. Nevertheless, based on the results of previously performed tests, all of these three species can be consider potent immunomodulatory agents.

As far as the anti-inflammatory activity is concerned, *E. purpurea* showed interesting *in vitro* properties, when even 2 μg/ml significantly inhibited the cyclooxygenase-2 activity (Benson et al. 2010). *B. aegyptiacus, O. sanctum, P. emblica* and *Z. officinale* possessed significant *in vivo* anti-inflammatory activities (Ganju et al. 2003; Ramadan et al. 2011; Shetty et al. 2008; Speroni et al. 2005). The most effective plants seem to be *B. aegyptiacus* and *P. emblica*, with the lowest doses ranging from 25 mg to 200 mg/kg when significant paw edema inhibition was observed (Speroni et al. 2005; Ganju et al., 2005). However, it is difficult to compare these results between each other because of different experiment designs (e.g. observation times, excision wound model, paw edema model), therefore, all of these four species can be consider potent anti-inflammatory agents.

Interestingly, *Z. officinale* showed growth promoting effects in fish in an experiment carried out by Nya and Austin (2009). Feeding with ginger led to significantly better feed conversion in all tested doses (50, 100, 500 and 1,000 mg of ginger per 100g of feed) compared with the control group. Best feed conversion ratio of 0.1 was observed when fish were fed the highest dose of ginger (control group had feed conversion ratio of 0.5). Weight gain was significantly increased at the doses of 100, 500 and 1,000 mg of ginger per 100g of feed compared with the control group. The highest percentage of weight gain was observed at the highest dose of ginger (31%, control group 18%). Moreover, when fish were challenged with the *Aeromonas hydrophila* infection, the use of ginger for 14 days led to a significant reduction in mortality. In the control group there was the mortality of 64% compared to 16%, 4% and 0% mortality rates in the groups which received 1000, 50 and 5 00 mg of ginger per 100 g of feed respectively. Interestingly, the surviving fish from all the treated groups did not show any signs of disease at the end of experiment.

The most potent plants from Table 3 are *O. sanctum* and *Z. officinale*, possessing marked effects in most biological characteristics previously tested. However, to evaluate immunomodulatory and anti-inflammatory activity and to decide which plant is more effective, it is recommended to test *O. sanctum* and *Z. officinale* in the same type of test and under the same conditions. As mentioned above, Nya and Austin (2009) previously proved beneficial effects of *Z. officnale* for growth promotion in fish. Furthermore, ginger rhizome powder was found not to be toxic in hens fed up to 3% of ginger in feed (Malekizadeh et al., 2012). Regarding *O. sanctum*, the ethanol extract was observed to be safe up to 4 g/kg of body weight in rats (Shetty et al., 2008). Therefore, further studies of *Z. officinale* and *O. sanctum* on livestock animals are recommended to test their growth promoting effects.

From Table 4 showing plants possessing three of the required properties, *B. dracunculifolia, L. nobilis, M. officinalis* and *N. sativa* previously exhibited significant *in vitro* growth-inhibitory effect against various species of pathogenic microorganisms (*Bacillus cereus, Candida albicans, Cryptococcus neoformans, Dekkera anomala, Staphylococcus aureus, S. epidermidis,* and *Torulaspora delbrueckii*) with lowest MICs ranging from 4 to 200 µg/ml (Araujo et al. 2003; Filho et al. 2008; Kokoska et al. 2008; Ozcan et al. 2010). This corresponds to the parameters proposed by Rios and Recio (2005)

for antimicrobially effective plant-derived products (MIC < 1 mg/ml for extracts). Furthermore, two other plant species *D. viscosa* and *T. capitata* possessed interesting antimicrobial effect with MICs ranging from 0.05 to 1 μl/ml against various pathogens (*Listeria monocytogenes, Proteus vulgaris, Staphylococcus epidermidis* and *Streptococcus faecalis*) (Blanc et al. 2006, Faleiro et al. 2005).

Considering antioxidative activity, the extracts of *A. nilotica, D. linearis M. officinalis, P. vulgaris* and *T. capitata* exhibited significant *in vitro* antioxidative properties in DPPH assays, with lowest IC_{50} ranging approximately from 4 to 61 μg/ml (Albano et al. 2012; Kyung-A et al. 2011; Mimica-Dukic et al. 2004; Sultana et al. 2007; Zakaria et al. 2011). *A. nilotica, M. officinalis* and *P. vulgaris* had IC_{50} lower than 10 μg/ml (Kyung-A et al. 2011; Mimica-Dukic et al. 2004; Sultana et al. 2007), which are values comparable with the effectiveness of a standard antioxidant ascorbic acid.

From Table 4, only three plants, namely *A. nilotica, B. dracunculifolia* and *P. vulgaris* were previously tested for their immunomodulatory activity and that in *in vitro* experiments (Harput et al. 2006; Koko et al. 2008; Missima et al. 2007). The most potent effect was recorded for *A. nilotica*, since the smallest concentration was needed to cause a significant immunomodulatory effect.

Considering anti-inflammatory activity, five plant species were tested *in vitro.* In the 5-lypoxigenase assay the IC_{50} of extracts were 93 and 291 μg/ml for *T. capitata* and *D. viscosa* respectively (Albano et al. 2012; Azah et al. 2000). Another *in vitro* test examined the inhibition of nitric oxid production when IC_{50} ranged approximately from 4 to 243 μg/ml for *C. spinosa, N. sativa* and *P. vulgaris* (Bourgou et al. 2010; Harput et al. 2006; Panico et al. 2005), where *N. sativa* exhibited the best activity. This anti-inflammatory activity of *N. sativa* was also observed *in vivo* when the extract of *N. sativa* at the dose of 10 μl/kg significantly inhibited induced ear edema in mice by 39% (Hajhashemi et al. 2004). In the rest of the plant species the anti-inflammatory activity was proved by *in vivo* experiments, observing paw edema inhibition. The best activity, where the lowest doses significantly inhibited paw edemas, were observed for *D. linearis, M. officinalis* and *V. vinifera*, with doses ranging from 50 mg/kg to 100 mg/ kg (Birdane et al. 2007, Kosar et al. 2007, Zakaria et al. 2008).

The most potent plants from Table 4 are *M. officinalis* and *T. capitata*, since they exhibited the lowest concentrations to cause a significant biological effect in all three

activities for which they were previously tested. However, the data is lacking on their immunomodulatory properties, therefore, further investigation is necessary. *M. officinalis* aqueous extract showed low toxicity *in vitro* on African green monkey kidney cells, with maximum nontoxic concentration of 150 μg/ml (Astani et al., 2012). Toxicological data for *T. capitata* is lacking, therefore, further investigation on toxicity is neecessary.

5. Conclusion

Investigation of available scientific literature has led to the conclusion that plants contain biologically active products, which exhibit biological effects (antimicrobial, antioxidative, immunomodulatory and anti-inflammatory) and are capable to promote growth in livestock animals.

I have identified 16 plant species previously exhibiting either all four required effects (antimicrobial, antioxidative, immunomodulatory and anti-inflammatory) or at least three of them. There were five plant species previously tested on their antimicrobial, antioxidative, immunomodulatory and anti-inflammatory activity and from these I have identified two plant species, *O. sanctum* and *Z. officinale* (leaves and rhizomes respectively), with the most promising biological effects. Further research on livestock animals is recommended to prove the potential growth promoting effect of these plant species.

Furthermore, from the total number of 16 plant species there were 11 plant species previously tested on three out of four of the above mentioned biological effects and from these I have identified two plant species, *M. officinalis* and *T. capitata* (aerial parts for both plant species), with the most promising biological effects. These plant species, however, have to be further investigated for their immunomodulatory effect and *T. capitata* should be tested on its toxicity.

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7. Abbreviations

MIC	Minimum inhibitory concentration
MFC	Minimum fungicidal concentration
DPPH	2,2-diphenyl-1-picrylhydrazyl
IC_{50}	Half maximal inhibitory concentration
COX	Cyclooxygenase
EC_{50}	Half maximal effective concentration
NO	Nitric oxide