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A Survey of Recent Data of the Last
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Sophie Barelli

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Abstract

The aim of this master thesis was to provide an overview of the published data on the potential of essential oils and their components that are used in veterinary aspects. Therefore, studies from 2000-2012 that dealt with different ways of aromatic extracts in reference to animals, were discussed. However, the focus of this master thesis lies on the application of essential oils as food additives instead of antibiotics as well as their different fields of application. In the future, more studies are necessary to reveal the benefits of aromatic extracts on animals, especially when they are used for treating diseases. Considering, that essential oils cannot only be used in aromatherapy, but can also be applied orally or topically.

Zusammenfassung

Hiermit soll ein Überblick über veröffentlichte Daten zum Thema „Ätherische Öle und ihre Komponenten, welche für veterinäre Zwecke angewendet werden“ gegeben werden. In diesem Zusammenhang wurden Studien (2000-2012) untersucht, die sich mit aromatischen Extrakten und Tieren befassten. Der Fokus dieser Diplomarbeit liegt dabei auf dem Einsatz von ätherischen Ölen als Futterzusatzmittel anstelle von Antibiotika, aber auch auf anderen Einsatzgebieten. In der Zukunft werden weitere Studien nötig sein, die sich genauer mit dem Thema befassen werden, vor allem im Bereich der Tiermedizin. Es wäre wünschenswert, mehr Informationen über die Auswirkungen von ätherischen Ölen bei Tieren zu bekommen.

Table of contents

1	Introduction.....	11
1.1	Essential Oil definition:	11
1.2	Essential Oils and Animal Aromatherapy	12
2	Oils Attracting and Repelling Animals.....	14
3	Oils Used in Animal Feed	15
3.1	Essential Oils in Poultry nutrition	16
3.2	Essential Oils in Ruminants.....	20
3.3	Essential Oils in Pigs.....	36
4	Oils Used in Treating Diseases in Animals	43
4.1	Treating Diseases	43
4.2	Usage of Essential Oils in Psychology	46
5	New use of essential oils	50
6	Olfactory stimulation with Essential Oils	53
6.1	Essential Oils as olfactory stimulation	54
6.2	Pheromone stimulation.....	57
7	Conclusion.....	59
	Tables	60
	References.....	61
	Curriculum vitae	71

1 Introduction

1.1 Essential Oil definition:

Essential Oils are volatile, lipophilic substances obtained from plant materials by steam distillation mechanical separation, except for citrus oil, which is obtained by cold pressing of the peels. These oils are complex mixtures of secondary plant metabolites and are largely based on mono-, sesqui- and sometimes diterpenoids and phenylpropanoids.

Estimated 3000 essential oils are known to man, of which approx. 300 are commercially valuable. They can be used in many different fields, like pharmaceuticals, food flavoring and perfumery.

Their spectrum of biological activities is very broad, ranging from antiviral, antibacterial, antifungal, analgesic, sedative, expectorant, antispasmodic, stimulant to skin-regenerative, etc. Consequently, the indication area is widespread as well. Essential oils are frequently applied to cure common colds, skin problems, stomach and intestinal problems, headaches, burns, and poor wound healing.

They possess the ability to directly affect the brain and, from there, many psychological and physiological processes can be observed.

There are many different ways how an essential oil can pass its active pharmaceutical ingredients into the body; the most common ways are through aroma lamps, sprays or by inhalation. Oils can also be absorbed by the skin, e.g. by a massage, reaching the organs to be treated by the way of the connective tissue and the circulatory system.^[1-5]

1.2 Essential Oils and Animal Aromatherapy

Aromatherapy is an independent field of phytomedicine and records indicate its' use already approximately 6000 years B.C. in India, Mesopotamia and Egypt. Ancient Egyptians not only used an apparatus to distill cedar wood oil, they were also acquainted with clove, cinnamon, nutmeg and myrrh oils. However, while phytotherapy uses the whole plant, the effectiveness of aromatherapy only lies in its very specific and highly potent plant compounds, the essential oils. Unfortunately, synthetic fragrances or ingredients can also be labeled "aromatherapy", although they are not true essential oils, even when they will smell like them.

Essential-oil-therapy as a special part of phytotherapy for animals is the traditional use of essential oils to promote and maintain balance in all systems of the body. In the wild, animals seek out a natural remedy from various herbs and plants, instinctively picking out their own medication, scientifically known as zoopharmacognosy. However, in a domestic environment they are deprived of this choice. We can offer them a natural choice of relief by treating them and their physical or behavioral problems with essential oils. Aromatherapy is a suitable "self-treatment" for minor or short term problems and is compatible with conventional medicine. Nevertheless, it is always better to consult a specialist before starting with a therapy on your own, although there are not too many veterinarians, who use essential oils to treat or calm animals.

Generally, it is known that not every animal likes essential oils; especially cats do not digest them like humans, so they should be used with caution. The most important safety tip is, to never use or spray an essential oil in or around an animal's nose or eye.

A dog's system of smell, for example, is much keener than the humans. Although the aroma will not be smelled by humans, it will be pretty potent to a dog; consequently the scent has to be diluted.

Nonetheless, there are benefits too, such as the natural oils will not harm the sheen of the animal's coat or dry its skin.

There are multiple levels of how aromatherapy works on animals: conditionally, emotionally, evolutionary, physically and spiritually. These levels also apply to the human's use of natural aromatics; however the experience is dissimilar due to these documented emotional responses to various odors. Smells are processed by the limbic system, which is the simplest part of the human brain and is connected to the recall of certain emotions. Neither is there scientific proof that animals have this type of reaction to an aroma, nor do we know if their brains process scents in this manner.

Nevertheless, smell is a very powerful sense and is even more pronounced in animals. They will inhale a small amount of essential oils into the ideal conditions of the warm and moist nasal cavity, some of the active components will directly route into the bloodstream via capillaries very close to the surface.

Normally, aromatherapy for animals is given either by inhalation or by ingestion of the oil whilst topical application is more reserved for the treatment of physical problems (wounds or disorders of the musculoskeletal system).

Essential oils are easily absorbed either through the olfactory system or the skin and are so transported throughout the body.^[3-7]

"The potential for direct psychological and physical effects becomes clear when the anatomy of the olfactory system is examined. In the nasal mucosa, chemoreceptors give rise to axons that synapse on the olfactory bulb. Olfactory neurons then run through the olfactory tract to the olfactory cortex. Behavioral reactions to scent are mediated by direct connections to the limbic system."^[7]

Essential oils are often used as feed additives nowadays and this topic is becoming more and more important, because of the negative effects of antibiotics. During the last decades, synthetic growth promoters (e.g. antibiotics) were widely applied in the livestock

industry for their outstanding health and growth promoting possessions. However, possessing a declined therapeutic efficacy of antibiotics for treatment of bacterial infection in humans, their usage as feed additives for livestock was completely outlawed. Consequently, extensive efforts have been made toward developing alternatives to stabilize the health and growth performance in animals.^[8]

The use of essential oils, not only in aromatherapy, is getting more and more popular nowadays. However not every oil is supposed to help every animal. Especially cats cannot be easily treated with aromatic extracts, because they do not like the intense smell of most oils. In general, almost every animal tends to know which aroma it likes and which odors it does not prefer. There is a wide range of essential oils that can be applied to animals. Nevertheless, it is important to know, which one is suitable for which animal and what effects the essential oil has.^[6, 7]

2 Oils Attracting and Repelling Animals

The subject of essential oils, attracting and repelling animals, is quite popular and has been discussed in other theses, such as Marianne Müller^[9] sufficiently. Hence this chapter only gives a short overview.

There are many different plants containing essential oils. Their aromas are the fragrant messengers that, among other things, attract or repel animals.

Although they have a long history and are used in many different ways to appeal animals, only a few studies are published about the appealing effect of essential oils to animals,

However, one abstract was found about horse care products that contain essential oils and were exposed on them. This investigation showed that horses were especially tempted to peppermint, violet

leaf, valeriana and lavender, although only a limited number of ten oils were tested.^[10]

It is also well-known that especially cats seem to love the aroma of valeriana or nepeta oils, while dogs prefer to inhale floral ones, such as bergamot oil.^[1]

On the other side, essential oils can also be used to repel various animals, such as cats, dogs and mice. Most studies deal with the use of essential oils as repellents against insects and pest. Besides essential oils pheromones can also be used to repel bigger animals.

3 Oils Used in Animal Feed

In the last decades, antibiotic and antimicrobial compounds produced by microorganisms have been used in animal diets to increase their performances and decrease morbidity. Nevertheless, they have been more and more criticized, because of the potential development of antibiotic-resistant or cross-resistance in animals and humans. As a result, the development of non-antibiotic feed additives, such as aromatic plants and their extracts, have received attention as growth and health promoters. Especially essential oils and other secondary plant metabolites are responsible for most of the properties that lead to better performances. The effects of aromatic extracts in livestock is not constant, since the quality of natural products can alter greatly due to the dissimilar herbal materials, selection of specific plants and forms of their application (Windisch et al.).^[11] Above that, Wagner and Ulrich-Merzenich^[12] implied that a complex blend of herbal extract has a better biological benefit than single extracts do.

Moreover, the European Union (EU) strongly proceeded with regulations affecting the feed additives market due to human health and safety concerns. Since the end of 1998 only four antibiotic based growth promoters were allowed. The Regulation (EC) 1831/2003^[13] followed and finished the phasing-out of antibiotics, which were hasty

fed to animals before. Moreover, since 01.01.2006 there has been a new EU-wide prohibition against the last allowed antibiotic feed supplements.^[13] Although there is a less legislative regulation in the USA, lobbies by the American Medical Association and other mechanisms achieved a decline in the application of antibiotics in agriculture and particularly in food-producing animals.^[14]

3.1 Essential Oils in Poultry nutrition

In the limited number of controlled studies particularly in broiler chickens, most were about the effects of essential oil components on growth performance, macronutrient digestibility and digestive enzymes activities. However, some authors document a more positive influence on broiler performance, while alternative results report unchanged weight or other parameters, such as Windisch et al..^[11]

Because of the ban of antibiotics the use of essential oils in poultry production is becoming more common. Nevertheless, the mode of the active compounds in birds cannot be fully analyzed. There are four different mechanisms, how aromatic plants interfere with them. Essential oils can influence these animals through antimicrobial, antioxidant, sensorial or metabolic systems.

The most frequently experienced effects of aromatic plants are found through oral and nasal sensing, often referred to as “oronasal”, and digestive stimulation.

“Oronasal sensing prepares the gastrointestinal (GI) tract for food reception and stimulates digestive secretions and gut motility. The somatic sensing in the oronasal cavity is linked to the cranial nerve V (the trigeminal) and covers all the oronasal epithelium and detects touch and proprioception (A β -fibers) noxious temperatures (low or high) and chemesthesis (A δ -fibers) and pain (C-fibers) including pungency by acids and spices. Noxious, high and low temperatures but also pungent and spicy compounds may lead to mucosal

damage in the GI. The trigeminal stimulation consequently leads to an alarm response characterized by feed avoidance, followed by an integrated mucous secretory response of the GI tract and an increase in the intestinal motility aimed to protect the digestive epithelium. Spices or their active principles have been reported to stimulate digestive secretions such as saliva (and salivary amylase) in humans and gastric, bile acids, pancreatic enzymes (lipase, amylase and proteases) and intestinal mucosa in rats”.^[15]

The second mechanism is the antioxidant effect of essential oils, which prevents tissue oxidation. Antioxidants have been used since ancient time. However, because of the new trend to use more natural products and question the synthetic ones, such as butylated hydroxytoluene (BHT), antioxidants of plant origin have become more popular again. Investigations about aromatic plants and spices that revealed an effect in retarding the process of lipid peroxidation in oils and fatty foods, gained the interest of many experimental groups. Especially herbs of the Labiatae family, such as oregano and rosemary are well documented in this aspect. Oregano has high antioxidant activity and shows a respectable reaction on delaying or preventing the process of large oxidation, because of its main phenols: carvacrol and thymol.^[15]

Antimicrobial activity and modulation of gut microbiota is the third effect of essential oils, which is also the most reasonable intrinsic one. There are many scientific references about aromatic plants and their antibacterial, antifungal and antiviral activities against foodborne pathogens and animals.^[15]

In general, most studies showed that essential oils are more efficient against gram-positive bacteria, while gram-negative are more resistant to them. Nevertheless, some oils, like rosewood and cinnamon bark, are active against both bacteria forms.

Essential oil	Zone of inhibition							
	Gram-positive bacteria				Gram-negative bacteria			
	Bc	MI	Sa	Ef	Ec	Af	Ecl	Pa
Angelica	2	4	4	4	2	7	1	-
Bergamot	4	6	2	4	2	3	1	-
Cinnamon	17	27	9	12	12	24	18	6
Coriander	>33	>33	25	33	-	10	-	-
Dill	4	4	5	3	4	7	1	1
Eucalyptus	>33	4	-	-	1	7	2	-
Ginger	2	2	-	1	-	-	-	-
Juniperberry	4	7	3	2	2	10	1	-
Lime	4	2	2	2	-	7	-	1
Mandarine	3	-	4	4	1	6	-	-
Savory	15	>33	10	15	18	30	17	-
Nutmeg	2	2	2	1	5	5	3	-
Orange	-	3	-	2	1	5	-	-
Pepper	1	2	-	-	-	8	-	-
Pine	5	5	3	4	3	14	-	-
Rosemary	3	2	-	-	6	6	3	-
Rosewood	13	17	7	5	12	19	15	-
Sage	2	4	2	1	2	12	2	-
Tarragon	4	6	6	7	3	12	1	-

Bc, *Bacillus cereus*; MI, *Micrococcus luteus*; Sa, *Staphylococcus aureus*; Ef, *Streptococcus faecalis*; Ec, *Enterobacter cloacae*; Af, *Alcaligenes faecalis*; Ecl, *Escherichia coli*; Pa, *Pseudomonas aeruginosa*.

Table 1: Effect of essential oils on the growth inhibition of bacteria (adapted from Brenes et al.^[22])

Table 1 represents a selection of minimum inhibitory concentrations (MIC) of essential oils and their tested components. MIC is a measure of essential oils antibacterial capacity. It was often concluded that the whole oil has more potential than its major components. Lambert et al. revealed that the two structurally similar principal components of oregano essential oil, carvacrol and thymol, were found to give additive effect when tested against *S. aureus* and *P. aeruginosa*.^[16]

Mitsch et al.^[17] tested two different blends of essential oils and the results imply that different mixtures of aromatic plants can reduce *C. perfringens* colonization and proliferation in the broiler gut. On the one hand all blends are direct inhibitors of this bacterium, on the

other hand digestive enzymes increase nutrient digestibility and boost the whole gut micro biota.

The last way how essential oils can influence birds is through the metabolic pathway. After dermal, oral or pulmonary absorption the bioactive substances are often metabolized or eliminated by the kidneys or exhaled as CO₂.

Michiels et al.^[18] reflects that carvacrol, thymol, eugenol and trans-cinnamaldehyde are effectively absorbed in the stomach and the proximal small intestine. Moreover, this study demonstrates that the intestinal microbiota doubtfully can metabolize the essential oils.

Amerha et al.^[19] carried out a study about the benefits of a blend of essential oils and whole wheat inclusion on the performance, digestive tract development, ileal microbiota profile and the nutrition utilization of broiler chickens. 192 day-old male broilers were divided into different groups, one was fed with a basal diet on ground wheat (GW) and the other with whole wheat (WW). Both clusters fulfill the Ross 308 strain recommendations for major nutrients for broilers. The active components of the essential oil blend were cinnamaldehyde and thymol. Essential oils have a positive effect against the colonization and proliferation of pathogenic bacteria and can boost nutrition digestibility and boiler achievement. Moreover, they can increase the intestinal health and digestive efficiency of broiler chickens (Amerah et al.).^[20]

The results showed that during the starter phase (1-21 day) the combination of essential oils and wheat form had a significant effect on the weight gain. However, during the trial period (all 35 days) wheat form had no real impact on weight gain or feed intake, although WW inclusion increased feed per gain. On the other hand, the essential oil mixture had a positive influence on weight gain in the two diet groups, while the benefit was more significant in the GW one. The essential oil and wheat form supplementation showed no indicative effects on the relative weight, length and digesta content of

different parts of the digestive tract. All the same, the results showed forceful interactions by the relative gizzard and caecal weights. Cinnamaldehyde and thymol had a significant impact on the relative gizzard weight and reduced relative fecal weight in broilers fed with GW, while there was no benefit for those fed with WW. The combination of essential oils and whole wheat inclusion had a significant improvement on the apparent ileal nitrogen digestibility, while they could not influence the apparent ileal digestible energy. A denaturing gradient gel electrophoresis revealed the ileal microbiota profile, which implied that the feed form influenced the composition of bacterial species. Nevertheless, broilers, which received the GW diet with the essential oils, had a higher mean number of bacterial categories in the ileal contents, than the chickens fed on unsupplemented GW based diet.

The conclusion of all these presented data is that a dietary adding of essential oils boosts broiler weight gain and ileal nitrogen digestibility in both diet forms, however the dimension of the benefit of the blend of cinnamaldehyde and thymol was more significant in the GW based diet. Nonetheless, whole wheat feeding has a positive effect in terms of feed efficiency.

3.2 Essential Oils in Ruminants

The public interest over the use of antibiotics in livestock and their production has grown in recent years because of the possible contribution to emergence of antibiotic-resistant bacteria and their transmission from animals to human beings. Consequently, ruminant microbiologists and nutritionists have seen an investigating alternative, more natural ways of favorably altering ruminal metabolism to develop higher feed efficiency and animal productivity.^[21]

Wallace^[22] showed that plant extracts offer a unique opportunity in these fields, considering that a lot of plants can produce secondary

metabolites, such as essential oils, which for example have antimicrobial properties. Above that, essential oils and their active compounds can manipulate the rumen microbial fermentation, and so lead to an increased production efficiency of the animals. As a result, these aromatic extracts are potential alternatives to the often used antibiotics.

Essential oils are considered to be safe for humans and animals, even when consumed. Moreover, the Food and Drug Administration of the United States categorize them as safe (GRAS, FDA, 2004).^[23]

Their antimicrobial potency is well documented in many studies, such as Chao et al.^[24], and has a wide range against microorganisms, including bacteria, protozoa and fungi. Essential oils have also been applied because of their activity against an expansive variety of food-borne pathogens. Elgayyar et al.^[25], for instance, published that *Escherichia coli* was prevented by oregano oil and its two main compounds: carvacol and thymol.

A number of researches were induced by the well-documented antimicrobial effectiveness of essential oils, to examine their potential to manipulate ruminal fermentation and as a result improve their feed efficiency and nutrient use. There is a limited number of essential oils and mixtures of them, which seem to have some promise in these concerns. Nevertheless, the knowledge of plants, their constituents or how they work has not been fully uncovered and therefore, many of them have still to be explored for these intentions. Moreover, most published studies have been carried out only *in vitro*. Consequently, *in vivo*-analysis are desired to determine the mode of activity of numerous essential oils and some combinations that favorably modify ruminal fermentation.

There have been various publications about the effects of aromatic plants on the microbial fermentation in ruminants, which applied a broad range of essential oils and their mixtures, different dose rates and diets. The inconsistent results obviously reflect dissimilar

chemical structures in essential oils that influence the outcome on microbial activity.

At first, nutritionists only focused on essential oils because of their role in decreasing the palatability of a few plant species. Since the end of the 1960 even their *in vitro* benefits on ruminal microbial fermentation, like the gas production, were investigated.^[26-28] Nagy and Tengerdy^[28] showed that the extracts from Sagebrush (*Artemisia tridentata*, Asteraceae) distinctly inhibited the activity of ruminal bacteria. Above that, Oh et al.^[26] published that the essential oil from Douglas fir needles (*Pseudotsuga menziesii*, Pinaceae) was a general inhibitor on the animal's bacteria liveliness. Nevertheless, the amount of the inhibition relied upon the chemical structure mixture of the aromatic plants. It was even shown that the compounds evaluated, which were oxygenated monoterpenes, especially the monoterpene alcohols and aldehydes could powerfully prevent growth and metabolism of rumen microbes, whereas monoterpene hydrocarbons hardly had these effects or even excited the activity of rumen microbes. These were the first demonstrations that the chemical compositions of essential oils have intense influence on the effects and the activity of ruminal microorganisms. In current research with essential oils in ruminant nutrition the focus is often centered upon their potential to increase the animal's N and energy utilization.

The effects on the protein metabolism:

Because of the symbiosis between ruminants and their microflora they have the exclusive advantage of using non-protein origin of N as nutrients. The ruminant is well supported with the microbial protein that moves from the rumen to the small intestine and works as a unique source of amino acids (AA), which synthesizes milk and meat proteins. Nevertheless, these synthesized microbial proteins are not enough to reinforce the AA necessity of high-producing ruminants. Therefore, diets are frequently added with origins of feed protein, which often leads to increased costs. Additionally, the ruminal

inefficient N usage proceeds in excretion of N-rich wastes to the surroundings. Lapierre et al.^[29] guessed that a dairy cow excretes about 0.3 of the consumed N with its urine. As a result, enhancing N use shows positive results on the efficiency of ruminant production and on the environment.

In an early study^[30] it was revealed that the supplement of thymol to ruminal fluid (1g/l) including casein lead to an AA accumulation and a reduction of ammonia N (NH₃-N) concentration, which recommends the blockage of AA deamination by ruminal bacteria. Another older publication^[31] showed that thymol inhibited the deamination of AA to NH₃-N. McIntosh et al.^[32] noticed a 9%-decline in the rate of AA deamination, when for 48h casein acid hydrolysate was *in vitro* incubated in batch cultures of ruminal fluid, collected from cows that were fed with a silage-based diet added with 1 g/day of a commercial blend of essential oil compounds (MEO: mixture of essential oil compounds; Crina[®] ruminants; Akzo Surface Chemistry Ltd., Herfordshire, UK). The product Crina[®] holds 100-300 g/kg of phenolic combinations that contain: thymol, eugenol, cresol, resorcinol and guaiacol^[33]. Furthermore, Newbold et al.^[34] revealed a decline (-24%) in the ratio of AA deamination, after a 24h-incubation of casein acid hydrolysate with ruminal liquid accumulated from sheep, which got diets including 110 mg of MEO. Both studies show, that peptidolytic and proteolytic liveliness's in ruminal fluid were not influenced by the blend. Above that, McIntosh et al.^[32] published that there was no supplementary reduction in the amount of deamination when the ionophore monensin was enclosed to ruminal liquid as well. These result showed that the bacterial species impinged by MEO were the same as those prevented by monensin. The same study displayed that MEO inhibited the growth of some, like *Clostridium sticklandii* and *Peptostreptococcus anaerobius*, hyper-ammonia producing (HAP) bacteria, but a different group of HAP bacteria, such as *Clostridium aminophilum* was lacking this benefit. Although hyper-ammonia creating bacteria are only < 0.01 of the rumen bacterial

population, they occupy a colossal deamination power. Another study^[22] showed that the HAP bacteria number could be decreased by 77% in sheep, which were fed a low-protein diet in combination with MEO at 100 mg/das. Nevertheless, MEO lead to no effect on this bacterial group when the animals received a high-protein diet instead. Generally, the outcomes of all these studies imply that effects of essential oils on the ruminal protein metabolism are on AA degradation, e.g. mostly deamination, and that these results are probably due to the blockage of HAP bacteria.

Continuous culture systems were used in other experiments to explore the benefits of aromatic extracts and their constituents on the N metabolism in ruminants. Castillejos et al.^[35] worked with a dual-flow continuous culture fermenter, which maintained a constant pH through the whole incubation-time (8 days). This study showed that the supplementation of MEO at 1.5 mg/l had no reaction on the NH₃-N concentration, bacterial and dietary N flows, the degradation of crude protein or even the effectiveness of microbial protein synthesis. The deficiency of activity of MEO on the N metabolism was credited to the low dose of 1.5 mg/l, which may not be able to modify effects on ruminal bacteria. Regardless, there was still no effectiveness on the N metabolism (i.g. ruminal concentration of NH₃-N, small peptides and AA or large peptides) in ruminants, when Castillejos et al.^[36] worked under the same conditions with the identical MEO, but in higher concentrations (like 5.50 and 500 mg/l). It was suggested^[32, 37] that a concentration above 35 mg/l of this blend, which is problematic to achieve *in vivo*, would be desired to activate the N metabolism. However, Benchaar et al.^[38, 39] revealed that lactating dairy cows, which got a diet added with MEO at doses of 0,75 or 2 g/day, lacked adjustments in ruminal NH₃-N concentration, N retention and even N digestibility. With a rumen volume of 100 liters and an outflow rate of 0.1/h for each adult dairy cow the ruminal concentration of MEO should have been around 3.1 and 8.3 mg/l for the two mentioned doses. These results are certainly under the

range of concentration, like 35-360 mg, that is needed for MEO to alter the N metabolism of ruminal bacteria.^[32] Nonetheless, in the same study it was speculated that the local concentration of essential oils, which are often sparingly soluble, can be higher on the surface of swallowed plant extract, which can enhance the bacterial effectiveness of essential oils *in vivo*.

Another technique, named the “rumen *in situ* bag method”, examined the effects of MEO on the metabolism in ruminants in other studies. For instance, Molero et al.^[40] worked with growing heifers to classify the effects of this blend (700 mg/day) on *in situ* ruminal degradability of proteins in soybean meal, fish meal, sunflower meal, corn gluten feed, green peas and lupin seeds. MEO only decreased the effective ruminal protein degradability of three (lupin seeds, green peas and soybean meal) of the five tested protein additives. Moreover, their declines were too small to influence any rational nutritional impact on the ruminal protein metabolism in these animals.

Two studies^[34, 38] showed that sheep or dairy cows that were fed with 110 mg or 2 g of MEO had no adjustment in the kinetics of protein degradation from soybean meal, which was incubated in their rumen.

The absence of a real benefit of MEO on the N metabolism in long-term *in vitro*, ruminal *in situ* or *in vivo* experiments contrasted with a short-term *in vitro* batch culture study may be associated to the length that ruminal bacteria are exposed to essential oils. A longer duration of exposure could lead to fluctuation in microbial populations. Above that, it may be likely that some of the aromatic mixtures are subjected to degradation by the bacteria of ruminants. Cardozo et al. and Busquet et al.^[41, 42] published that a few effects of essential oils and their main parts on rumen microbial fermentation expended after 6-7 days of process in a dual flow continuous-culture system, which suggested that the rumen microbial population could adjust to essential oils. Consequently, the outcomes of *in vitro* batch cultures must be carefully clarified, because they declare reactions over a set of incubation time (like 24 or 48 h) and describe probable

shifts in microbial population that likely arise as a result of exposure of rumen microbes to aromatic blends.

In newer studies it was presented that e.g. the chemical structure or the dosage rate of essential oil could affect its effectiveness on ruminal N metabolism. For instance, it was revealed that the supplementation of 2.2 mg/l clove bud essential oil (*Syzygium aromaticum*, Myrtaceae) to a continuous culture fermenter powerfully decreased (e.g. -80%) the concentration of large peptides.^[42] Nevertheless, that showed no consequence on $\text{NH}_3\text{-N}$, which means it only reduced to peptidolytic activity of ruminal bacteria. Moreover, the supplement of the main constituent of this essential oil, eugenol, at the same concentration could not affect the N metabolism, recommending that the anti-peptidolytic activity of *clove bud* oil is not depending on its principal part, but results from unfamiliar components within the oil fraction. Contrary, Busquet et al.^[43] revealed that not only oregano oil but also its main component carvacrol could reduce the concentration of $\text{NH}_3\text{-N}$ in when they were added in the same concentration (e.g. 3000 mg/l). The study showed that the activity of oregano oil and its antibacterial effectiveness mainly consists of carvacrol.

The variable chemical structure of essential oils could also influence the way how they change the N metabolism in ruminants. Castillejos et al.^[44] examined a discrepancy in the effects of increasing dosage levels (5, 50, 500 and 5000 mg/l) of various essential oil combinations on fermentation products in 24 h *in vitro* batch cultures of rumen liquid. While vanillin, an aldehyde, had no effectiveness on changing $\text{NH}_3\text{-N}$ concentration in the different doses (5, 50 and 500 mg/l), the monoterpene limonene could reduce it with a dosage of 500 mg/l. Only three (5, 50 and 500) applications of eugenol, a phenol, too lowered this concentration, while another phenolic, guaiacol, could decrease in all doses the $\text{NH}_3\text{-N}$ concentration. Altogether, these outcomes have reflected that phenolic compounds have higher antimicrobial activity, because of the existence of a

hydroxyl group within the phenolic architecture.^[45-47] Dorman and Deans^[45] also showed that compounds with a phenolic anatomy have a broad spectrum of activeness against a variety of Gram-positive and Gram-negative bacteria too.

Essential oils and their mixtures have been shown to influence the N metabolism in ruminants depending in a dosage way. Busquet et al.^[43] for example, revealed that some essential oils (like: anise, cade, capsicum, cinnamon, clove bud, dill, garlic, ginger, oregano and tea tree oil) and their main parts (e.g. anethol, benzyl salicylate, carvacrol, carvone, cinnamaldehyde and eugenol) significantly inhibited the $\text{NH}_3\text{-N}$ concentration at high doses (i.e. 3000 mg/l), but the activities were insignificant with moderate concentrations (like 300 mg/l) and nonexistent at minimal dosages (e.g. 3 mg/l). Nevertheless, the reduced ruminal $\text{NH}_3\text{-N}$ concentration was combined with degradation in total volatile fatty acid (VFA) concentration, suggesting a decline in complete fermentation of the diet. Considering VFA are the main source of energy in these animals, lowering ruminal VFA production could have antagonistic nutritional consequences, when this effect was shown *in vivo*.

There are only few data on the effectiveness of essential oils and their elements on ruminal bacterial N escape available. The supplementation of garlic oil or cinnamaldehyde^[48] did not change the flow of bacteria, but Fraser et al.^[49] showed that it could be decreased by cinnamon leaf essential oil. Two other studies^[34, 38] observed that a daily dose of 110 mg and 2 g of MEO had no influence in the duodenal bacterial N flow of sheep and dairy cows. Different dosages, altered experimental setups or unlike chemical structure of the aromatic extracts may be responsible for the discrepancy between the analyses.

In ruminants the utilization of N is negatively affected by ruminal protozoa, which absorb and digest enormous numbers of ruminal bacteria and therefore decline net microbial protein flow from the rumen to the duodenum.^[50] Above that, protozoa own deaminating

and proteolytic activeness. Consequently, the disposal of protozoa from the rumen (e.g. defaunation) impedes the recycling of N between bacteria and protozoa that leads in an expanded flow of microbial N in this area. For example, the bacterial protein flow to the intestine in defaunated sheep reached a higher percentage (35%) than in faunated ones (Ivan et al.).^[51] The boosted bacterial protein synthesis in the defaunated rumen could improve the host by providing extra AA for absorption. Additionally, the progressed effectiveness of the N metabolism in the rumen could decrease N losses in feces and urine. Due to the absence of an appropriate defaunating agent as well as spontaneous refaunation, this process has not been useful in commercial ruminant production systems. In many studies (e.g. Wang et al., Min et al.)^[52, 53], plant extracts, such as condensed tannins and steroidal saponins, were extensively observed for their inhibitory benefits on ciliate ruminal protozoa. Still there are only a few studies that address the outcomes of essential oils and their combinations on protozoa in ruminants. Ando et al.^[54] published that a 200 g/day supplementation of peppermint (*Mentha X piperita*, Lamiaceae) to Holstein steers reduced the total number of protozoa and even the ones of *Entodinium*, *Isotrica* and *Diplodinium*. On the other hand, Mohammed et al.^[55] reported that an admixture of cyclodextrin encapsulated horseradish oil could not influence the count of protozoa in ruminal liquid of steers. McIntosh et al.^[32] too documented an unaffected bacteriolytic action of the rumen ciliate protozoa in dairy cows, which were fed with 1 g/day of MEO. Newbold et al.^[34] and Benchaar et al.^[39] showed that the ruminal protozoa numbers of sheep or dairy cows, which got a 110 or 750 mg/day adding of MEO, were not influenced.

The admixture of 1 g/day of cinnamaldehyde in the diet of dairy cows had no consequences on the count or common structure of ciliate protozoa (Benchaar et al.).^[56] Cardozo et al.^[57] discovered that the supplementation of a cinnamaldehyde (180 mg/day) and eugenol (90mg/day) blend to the diet of beef heifers improved the number of

holotrichs and had no impact on entodiniomorphs. Moreover, the same study revealed that increased concentrations of cinnamaldehyde (600 mg/day) and eugenol (300 mg/day) in the combination did not change the counts of these protozoal species. In comparison, 2 g/day anise extract, which included 100 g/kg anethol, added to the diet of beef heifers, lowered the numbers of holotrichs and entodiniomorphs (Cardozo et al.).^[57] In general, essential oils and their parts seem to have no real effectiveness on the counts and/or activeness of ruminal ciliate protozoa.

Effects on volatile fatty acid production

A confine number of studies published that the supplementation of essential oils or an aromatic mixture has progressed the ruminal total VFA concentration. This fact may signify a better feed digestion. For instance, Castillejos et al.^[35] reported that the adding of 1.5 mg/l MEO raised the total VFA concentration in continuous cultures with constant pH, whereas there were no signs of concomitant growth in organic matter digestibility. A diet including MEO (110 mg/day in sheep or 1 g/day in cattle) had no benefits on the absolute VFA concentration or rate (Newbold et al.; Beauchemin and McGinn)^[34, 58], which suggests that the effectiveness of this blend on the total VFA concentration may rely upon the balance of the whole diet. For instance, Benchaar et al.^[39] revealed that MEO (750 mg/day) had an inclination to enhance absolute VFA concentration in the rumen of lactating cows that were fed with a diet including alfalfa silage, but when it was changed to corn silage the outcome was rather lowered.

In general, most studies demonstrated that the addition of essential oils or their components has furnished either a decline or no effect in total VFA concentration. In case, the VFA concentration was diminished on account of the antimicrobial effects of the aromatic plant may be dose dependent. For instant, one study (Busquet et al.)^[43] reported the benefits of several plant extracts (like: anise oil, capsicum oil, cinnamon oil, clove bud oil, dill oil, fenugreek, garlic oil, ginger oil, oregano oil, tea tree oil and yucca) and their secondary

metabolites (e.g. anethol, benzyl salicylate, carvacrol, carvone, cinnamaldehyde and eugenol) on the fermentation in ruminants in a 24 h batch culture. Each extract was added at diverged dosages up to 3 g/l of culture fluid. Nevertheless, no essential oil or even a mixture increased the absolute VFA concentration. On the other hand, most high measured supplementations declined this concentration, which could reflect a decreased feed digestion. Castillejos et al.^[44] published analogous effects for eugenol, guaiacol, limonene, thymol and vanillin, in doses up to 5 g/l. Altogether, they showed no effectiveness, with the exception of their highest dosage that declined the total VFA concentration in cultures for all essential oils.

The unchanged absolute VFA concentration could be noticed as desirable if it was accompanied with adjustments, such as a declined $\text{NH}_3\text{-N}$ concentration, a decreased methane production or a modification in molar proportions of VFA. Nevertheless, the decline in the total VFA production through an essential oil adding would mainly be seen as nutritionally unfavorable. Consequently, it will be a challenge to identify the dosage ranges for various essential oils or their active parts, which can positively alter some aspects of the rumen metabolism without decreasing the absolute VFA concentrations.

In some studies it was reported that certain essential oils and their components switched molar proportions of VFA in a similar way like monensin (e.g. declined acetate and boosted propionate rate; McGuffey et al.^[59]) that is linked as a desired benefit of essential oil supplementation. Busquet et al.^[48] conducted an experiment in a continuous culture with cinnamaldehyde and garlic oil, which were applied in two dosages (31.2 and 312 mg/l of culture liquid). The acetate proportion was declined and the one of propionate increased, with 31.2 mg/l cinnamaldehyde and 312 mg/l garlic oil. With the high dosage the molar proportion of butyrate was boosted as well. Hence the essential oils, or their active parts and the dose rates utilized,

lead to matching effects like monensin, with the only exclusion of the increased butyrate concentration. A subsequent study by this group (Busquet et al.)^[43] showed that supplemented garlic oil and benzyl salicylate (both administered the dosage 300 and 300 mg/l) had the same three outcomes as their first one. The increased butyrate concentration after the application of some essential oils suggests that the mode of action of these blends distincts from that of monensin.

Although many studies have reported the positive changes in the VFA profile, a few essential oils cause disagreeable adjustments in the proportions of individual VFA. Castillejos et al.^[44] for instance, published that 500 mg/l eugenol decreased the rate of propionate, but without influencing the absolute VFA concentration. Moreover, another *in vitro* study (Cardozo et al.)^[60] revealed that the effectiveness of essential oils or even their components on the VFA profile are pH dependent. For example, cinnamon oil and its active part cinnamaldehyde developed in higher acetate to propionate proportion at pH 7.0, while at pH 5.5 it resulted in the opposite effect.

The essential oil supplementation also seems to lead to an adaptive reaction at the bacterial and/or population level in the rumen. This modifying response is a major challenge to create aromatic feed additives with long-lasting effectiveness. In particular the adaptive reaction is apparent when the essential oils are added in low dosage. In a continuous culture Cardozo et al.^[41] applied cinnamon, garlic and anise oils (7.5 mg/kg dry matter: DM; or 0.22 mg/l) and discovered alterations in the FVA profiles only during the first 6 days of microbial adaptation. Busquet et al.^[61] likewise observed the effectiveness of garlic oil on *in vitro* rumen microbial fermentation in a 24h batch culture. A dosage of 300 mg/l was able to decrease the absolute VFA concentration, while in a subsequent study (using a continuous culture) the same essential oil (at doses up to 312 mg/l) had no influence on this concentration.^[48] These outcomes show that microbial populations are able to modify themselves to the aromatic

adding over time, which demonstrates a challenge for commercial utilization of such a feed supplementation technology.

Methane production

The reduction of the methane emissions from domestic ruminants is an expanding worldwide concern, because it is a strong greenhouse gas. Moreover, its escape into the atmosphere is directly combined with animal agriculture, specifically ruminant production. The antimicrobial liveliness of essential oils has evoked curiosity if these mixtures could be applied to prevent methanogenesis in the rumen. Analyzing the oils, which only decrease the methane production and are not concomitant with reducing feed digestion will be a challenge. Evans and Martin (2000)^[62] published that 400 mg/l thymol (obtained from thymus and oregano) was a powerful inhibitor of methane; however the rates of acetate and propionate were reduced too. Another study^[63] observed the effectiveness of some methanol and ethanol aromatic extracts (like fennel, clove, garlic, onion and ginger) on the *in vitro* methane production. The methanol extract of garlic was the most potent one and lead to a 64% decline and no unfavorable effects on feed digestibility. Busquet et al.^[48] also published that 312 mg/l garlic oil declined acetate and increased propionate rates in a way consistent with a decrease in the *in vitro* methane production too, even though it was not directly calculated. Classifying the outcomes of garlic oil and four of its active parts (diallyl sulfide, diallyl disulfide, allyl mercaptan and allicin) Busquet et al.^[61] noticed that the essential oil as well as diallyl disulfide (300 mg/l of ruminal liquid) lowered methane production by 74 and 69% appropriately, without modifying digestibility. However, monensin was not able to decline the methane production to the same range as the oil or its active component. In the same study it was suggested that garlic oil and diallyl disulfide were not using their effects through the same manner of action as monensin. Nevertheless, the inhibition of the methane production by these combinations was due to the direct prevention of rumen methanogenic Achaeta. Patra et al.^[64]

documented that ethanol and methanol extracts of cloves and the methanol one of fennel too restrict the methane production and unfortunately declined the digestibility of the feed as well. The ground root from rhubarb (1.6 g/l *Rheum officinale*, Polygonaceae) could reduce the *in vitro* methane production by 20% without influencing digestibility, while the bark from buckthorn (1.6 g/l *Rhamnus frangula*, Rhamnaceae) had no impact on methane.^[65]

Maybe there is a potential to pick out essential oil compounds, which decrease methane by selectively inhibiting protozoal numbers. This process would be anticipated to reduce the methane production because ruminal protozoa support a habitat for methanogens, which live on and within them. Still, the antiprotozoal effectiveness of essential oils has been discordant and variable among the aromatic oils and their main parts.

Only a few *in vivo* studies have evaluated the outcomes of essential oils and their active compounds on methane emission, but no studies were carried out about the long-term results of these additives on the methane production. Beauchemin and McGinn^[58], which fed MEO (1 g/day) to beef cattle that consumed a high forage diet, showed that the methane emissions were unaffected, even though feed digestibility declined. Nevertheless, McIntosh et al.^[32] showed in an *in vitro* study that the same commercial product inhibited the growth of *Methanobrevibacter smithii* when it was applied in a concentration, which was 33 fold above the one used *in vivo*. However, this application is impossible due to potentially harmful outcomes on diet digestibility. Through the use of another huge concentration amount (like 20 g/kg DM intake) of encapsulated horseradish Mohammed et al.^[55] discovered only a 19% declining in methane production in steers, while the numbers of protozoals were not changed. Based on these studies, it is obvious that there is a potential benefit in using essential oils to selectively decrease methane. However, the aromatic supplementation has to be used at levels that will not

reduce feed utilization. Consequently, further researches are fundamental to estimate such compounds *in vivo*.

Effects on ruminants

There are not many analyses released on the consequences of essential oils or their active substances on milk production and composition of dairy cows. For instance, Benchaar et al.^[38, 39] examined no influences in DM intake, milk production or its components, when dairy cows were fed 750mg or 2g of MEO per day. Equivalently, Hosoda et al.^[66] showed that the addition of 20 g/kg peppermint to dairy cows had no effectiveness on milk yield and its structure. Another study^[67] revealed that the supplementation of garlic (5g/d *Allium sativa*, Amaryllidaceae) and juniper berry (2g/day *Juniperus communis*, Cupressaceae) oils to the diets of dairy cows had no impact on their DM intake, milk production or its composition. Spanghero et al.^[68] examined the influence of an essential oil blend (RumaXol Feed®, Soda Feed Ingredients, MC 98000 Monaco) , which was microencapsulated and fed at growing levels in diets of high yielding lactating cows. The outcomes presented no adjustments in DM intake, water consumption and fecal DM, despite urine density was smaller at intermediate dosage. Digestibility and biometric measures of the Holstein heifers were not affected by the supplementation as well. Moreover, milk and its components were not altered by incremental adding of essential oils, although the protein content of milk inclined to be higher at average doses. This effect was combined with a numerically comparable effect for fat and resulted in superior milk energy concentrations at intermediate dosages. These studies not only reflect the consistent lack of benefits of essential oils and their active parts on milk performance but also demonstrated that the aromatic extracts had no effects on feed intake and ruminal fermentation.

It is known that essential oils have an antibacterial activity against gram-negative and gram-positive bacteria^[69, 70] and various Gram-positive ones are included in ruminal biohydrogenation of

unsaturated dietary fatty acids.^[71] Therefore, the adding of essential oils could minor biohydrogenation of fatty acids by decreasing the number as well as the activity of bacteria, which are involved in these processes. Although Benchaar et al.^[39] published no benefits in the milk fatty acid profile, when cows were fed with 750 mg/day of MEO, a higher concentration of this blend (e.g. 2 g/day) raised the concentration of conjugated linoleic acid (CLA), which is a health-promoting fatty acid, in milk fat.

Data on the outcomes of essential oils and their mixtures on the performance of beef cattle are not really existent. Benchaar et al.^[76] evaluated their growth performance, when the animals were fed a silage base diet added with 2 or 4 g/day of a commercial blend of essential oils (Vertan®, IDENA, Sautron, France) consisting of thymol, eugenol, vanillin and limonene. The study revealed that DM intake and average daily gain were not influenced by the supplementation. Still, the rise to DM intake ratio was altered quadratically with a dosage of 2 g/day magnifying feed efficiency.

Conclusion:

It was displayed that plant-derived essential oils can be beneficial to progress the efficiency of nutrient utilization in ruminants and decline the force of their production on the environment. Although, the majority of the studies only were laboratory based ones and of a short term-nature, they point out that essential oils or their active parts may favorable alter the ruminal fermentation. It was revealed that high dosages can prevent restrict deamination of AA and decrease the methane production. Nevertheless, in long-term *in vitro* or *in vivo* studies it is indicated that the benefits associated with the aromatic plants decline over time due to transformations in microbial populations or adjustments of individual microbial species. Therefore, it seems formidable to comprehend the benefits of essential oils throughout the feeding or lactation period. Above that, because of the span of essential oils and their active parts it is problematic to achieve constant positive feedbacks in ruminant production.

3.3 Essential Oils in Pigs

In livestock production many essential oils and aromatic herbs are used for advancing the flavor and palatability of feed or to influence other parameters. In most of the feeding trials that have applied these additives, the outcome was reduced to the growth parameters: feed intake, weight gain and feed conversion rate.

The improvement of the performance in pigs was ordinary 2% increase in weight gain and 3% in feed conversion efficiency, while the range in weight gain was from -5% to +9% (as an extraordinary exception published a 23% growth)^[73] and in feed conversion rate from +4% to -10%. These results are comparable to the potential of the “older” growth promoters, like antibiotics, probiotics and organic acids. Not only the type and origin of the essential oil or herb species, but also the quantity supplied to the diet and the environmental conditions of the trial could have influenced the outcomes. The studies, which were carried out under practical conditions of large-scale animal production, have exposed more benefits than more recent analysis under controlled experimental and even more hygiene conditions. It is regularly adduced that aromatic herbs and essential oils improve the flavor and taste of feed and therefore lead to a zootechnical performance-enhancing. Of course, there are investigations that proof a higher feed intake of piglets with the help of aromatic supplementation. Still, these improvements are generally examined with growth-promoting feed enhancer and primarily reproduce higher consuming capacity of large grown animals in comparison to the control groups, but not certainly a specific improvement of voluntary eating due to augmented palatability. Only a few evaluations of feed acceptance, preference and palatability stimulated by essential oils have been described so far, displaying a decline of chosen feed intake in these animals through increasing quantity of fennel and caraway oils^[74] and thyme and oregano herbs.^[75]

Ungerhofer^[76] studied the approval of thyme and oregano herbs, which were fed to pigs in a randomized block design. The animals had to option to select a standard diet without any aromatic adding, two different concentrations of the single spices (nearly 0.12% and 1.2%) or a blend of both ones (0.06% and 0.6% of each herb) equivalent to 20 and 200 mg essential oil/kg feed. This dissertation revealed that the pigs preferred the conventional diet notably without any aromatic supplementation. Nevertheless, within the other groups, the thyme one was favored compared with oregano, which generally has a `heavier` flavor as well as taste. Still, an appraisal on the pig`s performance was not available, because they could consume whatever they wanted. In total, the study showed that the added herbs could not acquire an improved palatability.

Allan and Bilkei^[77] published that oregano improves the reproductive performance of sows. Their study was carried out under field conditions and the exact aim was to conclude the benefit of a strategic supplementation of oregano to prefarrowing and lactation diets in sows. Alternate farrowing groups of the animals received a lactating diet in prefarrowing and lactation diets, which had 1000 ppm oregano (*Oregpig*® Pecs, Hungary) added. The supplementation was made of dried leafs and flowers of *Origanum vulgare* (Lamiaceae), enriched with 500 g/kg of its cold-pressed essential oil to be specific. Each kg of *Oregpig*® was made of 60g *carvacrol* and 55g *thymol*. In general, 801 sows had an oregano treatment (601 primiparous and 1200 multiparous), while the control-group consisted of 1809 untreated ones (705 primiparous and 1104 multiparous). The treated animals had a lower annual mortality rate as well as a lower culling rate during lactation. Moreover, this group had an enlarged farrowing rate, a higher number of live born piglets per litter and the stillbirth rate was declined. It was also shown that multiparous sows with the oregano diet had a better daily voluntary feed intake than the one with the supplementation. However, Allan and Bilkei^[77]

suggested to investigate the benefits of oregano on the gastrointestinal, immune and urogenital system in swine.^[77]

Schöne et al.^[74] examined the effects of the essential oils of fennel (*foeniculi aetheroleum*) and caraway (*carvi aetheroleum*) oils in pigs to find out if they can stabilize the health and even lead to a better performance especially in young animals. In 64 piglets a diet with the two essential oils was tested compared with either a diet without any adding or with the proven combination of formic acid and copper (the positive control). The used fennel oil contained nearly 2/3 anethol, almost 1/5 fenchone and the remaining share existed of *alpha*- and *beta*-pinene, limonene (p-mentha-1,8-diene) and estragol, while one half of the caraway oil consisted of limonene and the other of carvone. The animals were divided into four groups of 16 piglets (live weight 7kg at age 26 days) and were fed with diets without (1) or with a supplementation of 7.5g formic acid and 160mg Cu/kg (2), 100mg fennel oil/kg (3) or 100mg caraway oil/kg (4) during three weeks after weaning. In the following four weeks, all animal offspring received a diet without any additions. Through the whole study no piglet died and only a few diarrhea-cases appeared. A 27% increase of feed consumption and a 25% of daily weight gain were achieved with the proven combination of formic acid and copper. No dissimilarities were found in the performance between the control group and the animals that received the fennel oil supplementation. In contrast, the caraway oil-based diet influenced a lower feed consume and therefore led to an almost 10% less gain. In feed choice experiments the pigs received the same two diets from two different troughs with a 50% of total feed amount, as it was assumed. Interestingly, the fennel or caraway oil-based diets were less than 50% consumed. The most significant decline of percentage feed intake was displayed when the adding consisted of 100mg fennel oil/kg. Consequently, the outcomes of the feeding experiment and of the feed choice one question the categorization of the two applied essential oils as flavor additives or as 'appetite promoters' in diets for weaned piglets.

Ariza-Nieto et al.^[78] classified the efficiency of enriched sow diets with oregano oil during gestation and lactation on sow colostrum and milk configuration. Moreover, the study displayed the outcomes of this essential oil on the growth pattern and immune status of the suckling pigs. A total of 70 second-parity sows were randomly selected and divided into one of two gestation dietary treatments in a period of 24h after service: control (CON) or control added with 250 mg/kg oregano oil (OREG). The animals were assigned again to the CON or OREG diet in their lactation. As a consequence, the lactation treatments were: CON-CON, CON-OREG, OREG-CON and OREG-OREG. Per lactation dietary treatment the blood and colostrum samples of six sows were collected. T lymphocyte subpopulations ($\gamma\delta$, cluster of differentiation 8, and 32 cluster of differentiation 4) were counted in blood as well mammary secretions along with the concentrations of IGF-1, IgG and IgA. Respectively, the growth rate of piglets from 18, 17, 17 and 18 litters from the CON-CON, CON-OREG, OREG-CON and OREG-OREG lactation dietary treatments were calculated. In 630 piglets the growth ratios were calculated and the animal offspring was individually classified and weighed on day 1, 5, 9, 12, 16 and 19 of age. It was shown that the oregano oil adding during the gestation or lactation had no impact on GE, CP, GE: CP, GE: fat and IGF-1 in sow milk. A decline of the fat percentage in milk on day 7 and 14 was discovered in sows with the aromatic diet during lactation compared with the CON treatment ones. Moreover, the milk from the sows with the essential oil supplementation during their lactation had the best total of T lymphocytes on day 14 of lactation in comparison to the CON treatment. For sows the number of T lymphocytes in milk was greater in the CON-OREG treatment on day 14 of lactation. On day 1 to 5 the energy absorption was higher in piglets from sows receiving OEO during gestation than those from the CON treatment. Above that, there was a tendency for a better milk intake and a growth in average daily gain (on day 1 to 5) in piglets from sows, which had the aromatic diet, during gestation compared with the animals in the CON treatment. There was no

distinctness from the insulin-like growth factor-1 at birth and on day 7 and 14 of lactation among the diverse piglet-groups. The supplementation with the essential oil did not influence the immunoglobulin concentration in piglets after suckling. Furthermore, the aromatic diet during gestation or lactation had no impact on T lymphocytes, the percentage of the T-lymphocyte subpopulation or the natural killer cell activity of piglets during lactation. In the main, the supplementation with 250 mg/kg of OEO to the diets of sows during both phases did not lead to an enlarged growth potential or immune response in their suckling piglets. Nevertheless, more studies are necessary to explore the benefits of different amounts of oregano oil and the effectiveness of such an adding during more than one reproductive cycle.

Yan et al.^[8] studied the effect of a blend consisting of 5 herbal extracts (including buckwheat, thyme, curcuma, black pepper and ginger) on the growth performance, nutrient digestibility, blood characteristics and the fecal microbial shedding in weanling pigs. In the 6-week carried out experiment a total of 144 pigs were randomly assigned to four treatments according to their BW and sex (9 replicates of 4 animals per each treatment). The treatments pursued a 2×2 factorial design with two different concentrations of the aromatic extracts (0 or 250 mg kg⁻¹ diet) and the antibiotic apramycin (0 or 30 mg kg⁻¹ diet). Throughout the first week, the animals fed the herbal extract mixture diets conducted to a lower average daily intake but to a better increase in feed ration (G:F) compared to those without the aromatic supplementation. The dietary herbal adding raised the average daily gain and gain:feed ratio as well during the weeks 4 to 6. In the antibiotics treatments, compared to the non-antibiotics one, revealed a higher average daily gain and gain:feed ratio from weeks 0 to 1, 4 to 6 and the complete testing-period. However, in the same group a decline in average daily feed intake was noticed from weeks 4 to 6. A supplementation with the herbal extract mixture or the antibiotic directed to a greater dry matter

and nitrogen digestibility in pigs at day 7. The nitrogen and energy digestibility was raised in the antibiotic group at day 21 and also led to a higher dry matter of digestibility at day 42 in the animals. The study also showed that the coefficient of apparent total tract digestibility of dry matter and nitrogen was boosted by the herbal extract blend treatments at day 42 compared to the ones without this diet. Additionally, in the groups fed the aromatic diet the blood lymphocyte concentration was better at day 42 and they even had a declined fecal *Escherichia coli* concentration at the end of this study. Finally, the application of the herbal extract blend to the diet of weaning pigs had no impact on their growth performance during the whole experimental-period. The beneficial outcomes on the nutrient digestibilities, lymphocyte and fecal *E. coli* concentration implied that those chosen herbs have a positive effect in weaning pigs.

Clouarda et al.^[79] tested the effects of sensory functional additives on food preference, intake and the weight gain in piglets. Pigs often voluntarily decline their food intake when they are exposed to a new diet during food transition and that can force a lower growth. The presented study's request was to analyze the palatability of eight unknown food additives (blends of different essential oils and other plant extracts) and to determine the supplementations, which were likely to advance the food preferences, intake and weight gain in juvenile pigs. The first experiment consisted of 12 juvenile female pigs that were subjected to one- and two-way choice test to examine their spontaneous choice between a standard grower diet and nine experimental ones, where the familiar grower diet was combined with the sensory functional ingredients (N1-N8) at predefined applications. Since the control diet was chosen over the N5, N6, N7 and N8 ones, only the N1-N4 additions were picked for Experiment 2. In the second investigation, 12 juvenile female pigs were subjected to two-choice feeding test to research their relative choices between the four remaining experimental diets. In general, the tests of individual and collective consumption and picks offer that some supplementations

might have a better palatability than others, at the adapted concentrations, and that preferences are dealt with enormous inter-individual variability. Through the second experiment the best candidates for the third investigation were analyzed, which were N and N3. In Experiment 3 the spontaneous food intake of 24 juvenile female animals was figured out during a food transition. Following nine days, in which the pigs received the familiar starter diet, they were split into three groups and each one were fed with a different diet based on an unfamiliar grower diet: one group's diet had the N1 supplementation added, another the N3 and the control group had no additives. Although the adjunction of the useful components in the novel diet did not boost food intake or weight gain in these particular experimental conditions, it might allow to carry on with a common consumption the day of the food transition, contrary to the control diet, despite the outcomes were not significant. Additional complementary investigations, where a unique food is supplied in controlled conditions, added to two-choice tests, might explore more exactly the effects of the sensory supplementations to improve food palatability, preference and intake in pigs.

Nucleus	Composition	Concentration (ml/kg)
N1	Extract of <i>Stevia rebaudiana</i> (stevia) and high-saponin plant	3,7
N2A	Extract of <i>Citrus sinensis</i> (orange)	0,12
N2B	Extract of <i>Citrus sinensis</i> (orange)	0,03
N3	Extract of hot-flavoured spices	0,045
N4	Molecules of the family of aromatic aldehydes and short-to medium-chain fatty acids	0,58
N5	Extract of <i>Cinnamomum camphora</i> L. (camphor)	0,23
N6	Extract of <i>Cinnamomum aromaticum</i> Nees (cinnamon)	0,2
N7	Extract of <i>Illicium verum</i> (star anise)	0,11
N8	Molecules of the pyrazine family	0,02

Table 2: Brief description of the functional nuclei composition and concentration tested (ml/kg of food); Clouard et al.^[79]

4 Oils Used in Treating Diseases in Animals

Traditionally, plants with possible medicinal or therapeutic values have been successfully used for preventing and treating various ailments and diseases. Since ancient times, various plants and their products have been applied in traditional medicine to cure a number of common disorders and degenerative illnesses in animals as well in humans (like ayurvedic and traditional Chinese medicinal practices).

Recently, natural-based medicine is growing in popularity, due to the rising concerns of customers with consciousness to the usage of synthetic chemical preparations and use of artificial antimicrobial protections, in particular current food preservation practices. For example, new antimicrobial agents that are established from plant materials are often considered as natural and even safer in comparison to industrial chemicals. Above that, increasing market trends imply a rapid development in the number of natural plant-derived preparations, like herbal medicine and decoctions. Especially, floral extracts and their isolated essential oils are traditionally considered to be satisfying in phytochemicals proclaiming rich bioactivity. Not merely the industry but also the general society is interested in these compounds and is investigating variant commercial applications.^[80]

4.1 Treating Diseases

Medicinal plants and essential oils have become a bigger part of everyday therapy because of the exaggerate use of conventional medicine. Antibiotics for example often develop a resistance, trigger other infections or lead to an allergy. Herbal preparations can be as effective as the conventional ones. Moreover, because of their non-toxic nature they can be applied over extended periods.^[81]

M.R. Farahpour and M. Habibi^[82] demonstrated that Ceylon cinnamon may have a stimulating effect on the enclosure of excision

wounds. They tested thirty-two rats, which were divided into four groups, each one receiving a different application. Group A was treated with a placebo consisting of 1.5% cinnamon, Group B's placebo contained 3% cinnamon, group C was the control group without any treatment and group D acquired a blank placebo as the reference standard group. Wound healing was observed on days: 3, 6, 9 and 14 and histological evaluation was accomplished on the samples. The outcomes revealed that the cinnamon extract helped to accelerate the wound healing process; in particular it increased the epithelialization. Above that, the investigation displayed that Ceylon cinnamon may be efficient in animating the enclosure of injuries.

Bensignor et al.^[83] evaluated the beneficial properties of a spot-on (Dermoscent® Essential 6, LDCA laboratory, France) for improving skin status of cats and dogs in various countries unconcerned of climate condition or regional differences. 210 dogs and 79 cats were treated with a spot-on formulation consisting of polyunsaturated fatty acids and essential oils in an open study. The formulation improved skin and coat cosmetological aspects. Its assessment was based on scoring the following five criteria: hair shine and loss, dandruff, odor and skin balance. After 4 weeks use, which means that the spot-on was applied once weekly, scores were calculated. For each criterion the improvement percentage (I%) and the percentage of positive response (PR%) were evaluated. *“For dogs, hair shine score was improved by 80% with a PR% of 85; hair loss score was improved by 65% with a PR% of 80; dandruff score was improved by 79% with a PR% of 84; odor score was improved by 70% with a PR% of 80; skin balance score was improved by 74% with a PR% of 87%. For cats, hair shine score was improved by 76% with a PR% of 92; hair loss score was improved by 53% with a PR% of 79; dandruff score was improved by 79% with a PR% of 90; skin balance score was improved by 74% with a PR% of 93.”* These outcomes indicate that the spot-on formulation may improve skin and coat condition.

Parente et al.^[84] studied the wound healing and anti-inflammatory effects of *Calendula officinalis* (Asteraceae), which grew in Brazil, in animal models. In this investigation the ethanolic extract, the dichloromethane and hexanic fractions of the flower were produced. The evaluation of the angiogenic activity of the extract as and fractions was assessed through the chorioallantoic membrane and cutaneous wounds in rats. The healing benefit of the extras was judged by the same cutaneous wounds through macroscopic, morphometric, histopathologic and immunohistochemical analysis. Moreover, in this study the antibacterial efficiency of the extract and fractions was tested too. The outcomes revealed that *C. officinalis* (Asteraceae), exerts anti-inflammatory and antibacterial activities as well as angiogenic and fibroplastic properties, which are acting in positive ways on the inflammatory and proliferative phases of the healing process.

Komiya et al.^[85] researched the effect of the topical application of undiluted lavender oil on autonomic nerve activity in dogs. The sympathovagal activeness of 5 healthy adult male beagles was recorded with an ambulatory ECG monitor (Holter recorder) that was placed on each animal (day 0) for 48-hours (starting at 8:00 the next day = day 1). To the inner pinnae of both ears of each dog lavender oil (0.18ml) or saline (0.9% NaCl) solution (0.18ml) was topically applied at 8:30, 12:00, 15:30 and 19:00 on day 2. Each trial was duplicated in every animal, with a pause of 3 to 4 days between experiments. As an indirect estimate of autonomic nerve activity spectral indices of heart rate variability, power in the high-frequency range and the ratio of low-frequency to high-frequency were categorized. After the lavender oil treatment the average heart rate was notable lower during the period of 19:00 to 22:30 on day 2 in comparison with the average heart rate during the same interval when the beagles were treated with saline solution. While high-frequency power during the period 15:30 to 19:00 was considerably higher when dogs received lavender oil compared with the high-

frequency power during the same interval with saline solution. The investigation revealed some proof that the topical application of lavender oil altered vagal activity in dogs. Nonetheless, whether such effectiveness exists and whether this essential oil has a calming effect on dogs stays equivocal and requires extended investigation.

Perrucci et al.^[86] examined the therapeutic efficacy of linalool as a topical treatment of parasitic otitis caused by *Psoroptes cuniculi* in rabbit and goat. 45 five-month old rabbits with no clinical signs of ear mange were experimentally infected. The animals were divided into groups, which received various concentration of linalool (e.g. five rabbits were treated with 10% linalool) or served as a control group (five animals were untreated). The activity of linalool was compared to AcacerulenR (Teknofarma S.p.A., Turin, Italy) and IvomecR (Merck Sharp & Dome S.p.a., MSD-AGVET Division, Rome, Italy), two commonly used commercial drugs for treating ear mange, which were each applied to five rabbits. Moreover, during an investigation on the presence of mange in sheep and goat in farms of Tuscany, ear mange caused by *P. cuniculi* was diagnosed in five dwarf goats, which were therefore also used for the evaluation of the therapeutic efficacy of linalool for the topical treatment. The examination revealed the efficiency of linalool and therefore its possible use for therapy of animal diseases caused by mites. A further study of new active compounds could increase the number of available natural-based chemotherapeutic agents and thereby decline the frequency of resistance phenomena of pathogen arthropods.

4.2 Usage of Essential Oils in Psychology

Various essential oils are known not only to calm and relax animals as well as human beings and are often used in aromatherapy. Moreover, such olfactory stimulation can lead to an antidepressant effect, for instance. Valeriana is definitely the most popular and common plant in human treatment, followed by lavender and one of its main parts, linalool.

Lavender is listed as a calming factor in some herbal texts; on the other hand the eclectic *materia medicas* characterize it as a stimulant. Motomura et al.^[87] examined the effects of inhaled lavender oil in a clinical trial. The outcomes imply that the aromatic extract can increase arousal but declines stress. Another study^[88] investigated the behavioral effects of essential oils in mice and revealed that the anxiolytic diazepam as well as lavender oil increased the response rate during the alarm period and exposed an anticonflict benefit too.

Tanida et al.^[89] observed the olfactory stimulation with scents of lavender oil and how it affects autonomic neurotransmission and blood pressure in rats. It was shown that this aroma suppressed sympathetic nerve activities and raised gastric vagal (parasympathetic) nerve activity, while it declined the plasma glycerol concentration and body temperature. Moreover, the essential oil improved the animals appetite. Finally, the study concluded that olfactory stimulation with Lavender oil or its active part, linalool, influenced autonomic neurotransmission and decreased blood pressure through the central histaminergic nervous system and the SCN.

Linck et al.^[90] described a sedation in mice after the inhalation of the monoterpene, linalool. Linalool is an active part of many essential oils, like lavender oil, and often used as a hypno-sedative. The psychopharmacological utilization of this component (i.p. and i.c.v.) disclosed marked sedative and anticonvulsant central effects in numerous mouse models. In the study mice were put in an inhalation chamber during 60min with an atmosphere saturated with 1% or 3% linalool. The animals were evaluated regarding their locomotion, barbiturate-induced sleeping-time, body temperature and motor coordination (rota-rod test) instantly after the inhalation. Both concentrations boosted the pentobarbital sleeping-time and declined the body temperature. Only the 3% linalool lowered the locomotion, while the motor coordination was not influenced at all. Consequently,

after a 1h-inhalation of linalool appears to induce sedation without significant damage in motor abilities, a negative side-effect of many psycholeptic drugs.

Sah et al.^[91] studied the positive antidepressant effects of another plant, *Valeriana wallichii* (Valerianaceae). Mice, which received 10, 20 or 40mg/kg dichloromethane extract of *V. wallichii* patchouli alcohol chemotype p.o., had to individually undergo a forced swim test (FST), in an acute and chronic study. After the measurement of immobility period in FST, the neurotransmitter levels in mouse forebrain were estimated on day 14. Single application of 40 mg/kg extract significantly inhibited the immobility span in mice. Likewise, chronic utilization of 20 and 40 mg/kg extract considerably decreased the immobility period and significantly raised the levels of norepinephrine and dopamine in mouse forebrain. Consequently, the extract proved antidepressant effect and increased the norepinephrine and dopamine levels in forebrain.

The effects of *Valeriana wallichii* (Valerianaceae) aqueous root extracts on sleep-wake profile and level of brain monoamines on rats were investigated by Sahu et al..^[92] Transmitters and electrodes were implanted to document EMG and EEG in freely moving conditions and adjustments were recorded telemetrically after oral intake of extract in dosages of 100, 200 and 300 mg/kg body weight. In a dose dependent manner sleep latency was declined and duration of non-rapid eye movement (NREM) sleep was increased. Dosages of 200 and 300 mg/kg significantly decreased sleep latency and duration of wakefulness. Period of NREM sleep as well of total sleep was notably raised after treatments with 200 and 300 mg/kg *V. wallichii* extract. The same dosages increased EEG slow wave activity during NREM sleep too. After the 200 mg/kg treatment, level of norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and hydroxyl indole acetic acid (HIAA) were measured in frontal cortex and brain stem. NE and 5-HT level were significantly declined in frontal cortex and brain stem, while DA

and HIAA level were only notably decreased in cortex. Moreover, DOPAC level was not influenced in any brain region that was investigated. Therefore, *V. wallichii* water extract improves sleep quality that can be dependent upon levels of monoamines in cortex and brainstem.

Komiya et al.^[93] investigated the anti-stress effectiveness of the essential oils of lavender, rose and lemon using an elevated plus-maze task (EPM), a forced swimming task (FST) and an open field task (OFT) in mice. Because lemon oil had the strongest effect in all behavioral tasks, its regulatory mechanism was examined by pre-treatments with agonist and antagonists to benzodiazepine, 5-HT, DA and adrenaline receptors. It was concluded that the antidepressant-like effect is closely connected with the 5-HTnergic pathway, particularly via 5-HT_{1A} receptor. Likewise, lemon oil considerably accelerated the metabolic turnover of DA in the hippocampus and of 5-HT in the prefrontal cortex and striatum. Therefore, lemon oil possesses anxiolytic and antidepressant-like benefits via the suppression of DA activity related to improved 5-HTnergic neurons.

Seol et al.^[94] studied the antidepressant effects of essential oils and identified the regulatory mechanism of some selected ones. The essential oils of *Anthemis nobilis* (*chamomile*, Asteraceae), *Salvia sclarea* (*clary sage*; *clary*, Lamiaceae), *Rosmarinus officinalis* (*rosemary*, Lamiaceae) and *Lavandula angustifolia* (*lavender*, Lamiaceae) were evaluated using a forced swim test (FST) in rats. The animals received the aromatic extracts by intraperitoneal injection or inhalation. Enzyme-linked immunosorbent assay (ELISA) tested serum levels of corticosterone. 5% (v/v) Clary sage oil possessed the strongest anti-stressor benefit in the FST. Therefore, the antidepressant mechanisms of *clary sage* oil were investigated by pretreatment with agonist or antagonist to 5-HT, DA, adrenaline, and GABA receptors. The anti-stressor effect of this extract was definitely blocked by the pretreatment with buspirone (a 5-HT_{1A} agonist),

haloperidol (a D2, D3 and D4 receptor agonist) and a D1 receptor antagonist (SCH-23390). The outcomes indicated that clary oil could be developed as a therapeutic agent for treating depression and that its antidepressant-like effect is of the DAnergic pathway.

5 New use of essential oils

Nowadays, heart diseases and related illnesses with increased levels of plasma cholesterol, such as hypercholesterolemia or endothelium dysfunction, are becoming a real problem in our society. Consumers are looking for healthier ways of living and tend to avoid animal products with high fat content, because they trigger cardiovascular diseases. It is known that medical plants and their essential oils have a positive, depressing effect on the fat contents in animal meat and are therefore used as feed additives, especially thyme, rosemary and garlic.

Although, chicken meat is known to be a healthier protein source than e.g. beef for humans, there have been some efforts to reduce their cholesterol and fat levels even more.^[95]

An interesting experiment was carried out by Abdulkarimi et al.^[96] to calculate the benefits of *Thymus vulgaris* (thyme, Lamiaceae) extract supplementation in drinking water on abdominal fat weight, liver weight, color and plasma lipoproteins; like cholesterol, high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), very low density lipoprotein (VLDL-c) and triglyceride. *T. vulgaris* has antiseptic, antispasmodic, antimicrobial, antioxidant and hypocholesterolemic effects.

The study analyzed a total of 160-day-old broiler chickens, which were divided into four groups. Water and food were available *ad libitum*. The animals were fostered the same starter and grower diets in pellet form, however the groups received different percent of the alcoholic *T. vulgaris* extract (0.06% thymol and pH=5) in drinking

water. The clusters were defined: ZT (0%), LT (0.2%), MT (0.4%) and HT (0.6%) extract, which was prepared through a standard maceration method.

The chickens that were fed with thyme had lower plasma total cholesterol, LDL-s concentrations and proportional liver weights compared with the control birds ($P<0.05$). Above that, the broiler chickens with the extract supplementation, compared with the control group, decreased ($P<0.05$) the plasma triglyceride, total cholesterol, LDL-c and VLDL-c. Consequently, these effects lower the abdominal and liver lipids, debase the proportional liver and abdominal fat weights and boost the liver color intensity as well. The benefits of the thyme extract are assumed to be connected with the minor activity of HMG-CoA reductase enzyme, lower fat absorption from the gastrointestinal tract or the lipid catabolism for gluconeogenesis processes. The conclusion of this study is that broiler chickens, which are fed with *T. vulgaris*, can advance the carcass quality and therefore, are better for the consumers.

Treatment	Triglyceride, mg/dL	Total cholesterol, mg/dL	HDL-c, mg/dL	LDL-c, mg/dL	VLDL-c, mg/dL
ZT	47,2	231,3 ^a	94,4	127,5 ^a	9,44
LT	45,0	146,2 ^b	90,6	46,5 ^b	9,00
MT	44,9	140,9 ^b	92,3	39,5 ^b	9,00
HT	45,7	136,5 ^b	97,9	30,9 ^b	9,15
P value	0,06	<0,0001	0,45	<0,0001	0,06
Pooled SEM	0,34	7,23	1,64	7,4	0,07
Orthogonal contrast [#] T vs C	0,02	<0,0001	0,84	<0,0001	0,01

HDL-c, high density lipoprotein; LDL-c, low density lipoprotein; VLDL-c, very low density lipoprotein; ZT, 0% thyme extract treatment; LT, 0.2% thyme extract treatment; MT, 0.4% thyme extract treatment; HT, 0.6% thyme extract treatment; a,b means with no common superscript letter in each columns differ significantly ($P<0.05$); °two birds per replicate (8 per treatment) were used for the determinations; #orthogonal contrast: T vs C=contrasting birds supplemented with thyme vs control birds.

Table 3: Blood parameters in broiler chickens° receiving 0, 0.2, 0.4 and 0.6% thyme extract in drinking water; Abdulkarimi et al.^[96]

Ghasemi et al.^[97] studied the influence of medicinal herbs such as garlic (*Allium sativum*, Amaryllidaceae) and thyme (*Thymus vulgaris*, Lamiaceae) on laying hens, their productive performance and egg quality. These aromatic plants were used, because of their well-known and documented benefits, like the two main components of thyme, thymol and carvacrol, have antibacterial power and should have a positive effect on the broiler performances. Allicin, the active component of *garlic*, can lower serum levels of cholesterol, triglyceride and LDL. Therefore, garlic powder may have a benefit in reducing egg and serum cholesterol in hens.

The experiment found out, that a special diet with a mixture of garlic and thyme powder could improve a better egg quality and performance of laying hens. 108 chickens were divided into three groups, while 36 served as a control group, the two other received the special diet with 0.1% or 0.2% of the powder mixture from the beginning of week 32-39 of age. On two days of week 37, eggs of each group were taken and the egg quality, albumen weight, yolk weight and shell weight were documented.

The results showed that the mixed powder supplement had neither a real effect on the egg mass nor on the egg production. However, the group that was fed 2% had an increase in egg yolk color and a decrease in its shell weight.

In conclusion, Ghasemi et al.^[97] showed that a mixture of garlic and thyme can improve the yolk color and have a benefit on the performance of laying hens, especially on the egg production.

Yesilbag et al.^[98] conducted a study on broilers to assay the effects of a dietary supplementation with vitamin E (α -tocopherol acetate), dried rosemary leaves and rosemary volatile oil on the performance, meat quality (e.g. sensory variables, pH, color, malondialdehyde (MDA) level and bacteria count) and serum superoxide dismutase (SOD) activity. The animals were fed on a maize-soybean-based diet. A total of 800 broilers were randomly divided into 8 dietary

treatments, which consisted of 1 control group and 8 experimental ones. The control treatment (VitE1) received a basal diet added with 50mg/kg α -tocopherol acetate, while the 7 other groups were given 5x7 g/kg rosemary plant (R1), 8x6 g/kg plant (R2) 11/5 g/kg plant (R3), 100 mg/kg rosemary oil (RO1), 150 mg/kg oil (RO2), 200 mg/kg oil (RO3) or 200 mg/kg α -tocopherol acetate (VitE2). Despite the food consumption was not statistically influenced, live weight gain; feed efficiency and carcasse yield were significantly altered. The performance variables were even more affected with the oil supplementation than with the plant itself. As a measure of meat shelf life, TBA analyses were carried out on the meat samples on day 1, 3 and 5 after culling. The meat MDA levels of the rosemary treatments were considerably lower compared to the groups only fed with α -tocopherol acetate. A notable contrast between VitE1 and the experimental groups was examined in meat color and its pH values as well as for sensory analyses. Moreover, the *E. coli* counts in samples of these 7 treatments were significantly declined. Therefore, a dietary supplementation with rosemary and its oil enhance the meat quality. Additionally, the rosemary volatile oil had a positive effect on the growth performance in broilers.

6 Olfactory stimulation with Essential Oils

Naturally, wild animals are living in an ever-changing environment with many different sensory perceptions. By contrast, a captive surrounding is much more impoverished in these sensory stimuli, which often leads to a changed behavior in housed animals.

In the last years studies were carried out to explore the advantages of sensory cues, which were designed to trigger one or even more of their senses, and should lead to a better welfare. Most researches focused on auditory, olfactory and visual methods of sensory stimulation.^[99]

In general, enrichment strategies should encourage more in the way of species-typical behavioral patterns, enlarge the ability to face challenges, improve a positive use of the environment and or decrease or even eliminate atypical behavioral patterns, such as stereotypes (Young).^[100]

Hurst et al.^[101] described that many species are not only driven by their sense of smell, but also use olfactory signals to communicate with intra- and inter-specifics. Moreover, animals work with this sense to detect their prey, attract mates or to discover food.

Clark and King^[102] showed that despite the importance of odor cues for animals in their natural environment, in captive surroundings disinfectants and other used sanitizing preparations can lead to a scant olfactory stimulation and information.

Recent studies suggested some goals environmental enrichment through supplied odors, whether as olfactory impulses that are specific or indefinite to an animal's natural habitat, or pheromonal in nature.

6.1 Essential Oils as olfactory stimulation

Essential oils and other aromatic compounds have been used as olfactory stimuli in human treatments for decades. It is known that not only different plants but also one singular odor can imply various impulses in humans.

Despite biologically senseless to many animals, new studies showed that essential oils and other plant-derived scents can be beneficial for some species and their wellbeing. Similar to humans, where a lot of odors encourage relaxation and alleviate fear and stress. Many studies (Buchbauer et al.; Lim et al. and Shaw et al.)^[103-105] confirmed that an ambient scent of lavender can reduce motility in laboratory-housed rodents. Graham et al.^[106] showed that the same herb decreased vocalization and action in dogs, which were housed in

rescue shelters. Moreover, lavender essential oil was able to positively change their behavior by leading to a relaxation. Contrary to these positive experiences, Ellis^[107] described that sheltered cats have no benefits following exposure to the same olfactory impulse. However, the different modes of aroma presentation in the two experiments, may explain the discrepancy in the results. For dogs the essential oil was diffused into the air, while the scent was impregnated onto clothes for cats.

Bradshaw et al.^[108] showed that lavender is not only beneficial for pets, but also offers an improvement to animals in transit. The study published that the occurrence as well as the severity of the physical symptoms associated with travel-sickness in pigs, such as foaming, retching and vomiting, could be decreased significantly by the supplying of lavender-scented straw in the HGV. Above that, Wells^[109] evaluated the effectiveness of the ambient aroma of the same plant as a treatment for travel-induced excitement in dogs. The behavioral symptoms of the over-enthusiasm could be relieved through the attachment of lavender-impregnated clothes that were placed in the owners' cars, which happened to lower incidence of barking and hyperactivity and the dogs significantly rested for a longer period. Moreover, the experiment showed that diffused essential oil offers a practical alternative treatment for travel-induced excitement.

Besides to the aromas, which are calming down animals, there are scents that stimulate them. The essential oils peppermint or rosemary, for example, have been shown to boost the activeness of captive mice (Kovar et al. and Umeza et al.)^[110, 111], chimpanzees (Struthers and Campell)^[112], lions (Poweel and Pearson)^[113, 114] and dogs as well (Graham et al.)^[106] Graham et al.^[106] explored the influence of five various types of olfactory stimulation (lavender, chamomile, rosemary, peppermint and a control group) on the behavior of 55 dogs that were housed in a rescue shelter. As already mentioned, lavender oil as well as chamomile oil made the dogs

calmer (they spent more time resting and less time moving around) and encouraged less vocalization. On the other hand, the diffusion of rosemary and peppermint into the environment encouraged notable more standing, moving and vocalizing. This study suggested that the olfactory stimulation with lavender and chamomile oils is not only beneficial to the dogs itself (developing a more relaxed and peaceful behavior) but also may appeal to visitors by resulting in a higher desire to adopt a dog that is more easygoing.

Wells et al.^[115] described that most animals, which are housed in captivity for a long interval, tend to develop behavior symptoms of learned helplessness, like advanced sleeping or resting. In these cases it was useful to provoke the animals with stimulating scents and help them to promote mental incentive and psychological well-being. However, there could be the possibility that a depressed-like animal may develop a more alive type of aberrant behavior, like a stereotype, following exposure to activating aromas. Actually, supplied essential oils, which build up the agitation or stress, may do more harm than good and therefore more studies must explore the influence of such aromatic impulses.

Some other essential oils can enrich captive animals, because they work as sedatives or stimulant. The sedatives are listed in 5.3 and are often inhaled, like valerian which has an anxiolytic-like effect on mice and rats. On the other hand, captive felids have been found to gain exciting effects from the adding of spices like chilli, cinnamon, cumin, nutmeg and ginger (Schuett and Frase^[116]; Pearson^[114]; Wells and Egli^[117], Skiebiel et al.^[118]). Some other herbs, which include catnip, lemongrass, allspice and ylang ylang, have been shown to apply an excitatory benefit on captive lions (Pearson)^[114], black-footed (Wells and Egli)^[117] and domestic (Ellis)^[107] cats.

6.2 Pheromone stimulation

In recent studies the use of pheromones as a method of enrichment for captive animals has gotten some attention. Spielman^[119] published that Feliway® (*Ceva Sante Animal*, France) a synthetic analogue of domestic cat facial pheromones, showed to result in improved levels of head rubbing, which is suggestive of advanced behavioral repertoire, in zoo-housed tigers, although it resulted in no signs on lions.

Dog Appeasing Pheromone (DAP, *Ceva Sante Animal*, France) is the canine equivalent of the first mentioned product, showed some benefits in diminishing a few behavioral problems, like firework phobia or travel-associated complications, in pet dogs (e.g. Sheppard and Mills and Estelles and Mills).^[120, 121] Moreover, DAP can improve the quality of life in captive animals. Tod et al.^[122] revealed that this blend decreased the frequency of barking in sheltered dogs, after being exposed to DAP for a week, although maximum noise amplitude was not significantly changed.

Next to these species, livestock, especially pigs, have also been shown some benefits from “pheromonatherapy”. For example, older studies (McGlone et al. and Petherick and Blackshaw)^[123, 124] presented that the aggressive behavioral of pigs during the regrouping could be decreased by exposure to sexual pheromones. Above that, the nursing behavior in these animals could be managed by maternal pheromones. It was also published that piglets have been shown a less aggressive behavior and even gain more weight during their post-weaning, when they were exposed to synthetic mixtures containing elements of maternal pheromones (McGlone and Anderson).^[125] Driessen et al.^[126] revealed that pigs subject to the vibrations of a transport simulation, which is a known stressor, had a significantly better heart rate reduction after inhaling the scent of a synthetic combination containing maternal pheromones. This trial group was compared to conditions of a non-odor control or a non-relevant unfamiliar aroma, like hartshorn oil.

Pheromonal therapy could bring some benefit to horses too, which is shown in a few studies. For example, Falewee et al.^[127] published that 40 saddled horses, which were exposed to a fear-eliciting situation (they had to walk through a fringed curtain to enter their stable), were less frightened after the application of two nasal sprays of a synthetic Equine Appeasing Pheromone (EAP), than animals without this preparation-use. Nevertheless, EAP seems to lead to the optimum wellbeing in a foreseeable anxiety-eliciting circumstance, because of the small time lag (approx. 20 min) between the application of the pheromonal mixture and the obvious effects on behavior.

In the studies above the potential for certain scents to enrich the wellbeing of captive animals was introduced. However, the profit of olfactory stimulation as the modus of environmental improvement may rely upon the species under analysis. For instance, non-human primates that have more confidence in their sense of sight, showed no real benefits to exposed smells in comparison to more olfactory-stimulated species. To be specific, Ostrower and Brent^[128] published that the adding of “pleasant”, like vanilla, peach and orange, and “unpleasant”, such as cigar smoke and limburger cheese, aromas to fleece cloths caused no more attention from 5 groups of 21 captive chimpanzees than cloths without any olfactory stimulation. In a similar study Wells et al.^[129] showed that an olfactory provocation with the help of scent-impregnated (e.g. peppermint, orange, almond and vanilla) cloths had no real impact on the behavior of 6 zoo-housed gorillas. The sum of these experiments suggests that scent cues are not really important to such animals, at least in zoos. However, the environment of captive non-human primates can be enriched through visual stimulations, such as manipulable objects.

Above that, some of the used odors, especially those from potential predatory types, may lead to a higher stress-level and should carefully be sprayed in the captive surrounding, if at all.

There are, however studies showing that essential oils can have negative effects as well. For instance, moderate doses of lavender oil have an anxiolytic benefit; a higher application will cause sedation (Shaw et al.)^[105], which is a potentially unwelcome consequence in the locked up environment. In relation to the application of essential oils and plant products the issue of toxicity also needs to be discussed. For example, Richardson^[130] and Foss^[131] reported undesired physical side effects, like vomiting or skin irritation in pet cats that were exposed to potpourri. Moreover, Engel^[132] published that death arise in some non-human primates from nightshade plants. Lavender and other essential oils are not recommended for the use in pregnant women, there may too be concerns over the adding of these aromas in certain cohorts of animals.

7 Conclusion

Considering the outcomes of the previous studies, usage of essential oils in veterinary aspects has vigorously developed over the last years. Especially, their application as food additives instead of the well-known and frequently over-used antibiotics became very important in our society. Therefore, many investigations were found about aromatic extracts and plants as growth and health improvements. However, not every experiment proved the expected benefits of them, e.g. because of a vague dosage.

Unfortunately, the use of essential oils in a medical way for animals still is not well documented. It would definitely be interesting to find out more about established applications for treating our animals with these natural components.

Essential oils proved to be not only healthy and helping to human beings; they can also improve the wellbeing of animals.

In the future, it would be worthwhile to have a closer look at essential oils as a medicine that could support the constitution of animals as well as help them to cope with illnesses.

Tables

Table 1: Effect of essential oils on the growth inhibition of bacteria (adapted from Brenes et al. ^[22])	18
Table 2: Brief description of the functional nuclei composition and concentration tested (ml/kg of food); Clouard et al. ^[79]	42
Table 3: Blood parameters in broiler chickens° receiving 0, 0.2, 0.4 and 0.6% thyme extract in drinking water; Abdulkarimi et al. ^[96]	51

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Curriculum vitae

Sophie Barelli
Hafergrubenweg 10
A-2230 Gänserndorf
Mobil: +43 676 5371276
Mail: sophiebarelli@hotmail.com



PERSÖNLICHE ANGABEN:

Name:	Sophie Stephanie Barelli
Geburtstag:	18.10.1983
Geburtsort:	Wien (Österreich)
Staatsangehörigkeit:	Österreich
Familienstand:	ledig, keine Kinder

AUSBILDUNG:

1989-1993	Volksschule, Gänserndorf
1993-2001	Konrad-Lorenz-Gymnasium, Gänserndorf
ab WS 2001	Studium der Pharmazie, Universität Wien

BERUFLICHER WERDEGANG:

Juli 2001:	Ferialjob als Verkäuferin
August 2001 – Juli 2002:	Verkäuferin (Geringfügig) Tom & Mike
Juli 2002, 2003 & 2004:	Ferialjob als Kellnerin
August 2002 – Oktober 2004:	Kellnerin (Geringfügig – Teilzeit), AIDA
August 2005 & 2006:	Ferialjob, Rezeption und Personalbereich, Johnson & Johnson Med. Prod. GmbH
April 2006 – Februar 2008:	Administrative Mitarbeiterin im Bereich Qualitätssicherung (Geringfügig), OCTAPHARMA
Juli & August 2007 sowie 2011:	Ferialjob als Apothekeenaushilfe, Belvedere Apotheke

Dezember 08 – Dezember 11:	Apothekenaushilfe, Belvedere Apotheke
seit Juli 2012:	Apothekenaushilfe, Muttergottes- Apotheke

WEITERE QUALIFIKATIONEN:

Fremdsprachen:	Englisch : Sehr gut in Wort & Schrift Spanisch: Grundkenntnisse Latein: Grundkenntnisse
Auslandsaufenthalte:	Abnormal Psychology, Summer University 2008, UCLA (University of California Los Angeles)

PERSÖNLICHE INTERESSEN / FREIZEIT:

Pilates, Skifahren, Schwimmen
Theater, Kino, Literatur
Kochen