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Current use of phytogenic feed additives in animal nutrition: a review

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ABSTRACT: A rapid development in the potential use of phytogenic feed additives has occurred mainly due to the 2006 EU ban on the use of antimicrobial substances as growth stimulators; however, they are also used as sensory, technological additives or substances positively affecting the quality of animal products. The use of phytogenic additives in form of extracts, predominantly essential oils, has been tested in a number of animal species; however, there is still a lack of scientific findings on the safety and efficacy of their use, or on their effect on the quality of animal products. The aim of this review was to sum up current scientific knowledge on phytogenic additives in animal nutrition.

Keywords: phytogenics; essential oils; sensory, technological and zootechnical additives

INTRODUCTION

Farm animal populations are undergoing continuous selection to improve the economic efficiency of animal production (Svitakova et al. 2014). Animal production itself is affected by a number of both external and internal factors that unequivocally include nutrition. Phytogenic additives present a plausible alternative as they enhance a number of important processes in the animal body. Phytogenic feed additives may be included among supplements that are aimed to positively affect feed quality, health of animals as well as animal products by means of their specifically efficacious substances. They can be classified into several groups: sensory additives (feed additives affecting the sensoric properties of animal products), technological additives (antioxidants, substances decreasing mycotoxin contamination of feeds, etc.), zootechnical additives (immunomodulators, digestive stimulants, growth promoters of non-microbial origin, substances increasing performance or quality of animal products, etc.), and nutritional additives (vitamins, minerals, plant enzymes, etc.). Phytogenic additives are used mainly in the first three cases, however, a number of phytogenic additives have been demonstrated or are presumed to have more than one positive effect and cannot be strictly classified into the designated groups. The aim of this review was to sum up the current trends in the use of these additives in animal nutrition.

Sensory additives

Traditional sensory additives include substances affecting food odour and palatability, and colourings. Phytogenic additives are commonly used as colourings in laying hens to affect the egg yolk colour. Laying hens cannot synthesize egg yolk pigments; yet, the egg yolk colour is one of the main indicators of egg quality affecting consumer's preference (Englmaierova et al. 2014). Pigments of the egg yolk, xanthophylls, are dependent on fat soluble pigments that are present in the feed (Yildirim et al. 2013). The source of these pigments may be natural or synthetic colourings (ethyl ester of β-apo-8'-carotenoic acid and canthaxanthin known as Carophyll Yellow and Carophyll Red)

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(Englmaierova et al. 2014) that are more economical but also potentially dangerous to human health (the maximum dose of canthaxanthin should not exceed 8 mg/kg; at higher doses, minute crystal formation may occur in the retina by a reversible deposition process (Breithaupt 2007)).

Natural colourings are preferred; the most frequently used ones include carotenoids, the source of which are carrot, *Chlorella* algae, marigold (*Tagetes erecta* L.), or lutein, however, natural carotenoids are unstable and their use is also limited by their price (Englmaierova et al. 2014). The effect of 1 and 2% (i.e. 10 and 20 g) feed supplementation with biomass of *Chlorella* grown through heterotrophic fermentation on the concentration of total and individual carotenoids in egg yolk was studied by Kotrbacek et al. (2013). In this study, a significant increase ($P < 0.01$) in the deposition of total carotenoids in egg yolk was found (by 46% and 119%). The deposition of carotenoids significantly ($P < 0.01$) increased the colour characteristics, but Kotrbacek et al. (2013) also found a significantly decreased egg yolk weight in the group with 2% supplementation of *Chlorella*, which was probably related to lower feed consumption in these hens. However, in ISA Brown hens fed diets supplemented with the algae *Chlorella* (12.5 mg/kg) and lutein (250 mg/kg) Englmaierova et al. (2013) found significantly increased egg weight ($P < 0.001$), shell weight ($P < 0.001$), and shell thickness ($P = 0.017$), decreased yolk/albumen ratio ($P = 0.035$) of the egg, and *Chlorella* supplementation also significantly ($P < 0.001$) increased yolk colour.

Well known carotenoids include also astaxanthin, which is commonly added to fish feed to provide for a more attractive meat colour. Besides, this substance also has strong antioxidant effects (10× more effective than vitamin E); it is also a subject of recent studies for its effect against reactive oxygen species (ROS) (Gomez et al. 2013), or for its neuroprotective effect in subarachnoid haemorrhage (Zhang et al. 2014).

Egg yolk colour was also studied by Nobakht and Moghaddam (2013). The authors report that the addition of 1.5% and 2% costmary (*Tanacetum balsamita*) to the diet of laying hens has a positive effect not only on the egg yolk colour, but also on the overall production performance, and blood indicators (lower triacylglycerols and cholesterol levels). In contrast, no effect on the yolk colour and viscosity was demonstrated using *Beta vulgaris* L. ssp. *esculenta* var. *rubra* (Kopriva et al. 2014) or using a Korean ginseng exctract (*Panax ginseng* C.A. Meyer) (Yildirim et al. 2013).

Technological additives

Phytogenic additives are newly studied also in terms of decreasing the production of harmful gases in pigs. Alam et al. (2013) have concluded in their *in vitro* study that adding 0.1% various additives (red ginseng barn powder, persimmon leaf powder, ginkgo leaf powder, and oregano lippia seed oil extract) during anaerobic incubation of swine faecal slurries for a period of 12 to 24 h led in all groups to a significant decrease ($P < 0.05$) in propionate production, to higher production of volatile fatty acids ($P < 0.05$), and, with the exception of the group with the addition of oregano, to higher pH. In the group with the addition of oregano, they noted in contrast a decrease of pH and different bacterial population (uncultured bacterium clone PF6641 and *Streptococcus lutetiensis* (CIP 106849T)), and the dangerous gases ammonia-nitrogen (NH_3-N) and hydrogen sulfide (H_2S) were not detected in this group; compared to control and other additives tested, they noted lower amounts of "odorous compounds", and recommended the use of this additive in the grower category of pigs.

When using a *Quillaja saponaria* extract, Veit et al. (2011) noted by 38% and 32% lower ammonia concentrations in stables of pigs with the addition of 100 ppm of the phytogenic feed additive AROMEX® ME Plus and of 150 ppm of the phytogenic feed additive FRESTA® F Plus, respectively.

Lower ileum ammonia concentration in broiler chickens fed essential oil (125 ppm including the essential oil derived from oregano, anise, and citrus peel) was noted by Hong et al. (2012).

Various phytogenic substances or mixtures have the potential to reduce CH_4 emissions from ruminants (Flachowsky and Lebzien 2012). Methane is the second most important greenhouse gas, and a large part of it originates in animal production. Dong et al. (2010) found in an *in vitro* study after sampling the goat rumen fluid, that the addition of an *Artemisiae annuae* extract and a herbal mixture (60 g/kg diet; *Dryopteris crassirhizoma Nakai, Astragalus membranaceus* (Fisch.) Bge., *Crataegus pinnatifida* Bge., *Mentha haplocalyx* Briq.) significantly ($P < 0.05$) lowered the methane production. Gunal et al. (2013) found decreased

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($P < 0.05$) ammonia N concentrations using *in vitro* 24 h batch culture of rumen fluid with a 55 : 45 forage : concentrate diet supplemented with a Siberian fir needle oil, citronella oil, rosemary oil, sage oil, white thyme oil or clove oil at three doses (125, 250, and 500 mg/l), with the exception for the highest dose of white thyme oil. Cieslak et al. (2014) also achieved in their *in vitro* study a lowering of methane production by 29% compared to the control group when using *Saponaria officinalis* saponins, without any effect on rumen fermentation. However, Jayanegara et al. (2014) report that the methane mitigating properties of saponins in the rumen are level- and source-dependent; and they noted in their study the effectiveness of saponin-rich sources in mitigating methane in the order: yucca > tea > quillaja. Hristov et al. (2013) recorded when administering dietary supplementation of *Origanum vulgare* L. leaf material at the dose of 250–750 g/day, a decrease in rumen methane production in dairy cows within 8 h after feeding; however, the effect over a 24-h feeding cycle was not determined in their study. When using anise oil, cedar wood oil, cinnamon oil, eucalyptus oil, and tea tree oil at concentrations of 125, 250, and 500 mg/l, Gunal et al. (2014) noted in an *in vitro* study only moderate effects on rumen fermentation, but higher ammonia-N concentration in cultures incubated with essential oils regardless of the dose level. The authors (Gunal et al. 2014) report that it is unlikely that these moderate *in vitro* effects would correspond to any substantial impact on ruminal fermentation *in vivo*. After characterization of the substances, *in vitro* studies should only be the first step to identify substances with a CH_4 reduction potential. Feeding studies, especially long term studies, aimed to consider animal health and welfare, adaptation of rumen microbes, efficiency of additives over long feeding periods, animal performance, safety of the additive to the animals, consumer and environment, quality of animal products and CH_4 emissions, are essential prerequisites for the use of phytogenic feed additives in ruminant feeding practices (Flachowsky and Lebzien 2012).

Zootechnical additives

Immunomodulators. One of the main aims of using phytogenic additives is their potential effect on the immune system. Important immunomodulators include oligosacharides, in particular β-glucans. These substances are obtained mainly from fungi, Basidiomycetes and yeast of the genus *Saccharomyces ceverisiae* (Wojcik 2014a); their effects are studied in a number of animal species, e.g. in cattle (Wojcik 2014a, b), sheep (Milewski et al. 2013; Zabek et al. 2013) or pigs (Sorocinova et al. 2013). Beta-glucans can be found in plant components, too, traditionally in the aleuronic layer of barley and oat bran, or seaweed. Extraction of β-glucans from plant components is demanding not only financially (El Khoury et al. 2012); nevertheless, bamboo leaf extracts appear to be a new and promising source of β-glucans. Ohtsuka et al. (2014) evaluated the effect of an extract of β-glucans obtained from bamboo leaves (*Sasa sensanensis*) in cattle. The authors found mainly higher activity of $CD8^+$ T lymphocytes, i.e. cells that are crucial for the immune response to a number of viral infections. Beta-glucans are present also in the cell walls of pathogenic fungi, and plants themselves (*Nicotiana benthamiana*) have recently started to be used for the production of the so called therapeutic antibodies attacking β-glucans, thus providing important protection against *Candida albicans, Aspergillus fumigatus,* or *Cryptococcus neoformans* in animal models (Capodicasa et al. 2011).

A separate chapter is the use of essential oils that exhibit antimicrobial properties and at the same time have minimum impact on the health of the host organism (Rada et al. 2009). Essential oils are used in a number of animal species, among others, also fish and honey bees. Phytogenic feed additives (PFA) containing essential oils of thyme and star anise as main active components were studied by Cho et al. (2014) who examined their effect on the growth performance, energy, nutrient apparent total tract digestibility, blood metabolites, intestinal microflora, meat colour, and relative organ weight after oral challenge with *Clostridium perfringens* in broilers. They found improved growth performance, reduced blood total cholesterol, and also inhibited *C. perfringens* and *E. coli* proliferation in small and large intestines in the group of broiler chicks with the addition of PFA compared to the control group and the group with the addition of antibiotics after application of *Clostridium perfringens.*

Salvia officinalis essential oils (0.05% concentration) were used in a study by Ryzner et al. (2013) in broiler chicks with the aim to study their effect on the antioxidative status and blood phagocytic activity. The authors concluded that *Salvia offici-*

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nalis is an important source of antioxidants, and significantly enhances blood phagocytic activity.

The antimicrobial activity was also proved by Skrivanova et al. 2014 for the sucrose monoesters of capric and lauric acids. In their experiments, a significantly decreased number of viable cells of *E. coli* was found at all tested concentrations of sucrose monocapate (0.1–5 mg/ml). In the overnight incubation of *C. perfringens* with the sucrose ester of lauric acid at 0.1–5 mg/ml concentration the number of viable cells was reduced below the detection limit (2 log 10 CFU/ml).

The number of piglets born and weaned by sows per year is currently one of the most important characteristics affecting the effective production performance of pigs; and pig reproduction can be influenced by nutrition (Kapelanski et al. 2013; Nevrkla et al. 2014). Broccoli extract (0.15 g/kg sulforaphane) and the essential oils of turmeric, oregano, thyme, and rosemary (535, 282, 373, and 476 mg/kg, respectively) were used in pigs by Mueller et al. (2012a) with the aim to determine their potential effect on the intestinal and faecal microflora, on xenobiotic enzymes, and on the antioxidant system of piglets. The authors report that although the phytogenic additives had no effect on the intestinal microflora, they can nonetheless enhance the health status in pigs by up-regulating the antioxidant system either by direct or by indirect antioxidant effects. A high direct antioxidant potential was noted mainly in the oregano essential oils, meanwhile rosemary induced xenobiotic and antioxidant enzymes, and broccoli extracts improved the antioxidant status by indirect antioxidant effects. Similar conclusions were drawn by Mueller et al. (2012b) also in broiler chicks.

In many countries aquaculture represents the fastest growing animal husbandry. However, higher intensity of fish farming can lead to greater health hazards, too (Palikova et al. 2014). Phytogenic feed additives offer one of the ways of improving the health status of fish. In their study on tilapia (*Oreochromis mossambicus*) Gultepe et al. (2014) used 1% extract of thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), and fenugreek (*Trigonella foenum graecum*). After 45 days of feeding with admixed extracts, the authors found a significant ($P < 0.05$) increase of the phagocytic activity, higher counts of white and red blood cells and haematocrit levels in the fish, and in the group with the addition of fenugreek, also higher ($P < 0.05$) lysozyme and plasma myeloperoxidase activity; at the same time, after infection with 100 µl of *Streptococcus iniae* (8×10^8 CFU), they noted decreased mortality (22, 27, and 31% in fish receiving diets supplemented with 1% thyme, rosemary, and fenugreek, respectively, compared to 61% mortality in the control group). Gulec et al. (2013) reported a higher resistance to *Yersinia ruckeri* in trout after using a feed enhanced with the addition of plant oils (*Thymus vulgaris* and *Foeniculum vulgare*; 10 ml/100 g rates for one week). Beside increased bactericidal activity, they noted also significantly higher ($P < 0.05$) contents of total protein, albumin, cholesterol, triglycerides, bilirubin, K, Na, Ca, and Mg compared to the control. Giannenas et al. (2012) found after the addition of phytogenic additives (2 g/kg carvacrol and 6 g/kg thymol) to feed in trout (*Oncorhynchus mykiss*) not only improved ($P < 0.05$) feed efficiency, but also lower total anaerobe counts in the intestinal bacteria populations, significantly higher ($P < 0.05$) activity of glutathione based enzymes (glutathione reductase, glutathione-*S*-transferase), higher levels of lysozyme and total complement concentrations as well as catalase activity, and significantly reduced nitric oxide (NO) serum in the group with thymol supplementation. The authors demonstrated in their study that relatively low concentrations of carvacrol- and thymol-based feed additives had a positive effect on the growth performance of trout with apparent effects towards antioxidant defence and innate immunity status.

Kuzysinova et al. (2014) studied the inhibitory activity of sage (*Salvia officinalis*), anise (*Pimpinella anisum*), oregano (*Origanum vulgare*), caraway (*Carum carvi*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinum officinalis*), clove (*Syzygium aromaticum*), camomile (*Chamomilla recutita*), and fennel (*Foeniculum vulgare*) against *Paenibacillus larvae* attacking honey bees. They demonstrated in an *in vitro* study (disc method using 5 a 10 µl essential oil) that certain essential oils (*Thymus vulgaris, Origanum vulgare*) have inhibitory effects on *P. larvae* and may be therefore used for the prevention of this disease.

Addition of 3% microalgae spirulina (*Arthrospira platensis*) and/or 2.5% thyme (*Thymus vulgaris*) leaves in growing (7 weeks) dwarf rabbits did not prevent the animals from getting sick or dying during the 14 weeks of the study period, and there were no substantial effects on the growth performance and energy or nutrient digestibility, either (Dalle Zotte et al. 2013).

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The use of phytogenic additives appears to have no major effect against internal parasites. Van Krimpen et al. (2010) demonstrated a non-significant decrease in the number of pigs infested with *Ascaris suum* either when using a 3% addition of a phytogenic additive mixture (1% *Thymus vulgaris*, 1% *Melissa officinalis*, and 1% *Echinacea purpurea*), or when using a 4% (the mentioned herbs plus 1% *Camellia sinensis*), or when using a 3% addition of a mixture of *Carica papaya*, *Peumus boldus*, and *Artemisia vulgaris*.

Substances increasing production performance or animal product quality. In most European countries, the trend is to increase poultry meat consumption, where especially broiler meat is of satisfactory nutritive quality and acceptable to most consumers with respect to price and its organoleptic traits. Improvement of the nutritive quality of broiler meat as a functional product is the aim of many studies (Kralik et al. 2013). A whole range of phytogenic additives have been studied in broiler chickens with the aim to enhance their growth performance, to achieve more effective feed utilization, or just to enhance meet quality. Fascina et al. (2012) report that by using phytogenic additives in form of turmeric extract, citrus extract, and grape seed extract + Chinese cinnamon essential oil, Chile Boldo leaves, and fenugreek seed phytogenic additives (Imunostart® + Enterocox®; both Phytosynthese, Mozac, France), probably due to the effects of cinnamaldehyde and turmeric, the main active ingredients in cinnamon and curcumin, pancreatic and intestinal enzyme secretion is stimulated and concurrently, production of bile, bile salts, pancreatic and intestinal lipase is increased, leading to more effective nutrient absorption. In their experiment using these phytogenic additives in broiler chickens they noted significantly higher ($P < 0.05$) metabolisability coefficients of gross energy, dry matter, and ether extract. Significantly higher ($P < 0.05$) ileal digestibility of crude ash, crude protein, crude fat, calcium, and phosphorus, exhibiting a linear increase related to the increase of the dose of the phytogenic feed additive containing thyme and star anise essential oils (150, 750, or 1500 mg/kg) in the diet were reported for broiler chicks by Amad et al. (2011).

In a study in broiler chickens, Hong et al. (2012) used the addition of 125 ppm of essential oil from oregano, anise, and citrus peel powder. The authors reported that essential oil supplementation increased the survival rate by approximately 10%; serum levels of cholesterol were reduced, very low density lipoprotein (VLDL) levels decreased, total polyphenolic compounds and total flavonoids increased; breast muscles were more tender, and thigh muscles were juicier for birds in the essential oil group as compared to the control group.

Nasir and Grashorn (2010) studied the effect of supplementation with *Echinacea purpurea* and *Nigella sativa* on broiler performance, carcass and meat quality. The addition of *Nigella sativa* led in their experiment to significantly ($P < 0.05$) higher breast muscle percentage, and the addition of *Echinacea purpurea* led to significantly ($P < 0.05$) higher crude protein content in the meat samples.

Supuka et al. (2015) used the agrimony extract (*Agrimonia eupatoria* L., 0.2%) and a combination of agrimony and sage extracts (*Agrimonia eupatoria* L. + *Salvia officinalis* L., 0.1% + 0.1%) supplemented to water during the fattening period of broiler chickens. The authors report that in the agrimony supplemented group, they found a significant ($P < 0.05$) decrease of total cholesterol, low density lipoprotein, and malondialdehyde in serum, and a significant ($P < 0.05$) increase of dry matter and fats in meat. The authors also report that the activity of superoxide dismutase in blood and the amount of thiobarbituric acid reactive substances in thigh meat on the 1^{st} and 8^{th} day of storage under chilling conditions were lower ($P < 0.05$) in both experimental groups as compared to control. Mountzouris et al. (2011) tested the addition of a blend of essential oils from oregano, anise, and citrus on broiler growth performance, nutrient digestibility, and caecal microflora composition. These authors note that the phytogenic efficacy in broilers depends on the feed inclusion level used and on the broiler growth period, with most of the PFA overall beneficial effects seen mainly in the finisher growth period.

Mentha spicata (0, 1, 2, 3, and 4% of diet) in the diet of growing Japanese quail may have beneficial effects on the product quality rather than product quantity through improving the profile of blood metabolites, meat quality, and gastrointestinal environment (Ghazaghi et al. 2014).

The goal of feed additives in the nutrition of laying hens is mainly to increase the egg nutritional value. Galik et al. (2013) found in the Hy-Line Brown laying hens that using a 1% addition of sumac seed (*Rhus coriaria* L.) for the period of 21 weeks led to a significant increase of the

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content of fatty acids, such as linoleic, γ-linoleic, myristic, myristoleic, palmitic, and palmitoleic acids. On the other hand, Cherian et al. (2013) significantly enhanced muscle lipid stability in broilers after adding 2% and 4% sweet wormwood (*Artemisia annua*); concurrently, they achieved a pH lowering in the caeca.

The addition of peppermint (*Mentha piperita* L.) leaves (0, 5, 10, 15, or 20 g/kg for 12 weeks) in Hy-Line Brown laying hens (64-week-old) in the study of Abdel-Wareth and Lohakare (2014) led to significantly increased (linear, $P < 0.001$) egg weight, egg production, egg mass, and feed intake from 64–68, 68–72, 72–76, and 64–76 weeks of age. Moreover, the feed conversion ratio linearly decreased ($P < 0.001$) with increasing levels of peppermint in the laying hens' diet. Also eggshell percentage, eggshell thickness, and the Haugh units of hens fed diets supplemented with peppermint leaves were greater ($P < 0.01$) than those of hens fed the control diet.

Addition of lycopene (0, 20, 40, and 80 mg/kg) to the feed in hens was used by Sun et al. (2014). They found that lycopene supplementation significantly increased fertilization rates in hens. In the liver, lycopene supplementation significantly increased superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and reduced glutathione to oxidized glutathione ratio (GSH/GSSG). Additionally, lycopene supplementation increased serum SOD, serum T-AOC, serum glutathione peroxidase, and serum GSH/GSSG and significantly decreased total cholesterol and increased high density lipoprotein cholesterol and triiodothyroxine.

After addition of 1% (w/w) of dried oregano and sage leaves, Rotolo et al. (2013) found significantly higher ($P < 0.05$) growth performances in male Bianca Italiana rabbits; however, meat quality traits, oxidative lipid stability, and fatty acid profile were not influenced by the aromatic plants supplementation.

The use of phytogenic additives is tested in pigs for growth promotion during fattening. In their study Pastorelli et al. (2012) reported significantly faster ($P < 0.05$) growth in fattening pigs after the use of the *Lippia citriodora* extract at a dose of 10 mg/kg for a period of 56 days; they also demonstrated higher levels of serum IgA and lower levels of reactive oxygen metabolites (ROM) in these animals. In contrast, Bruno et al. (2013) using feeds supplemented with *Rosmarinus officinalis*, *Mentha piperita*, *Lippia sidoides*, and *Porophyllum ruderale* extracts noted no significant positive effect on the pigs' growth compared to control pigs or pigs supplemented with antibiotics (tylosin, colistin). Cho et al. (2012) found in a study in weaned pigs significantly lower ($P < 0.05$) occurrence of diarrhoea and improved growth performance using Japanese honeysuckle (*Lonicera japonica;* 0.5 g/kg feed), Houttuynia cordata Thunb (*H. cordata;* 1.0 g/kg feed), laquer tree (*Rhus verniciflua Stokes;* 1.0 g/kg feed), yellow ginger (*Dioscorea zingiberensis;* 1.0 g/kg feed), and hoantchy root (*Leguminosae;* 1.0 g/kg feed).

Lamb meat is considered to be a valuable food product due to its high quality and health-promoting properties and nutrition can affect not only the quality of lamb meat, but also the nutrient absorption or feed intake (Milewski et al. 2014). The effects of feeds containing a blend of essential oil compounds (BEOC, 1 g/kg feed; thyme leaf (*Origanum onites* L.), daphne leaf (*Laurus nobilis* L.), sage tea leaf (*Salvia triloba* L.), fennel seed (*Foeniculum vulgare* L.), orange cortex (*Citrus* sp.), and myrtle leaf (*Myrtus communis* L.)) on fattening performance, selected blood indicators, slaughter traits, and internal organ weights of Karya lambs were studied by Ozdogan et al. (2011). During a 56-day period, the body weights, body weight gains, total dry matter intake, and feed conversion ratios of males and females of the BEOC group showed better results; however, they were not significant ($P > 0.05$), only the conformation score and lung weight of female lambs were significant ($P < 0.05$).

Of interest is the use of caffeine. Chemically, caffeine is 1,3,7-trimethylxanthine ($C_8H_{10}N_4O_2$), which is an alkaloid found in tea, guarana, kola nuts, coffee, cocoa beans, and other plants (Jun 2009). Caffeine is not used in animal nutrition, but has important effects, which can be utilized in animals. Caffeine effects on the quality of cooling-stored sperm were studied by Spalekova et al. (2014) in rams. As the use of artificial insemination with preserved semen forms a large part of the reproductive technologies, production of high-quality insemination doses is of major interest (Prinosilova et al. 2014). Spalekova et al. (2014) found in their study that the addition of caffeine at a dose of 2 and 4 mmol/l significantly ($P < 0.05$) lowered the number of dead/necrotic and apoptotic sperm, and concurrently, motility was increased, for a period of 72 h.

Substances decreasing the negative effects of stress factors. A commonly discussed cause of chronic diseases is oxidative stress, which occurs

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in the body when there is imbalance between the formation and inactivation of the so-called reactive oxygen species (ROS) (Juadjur et al. 2015). Stress and stress-related diseases are currently a much discussed topic not only in animals (e.g. stress-related disorders are becoming subjects of growing interest for an increasing number of horse owners, breeders, and riders; Fejsakova et al. 2013), but also in humans, and animals frequently serve as models for the testing of various human diseases. The protective effect of phytogenic additives against stress-related disorders is studied both *in vitro* and *in vivo*. Juadjur et al. (2015) proved *in vitro* in Caco-2 cells that the ROS levels and the oxidative DNA damage were significantly reduced in the presence of the original anthocyanin-rich bilberry extract and phenolcarbonic acid-rich fraction.

Kim et al. (2015) evaluated the antioxidant potential and protective effects of *Celosia cristata* L. (family: Amaranthaceae) flower extracts (100 mg and 500 mg/kg of body weight given to rats for five consecutive days) on tert-butyl-hydroperoxide (t-BHP)-induced oxidative damage (a single dose of t-BHP (2 mmol/kg i.p.)) in the hepatocytes of Chang cells and rat liver. The results showed a significant ($P < 0.05$) protective effect by lowering serum levels of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase. The extract decreased the hepatic levels of lipid peroxidation and serum level of triglyceride against t-BHP-induced oxidative stress.

The neuroprotective effect of *Buddleja cordata* methanolic extract (50 or 100 mg/kg every 24 h for 14 days) in Parkinson's disease rat model was investigated by Perez-Barron et al. (2015). Rats were infused with an intrastriatal stereotaxic microinjection of 10 µg of 1-methyl-4-phenylpyridinium in 8 µl sterile saline solution to induce oxidative stress. Both methanolic extract doses led to a significantly lower ($P < 0.05$) number of ipsilateral rotations (75–80%). This behavioural protection was corroborated with a 60% level of dopamine preservation ($P < 0.05$) and a 90% decrease in the formation of lipidic fluorescent products in the striatum ($P < 0.05$), possibly due to the involvement of phenylpropanoids.

Labaque et al. (2013) suggested that dietary supplementation with thymol may help reduce fear responses in female Japanese quail (*Coturnix coturnix*) when they are exposed to stressful situation without affecting the birds' locomotor activity. Thymol can act as a positive allosteric modulator of the $GABA_A$ receptor, similarly to its analogue phenolic compound propofol. Hence, it is conceivable that thymol could also present anxyolitic and/or fear reducing properties (Labaque et al. 2013).

The antioxidative effect of evening primrose oil (EPO) administration (150 ml) on the oxidative stress of race horses during their regular training period was determined in the study by Mikesova et al. (2014). The evening primrose oil is unique for its content of a high percentage of gamma-linoleic acid and many antioxidants, such as catechin, epicatechin, gallic acid, and α-tocopherol. The authors of this study report that the mean values of total antioxidant reactivity after supplementation increased gradually and were detected at significantly higher levels ($P \leq 0.05$) in the sixth week in comparison with the control. On the other hand, the concentration of malondialdehyde, measured as thiobarbituric acid reactive substances (TBARS), decreased significantly ($P \leq 0.05$), so the results indicate that the total antioxidant activity of thoroughbred horses fed a diet supplemented with EPO was higher, and helped stabilize the permeability of the muscle cell membranes in the horses at full workload.

CONCLUSION

The possibilities of using phytogenic additives are various. Their use does not entail as many major hazards as for example the use of antibiotics or chemical compounds. Phytogenic additives and their wider practical application will undoubtedly be subject to further research. However, long-term studies will be crucial, proving mainly the efficacy of these additives, their safety with regard to animal health, the quality of animal products and environment, and, subsequently, their availability in terms of their anticipated regular use.

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