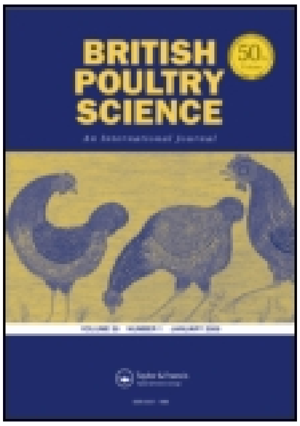

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Professor R.J. Wallace [a], W. Oleszek [b], C. Franz [c], I. Hahn [c], K.H.C. Baser [d], A. Mathe [e] & K. Teichmann [f]

[a] Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, AB21 9SB, UK

[b] Institute of Soil Science and Plant Cultivation, State Research Institute, Pulawy, Poland

[c] University of Veterinary Medicine, Vienna, Austria

[d] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

[e] Western Hungarian University, Department of Botany, Hungary

[f] BIOMIN Research Center, Tulln, Austria

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INVITED REVIEW PAPER

Dietary plant bioactives for poultry health and productivity

R.J. WALLACE, W. OLESZEK[1], C. FRANZ[2], I. HAHN[2], K.H.C. BASER[3], A. MATHE[4] AND K. TEICHMANN[5]

Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, AB21 9SB, UK, [1]Institute of Soil Science and Plant Cultivation, State Research Institute, Pulawy, Poland, [2]University of Veterinary Medicine, Vienna, Austria, [3]Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey, [4]Western Hungarian University, Department of Botany, Hungary, and [5]BIOMIN Research Center, Tulln, Austria

Abstract 1. Plants and their biologically active chemical constituents, sometimes called secondary metabolites or bioactives, present numerous opportunities for the improvement of livestock production by inclusion in the diet.
2. Many such plant derived materials have well established therapeutic values in man; however, their potential as feed additives in animal production, particularly of poultry, remains largely unexploited.
3. There is increasing evidence indicating that they can be efficient in controlling diseases, and plant bioactives may also influence production parameters such as feed efficiency and product quality.
4. It has been reported that they may even replicate some of the effects of antibiotic growth promoters, which were banned from use in Europe from 2006.
5. This review assesses the status of plant bioactives in poultry production and their mode of action on avian physiology, particularly in the digestive tract.

INTRODUCTION

Interest in plants, plant extracts and derived phytochemicals (botanicals) as components of livestock feedstuffs has increased during the last decade. Much of the impetus for revisiting the plant kingdom to look for new, useful additives that can enhance health and productivity results from concerns about the safety and sustainability of antibiotic growth promoters. If transmissible antibiotic resistance factors result from the use of growth-promoting antimicrobials (GPA) in animal production, the efficacy of similar antibiotics in therapy for human diseases may be compromised. Hence, the EU introduced a ban on GPA in 2006. Other nations may follow. Before the ban, poultry production had a high dependence on GPA to control intestinal pathogens such as *Escherichia coli*, *Clostridium perfringens* and coccidial infection. Since then, improved management has compensated for some of the production-benefit losses, but not all. Consumer pressure also plays a part in the move to more "naturally" produced foods (Rickard, 2004). The increasing demand for organically produced foods also drives the search for alternative feed additives (Griggs and Jacob, 2005).

Botanicals should not be considered only as replacements for GPA, however. They have useful properties not shared by GPA. Herbs, spices and their extracts can stimulate feed intake and endogenous secretions (Wenk, 2003). Many botanicals have antioxidant activities that can improve the oxidative stability of poultry meat and eggs, increasing their shelf life. They may stimulate immunity directly, improving birds' resistance to disease. They also have the potential to modify cholesterol metabolism, leading to a product with

Correspondence to: Professor R.J. Wallace, University of Aberdeen, Rowett Institute of Nutrition and Health, Bucksburn, Aberdeen, AB21 9SB, UK. E-mail: john.wallace@abdn.ac.uk
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healthier implications for human production. Even reproduction may be improved by increasing semen quality (Durape, 2007).

A final consideration, indeed the most important of all, is that many plants and phytochemicals have adverse effects on animals when ingested. Acamovic and Brooker (2005) remind us: ''The effects depend to a great extent on the chemistry of the compounds, their concentration in the diet and the amount consumed, and are further dependent on the health status of the animals. Traditionally, most studies of the effects of these compounds on animals have focused on their adverse effects and how to alleviate them. However, recent public concern about the use of synthetic compounds in animal diets to enhance performance and health and welfare issues, coupled with changes in regulations on the use of synthetic medicaments, has stimulated interest and research in the use and effects of phytochemicals in the diets of farmed animals. Phytochemicals vary in their chemistry but can be divided into hydrophilic and hydrophobic compounds, of which a wide variety of polyphenolic and terpenoid compounds, as well as alkaloids, carbohydrates and non-protein amino acids, invoke special interest''.

The use of botanicals in human and animal health undoubtedly has potential value – many widely used therapeutic drugs originate from the plant kingdom – but the use of botanicals in animal production is less well established. It is important to provide a good description of each plant, its active phytochemicals and their molecular mode of action against microorganisms or their interaction with the host. Given the vastness and diversity of the plant kingdom, variations between cultivars, variation in applications, and many other factors, such a complete description will never be attainable. Nevertheless, it might reasonably be expected that botanicals in common use or intended for widespread application should have a body of efficacy, safety and mechanistic information to enable users to assess and understand the biological activity of the botanical in question. The aim of this review was to report on the status of the botanicals field in poultry in relation to these benchmark criteria.

The review itself was prompted by two main drivers. One was the EC Framework 7 Specific Support Action, contract 43077, FEED-SEG, a consortium whose broad objective is ''to disseminate state-of-the-art research results in feed quality topics... (and to)... develop strategies and recommendations for European policies (e.g. research, health, agriculture)''. The second was the publication of an excellent review for botanicals and ruminants (Rochfort *et al.,* 2008). The challenge that we as authors of this article accepted was to attempt to replicate the review of Rochfort *et al.* (2008), with poultry rather than ruminants as the subject group of animals. Poultry production encompasses broilers, layers and breeders from different species – chicken, turkey, and duck principally. Much less is known about the ostrich (Vispo and Karasov 1996), in spite of its growing commercial importance. This review focusses on plant bioactives, poultry production and its underlying science, and does not repeat the comprehensive sections in the Rochfort *et al.* (2008) review on social and regulatory issues, to which the reader is referred.

THE STATUS OF PLANT BIOACTIVES AS FEED ADDITIVES

Generally speaking, feed additives are considered as being applied in the feed by the farmer to healthy animals not only for nutritional purposes but also additional functionality on a long-term basis (possibly along the entire production period of the respective species), in contrast to veterinary drugs used only to treat health problems under control of a veterinarian, applied for a limited period only. As per definition from Regulation (EC) No. 1831/2003, ''feed additives are substances or preparations – other than feed material or premixtures – which are intentionally added to feed or water in order to

- favourably affect the characteristics of feed, as e.g. flavouring or antioxidant,
- affect the characteristics of animal products regarding microbial load, shelf life or taste,
- affect the environmental consequences of especially large-scale livestock production e.g. by reduction of ammonia excretion or methane production,
- favourably influence animal production, performance or welfare by affecting the gastrointestinal microbiota and the digestibility of feeding stuffs, or
- have a coccidiostatic or histomonostatic effect''.

Thus, this review, for the most part (as benefits to productivity are inevitably linked with the control of disease) concentrates on plant bioactives as feed additives rather than therapeutics. Valuable effects on, for example, fowl typhoid (Waihenya *et al.,* 2002*a*) would not normally be covered.

Presently, herbal products are used by the feed industry predominantly as sensory additives, flavouring and appetising substances. Although an understanding of their mode of action would be a prerequisite for their optimal application in terms of efficacy, a full understanding of these aspects in animals is not yet achieved. For example, aromatic compounds and essential oils (EO) act along the animal digestive tract

	No. of natural products
With N	
1. Alkaloids	12 000
2. Non-protein amino acids	600
3. Amines	100
4. Cyanogenic glycosides	100
5. Glucosinolates	100
Without N	
6. Monoterpenes	1 000
7. Sesquiterpenes	3 000
8. Diterpenes	2 000
9. Triterpenes, Saponins, Steroids	4 000
Tetrapenes	350
10. Flavonoids	2 000
11. Polyacetylenes	1 000
12. Polyketides	750
Phenylpropanes	1 000

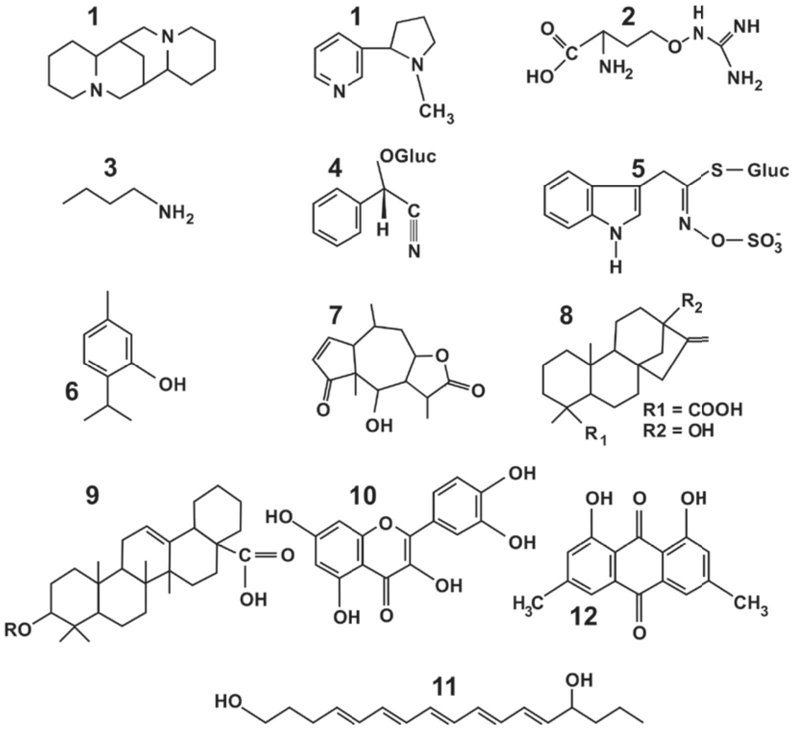

Figure. *Structures, estimated range and numbers of plant secondary metabolites (re-drawn from Acamovic and Brooker (2005), with permission).*

to improve appetite, bacterial modulation, and are able to induce a number of benefits on well being (Kamel, 2001). The antimicrobial properties of EOs and extracts can be dose-dependently bacteriostatic and/or bactericidal. In addition, several investigations have shown an antioxidative effect or changes in digestive physiology and digestion at weaning (Zabielski *et al.,* 1999), the microbiology of the gut (Jensen, 1998) and the implementation of test models in poultry (Hess, 2002). Another complication is that plant bioactive compounds occur in nature as complex mixtures rather than as single compounds, and synergy between individual components may be an important feature of their action.

Plant bioactives are often proposed as possible replacements for AGP. Their efficacy in achieving the same effects remains open to question, but an undoubted advantage of plant bioactives over GPA is that resistance is less likely to become a problem than with conventional synthetic compounds.

It must also be remembered that plants contain many poisonous compounds, including some of the most toxic known to man. Some plant extracts may therefore be detrimental for poultry and by numerous mechanisms may decrease body weight, feed intake (FI) and feed conversion ratio (FCR) and digestibility. They can also influence mortality, muscular conditions and in some instances can be neurotoxic.

BIOACTIVE COMPOUNDS AND THEIR EFFECTS ON PRODUCTION

Production efficiency and incidence of disease

Numerous feeding trials have been performed with plant extracts, aromatic herbs and EOs additives, to investigate production parameters

Table 1. *Performance effects of plant material and plant extracts used as feed additives in poultry nutrition*

		Performance effect			
Plant species/extract	Dose	Beneficial	No effect	Detrimental	Literature
Aesculus hippocastanum	0–10 mg/g body			Depression Muscular incoordination paralysis	Williams and Olsen (1984)
Alfalfa extract	0.06%	Thymus and spleen weight Bursal weight Immunity	FI FCR	Abdominal fat weight	Dong *et al.* (2007)
Aloe secundiflora extract	–	Reduced mortality in Newcastle disease infection, Increase of interleukin 6	Antibody levels	–	Waihenya *et al.* (2002*a*,*b*)
Artichoke water extract	5% DM	Humoral immune response Organ weight Protection against ochratoxin A	–	–	Stoev *et al.* (2000)
Balanites aegyptiaca saponins	5–250 mg/kg/day		Total cholesterol	Body weight	Nakhla *et al.* (1992)
Calendula officinalis extract	Drinking water	Mean weight	FCR Mortality	Immune response Bursal weight index	Barbour *et al.* (2004)
Cassia obtusifolia	2% body weight	–	Weight gain	FI	Hebert and Flory (1983)
Cassia occidentalis		–	–	Weight Muscular weakness	Graziano *et al.* (1983)
Citrus extract	10–1000 g/t	Live weight FI	Metabolisable energy	–	Juin *et al.* (2003)
Colchinchina momordica extract	5–80 μg/dose	Antibody level Daily weight gain	–	–	Rajput *et al.* (2007)
EO (thymol, cinnamaldehyde, commercial preparation)	100 ppm	–	FI Weight gain FCR Liver weight Ileal digestibility	–	Lee *et al.* (2003)
Fermented wheat germ extract	–	Suppression of *Mycoplasma gallisepticum* infection	Body weight gains FCR	–	Stipkovits *et al.* (2004)
Field bean tannin hulls		–	–	Digestibility TMEn	Flores *et al.* (1994)
Garlic extract	3.8% garlic paste	Cholesterol level Enzyme suppression	–	–	Qureshi *et al.* (1983)
Grape seed extract	2.59–5.18%	*Post mortem* meat parameters		Weight gain	Lau and King (2003)
Hemp seed meal	–	Concentration of palmitic acid and higher linoleic acids in eggs	Egg production FI		Silversides and Lefrancois (2005)

Various herbs (thyme, oregano, marjoram, rosemary, yarrow) and their EO	10 g/kg	Performance	Feed efficiency Intestinal microflora	Endogenous sialic acid secretion	Cross *et al.* (2007)
Labiatae extract (sage, thyme, rosemary)	5000 ppm	Apparent faecal digestibility Ether extract digestibility	Apparent metabolisable energy Digestibility CP digestibility	–	Hernandez *et al.* (2004)
Lentinus edodes *Tremella fuciformis*	0.5–4 g/kg	Body weight gain FCR FI	Weight of organs Relative weights of organs and GIT	–	Guo *et al.* (2004*b*)
Linseed meal	20%	Selenium toxicity	Growth rate	–	Jensen and Chang (1976)
Mangifera indica seed kernel meal	50–250 mg/kg meal	Body weight Body weight gain	FI Feed efficiency Organ weights	–	Odunsi (2005)
Myristica fragrance	–	–	–	Deep sleep in young chicken	Sherry *et al.* (1982)
Nepeta cataria	25–1000 mg/kg	–	–	Increased number if sleeping chicks	Sherry and Hunter (1979)
Oregano, cinnamon and pepper essential oils	200 ppm	–	FI FCR	–	Hernandez *et al.* (2004)
Oregano essential oil	50–100 mg/kg	–	Growth	Malondialdehyde in tissue	Botsoglou *et al.* (2002)
Plant extracts containing 5% carvacrol, 3% cinnamaldehyde, 2% capsicum oleoresin	100 mg/kg	Villi-related protective activity Adhesion of *E. coli* and *C. perfringens*	–	–	Jamroz *et al.* (2006)
Plant extracts containing capsaicin, cinnamaldehyde and carvacrol	100 mg/kg	Breast muscle proportion Empty body weight *E. coli* and *C. perfringens* numbers *Lactobacillus* spp. number Lipase activity	Ileal digestibility of nutrients	–	Jamroz *et al.* (2005)
Rosemary extract	500–1000 mg/kg	–	Lipid oxidation parameters	–	Galobart *et al.* (2001)
Rye extract		–	–	Weight gain	Day and Thomas (1980)
Saccharum officinarum	500 mg/kg/day	Phagocytic activity of peripheral blood leucocyte Antibody response Immunostimulating activity	–	–	Hikosaka *et al.* (2007)

(Continued).

Table 1. *Continued.*

Plant species/extract	Dose	Performance effect: Beneficial	Performance effect: No effect	Performance effect: Detrimental	Literature
Senna occidentalis seeds		Extensive axonal damage		Neurotoxic	Calore *et al.* (1998)
Silybum marianum fruit extract	40–80 ppm sylimarin	Decrease of slaughtering yields	Growth rate	–	Schiavone *et al.* (2007)
		Reduction of lipid content in meat	Hepatoprotection		
Synclisia scabrida extracts	–	Apomorphine-induced stereotype behaviour *Pseudomanas* and *Staphylococcus* infection	–	–	Sokomba *et al.* (1986)
Vicia faba tannin extract	8–16 g/kg	–	–	Mortality Body weight FI FCR	Ortiz *et al.* (1994)
Vicia faba proanthocyanidin extract	30 g/kg			Digestibility of protein Digestive enzyme	Yuste *et al.* (1992)
Yucca schidigera extract	–	FI	Viscosity of proximal ileal content	–	Preston *et al.* (1999)
	0.2% extract	Body weight gain Energy utilisation Net ammonia production	FI		Yeo and Kim (1997)

CP, crude protein; DM, dry matter; EO, essential oil; FI, feed intake; FCR, feed conversion ratio; TMEn, true metabolisable energy.

such as feed intake, weight gain and feed conversion rate (Table 1; see also Windisch *et al.,* 2008). Comparison of these data has often been very difficult, however, as different experiments were performed with different and widely ranged doses. Some authors reported the dose in mg per body weight, some in percentage in the feedstuff, while the others calculated based on the concentration of active principles. In many cases FI and FCR have been influenced by plant extracts. However, in a number of cases FI and FCR were not changed but the extracts still had positive effects on body weight, body weight gain, organ weight and/or energy utilisation. This results from the strong link between productivity and health. Plant extracts may stimulate the immune system (extracts from alfalfa, artichoke, *Saccharum officinarum*, Table 1 and elsewhere in this review), suppression of harmful microorganisms (*Pseudomonas*, *Staphyloccoccus*, *E. coli*, *Eimeria* spp., *C. perfringens*, *Mycoplasma gallisepticum*), stimulation of beneficial microbes such as *Lactobacillus* spp. (extracts containing capsaicin, cinnamaldehyde and carvacrol), the regulation of the activity of some enzymes (e.g. lipase), protection of gut villi and bacterial adhesion parameters. Plant extracts may also influence the post-mortem quality of meat, especially cholesterol concentration, lipids content, oxidative stability, as well as the quality of eggs, although sometimes better quality was accompanied by reduced weight gain (grape seed extract). An important parameter that can influence growth performance is the protection given by plant extracts against some toxins that can be found in feedstuffs, e.g. ochratoxin A and selenium.

An extract of sugar cane, which was the residue after removing glucose, fructose and sucrose from sugar cane juice was fed at the dose of 0.5 g/kg/d (El-Abasy *et al.,* 2002; Yamauchi *et al.*, 2006) to broilers. A higher body weight gain, gain in body weight/day and lower feed conversion ratio were observed under this treatment, but, like many similar studies, the phytochemical(s) responsible were not identified. Besides some alterations in intestinal histology (higher values of villus height, villus area, epithelial cell area and cell mitosis) promoting growth and showing immunostimulating effects were observed. Chinese herbs were shown to be effective feed additives replacing antibiotics in Pekin meat duck diets (Wang and Zhou, 2007), and a similar conclusion was drawn by Jamroz and Kamel (2002) who observed improvements in daily gain and feed conversion ratio in poultry fed on a diet supplemented with plant extracts. Plant extracts from milk thistle (90 and 180 mg/kg feed), yarrow (900 and 1800 mg/kg), garlic (8230 and 16460 mg/kg), oregano, juniper (450 and 6000 mg/kg) and horseradish (450 and 6000 mg/kg) showed beneficial effects on male broiler chickens (Lewis *et al.*, 2003). Based on feed conversion efficiency (FCE), two extracts e.g. yarrow and garlic were indicated as promising. Garlic (1 g/kg feed) and thyme (1 g/kg feed) were also most promising herb feed additive in the research performed using 5 commercial feeds supplemented with NorSpice® powders (Demir *et al.*, 2003). Two additional commercial phytogenic feed additives XTRACT™ containing carvacrol (5%), cinnamaldehyde (3%) and *Capsicum oleoresin* (2%) as well as Sangrovit® containing ground roots of *Sanguinaria canadensis* rich in the alkaloids sanguinarin and chelerythrin had no effect on chicken growth performance, nutrient utilisation or threonine efficiency, but slightly improved daily gain (+3.7%) and feed conversion ratio (+1.7%).

Dietary supplementation of an EO mixture Herbromix® (oregano herb (*Origanum onites*), laurel leaf (*Laurus nobilis*), sage leaf (*Salvia fruticosa*), fennel fruit (*Foeniculum vulgare*), myrtle leaf (*Myrtus communis*) and citrus peel (rich in limonene) to broilers significantly improved feed conversion rate above that of the control group (Alcicek *et al.*, 2004; Cabukt *et al.*, 2006*a*). In laying hens, cracked-broken egg rate was decreased with the dietary supplement of EO (Cabukt *et al.*, 2006*b*). Supplying *Oregano* EO reduced daily feed intake of broilers compared to control animals. Enrichment with EO significantly improved feed efficiency in broilers (Halle *et al.*, 2008). Most studies have shown no significant difference in feed intake caused by herbal or EO additives, but growth was often enhanced and FCR rate improved. Since poultry are known to adjust feed intake strongly according to the demand of energy, FCR is therefore a very sensitive parameter in responses to growth promoters. Published results are, however, contradictory. Lee *et al.* (2003*a*) fed broilers with 200 mg/kg feedingstuff carvacrol or thymol. Carvacrol reduced feed intake, weight gain and feed conversion rate, whereas thymol showed no effect. Addition of oregano herb in quantities of 2–20 g/kg feed or oregano oil (100–1000 mg/kg feed) resulted, in contrast, in all cases in better performance of broiler chicks (Halle *et al.,* 2004), whilst another trial of the same group (Westendarp *et al.*, 2006) using carvacrol, p-cymene and γ-terpinene as pure substances in approximate 50 (carvacrol) or 25 (terpinene, p-cymene) mg/kg had almost no effect. Recently, Haselmeyer (2007) studied the effect of thymol in 4 concentrations from 0.1 to 1.0% as a feed additive in broilers. No significant difference in performance was obtained over the whole growing period (35 d). Turkeys fed with 1.25–3.75 g/kg dried oregano leaves showed, in contrast, a clearly improved feed conversion rate (Bampidis *et al.,* 2005). Adding 60 mg/kg carvacrol-rich thyme oil to the diet resulted in significantly higher body weight gain and better feed efficiency as well as decreased abdominal fat weight in quails (Denli *et al.*, 2004). A dietary supplementation of oregano EO (300 mg/kg) showed a positive effect on performance of broiler chickens experimentally infected with *Eimeria tenella* (Giannenas *et al.*, 2003).

In conclusion, the number of trials on the effects of plant extracts on performance in poultry is large. Recently, Windisch *et al.* (2008) also evaluated some of the available studies in broilers, turkeys and quails and concluded that a majority of the studies showed a reduction in FI due to the use of dietary plant extracts, a largely unchanged body weight gain and as a result an improvement in FCR. However, for each response parameter (feed intake, body weight gain and feed conversion ratio) differences in the quantitative response were found in different studies. Whether such a general statement is valid is doubtful, as each plant extract or phytogenic plant compound will most likely have a different mode of action and result in different types of effects on animal performance. A number of studies have also shown no response or a positive effect on feed intake and/or body weight gain. In addition, the experimental conditions in which the compounds are tested may greatly influence the outcome of the evaluation, e.g. the nature of the negative control treatment, experimental conditions with regard to health status of the birds and/or challenges imposed to the birds during evaluation of the feed additives, or the concentration of the compounds tested. Moreover, it can be assumed that there will be a publication bias in this area, meaning that products or studies showing no or negative effects have less chance of being published in a refereed journal.

Product quality

Many plants or plant extracts contain bioactive compounds that improve the quality of poultry products, including both meat and eggs. The main quality indices of interest are organoleptic properties, storage stability and the ‘‘healthiness’’ of the product for consumption by man. Although including herbs in the diet might be expected to influence taste in particular, there seem to be surprisingly few structured reports on the influence of phytochemicals on the organoleptic qualities of poultry products (Rizza *et al.,* 2008; Windisch *et al.,* 2008). Thus, the quality aspects reviewed here will cover predominantly the effects on stability and healthiness.

Antioxidants

Many plants and phytochemicals, including EO plants and EO, are known for their antioxidative properties based mainly on phenolic compounds in the oil or in other phytochemical fractions. Some non-phenolic substances may show a remarkable antioxidative potential. Such substances contribute to antioxidative benefits in three respects. Firstly, they may protect feed components from oxidative damage, substituting partly the use of α-tocopheryl acetate and related compounds as feed additives or preservatives respectively. They may also affect oxidative metabolism in the animal: examples will be given below. Finally, oxidative stability to a large extent determines the shelf life of fat, meat and eggs (Botsoglou *et al.*, 1997; Govaris *et al.*, 2005), and many plant bioactive feed additives have been shown to benefit storage quality.

The dietary supply of thyme oil or thymol to ageing rats showed a beneficial effect on the antioxidative enzymes superoxide dismutase and glutathione peroxidase as well as on polyunsaturated fatty acid composition in various tissues (Youdim and Deans, 1999). Animals receiving these supplements had higher enzyme levels and higher concentrations of polyunsaturated fatty acids in phospholipids of the brain than the untreated control (Youdim and Deans, 2000). Oregano EO added in doses of 50–100 mg/kg to the diet of chickens exerted an antioxidant effect in the animal tissues (Botsoglou *et al.*, 2002). Such antioxidant effects would be expected to improve the health of poultry livestock as they do in other animals, including man.

Storage quality is generally linked to the oxidation of fats. Dietary thyme improved the oxidative stability of eggs (Botsoglou *et al.,* 1997; Liu *et al.,* 2009); although thymol is the EO compound most associated with biological effects in thyme, other components also appeared to be involved (Botsoglou *et al.,* 1997). Saffron (*Crocus sativus* L.; Botsoglou *et al.,* 2005), oregano (Radwan *et al.,* 2009), rosemary (Lopez-Bote *et al.*, 1998; Florou-Paneri *et al.,* 2006; Radwan *et al.,* 2009), sage (Lopez-Bote *et al.*, 1998), turmeric (*Curcuma longa*; Radwan *et al.,* 2009), tea catechins (Yilmaz, 2006), mulberry leaf, Japanese honeysuckle and goldthread (Liu *et al.,* 2009) had similar benefits to the oxidative stability of eggs. The effects of rosemary were not seen in another study (Galobart *et al.,* 2001). Also with rosemary and sage extracts, the concentration of total cholesterol oxidation products (COPS) was reduced, and a similar trend was observed in microsomal fraction isolates in which the rate of metmyoglobin/

hydrogen peroxide-catalysed lipid peroxidation was lower in birds receiving these plant extracts in comparison with the control fed on basal diet only (Lopez-Bote *et al.*, 1998). Thus, many plants can improve the oxidative stability aspect of product quality, although the phytochemicals responsible have not been identified.

Lipid metabolism

Fatty acid and cholesterol metabolism in the bird is influenced by many plants and phytochemicals, leading to improvements in the fatty acid composition and particularly cholesterol content of meat and eggs. Garlic is probably the best characterised plant to lower the cholesterol content of poultry meat (Konjufka *et al.,* 1997; Lim *et al.,* 2006) and eggs (Chowdhury *et al.,* 2002; Mottaghitalab and Taraz, 2004; Yalcin *et al.,* 2006, 2007). Other plants and herbs have also been reported to be beneficial in this respect, including green tea (Uuganbayar *et al.,* 2005) of which Chinese green tea was best (Uuganbayar *et al.,* 2006), and mixed herbs (Poltowicz and Wezyk, 2001). The mechanism whereby garlic decreases cholesterol involves lower serum concentrations of cholesterol (Horton *et al.,* 1991; Chowdhury *et al.,* 2002; Mottaghitalab and Taraz, 2004; Lim *et al.,* 2006; Yalcin *et al.,* 2006), which then presumably limits the cholesterol available to be taken up into muscle and eggs. The results of Santoso *et al.* (2005) suggest that *Sauropus androgynus* (Katuk) extract acts in a similar manner to garlic. Broiler chicks fed on *Codonopsis lanceolata* root (a plant used in Korean cuisine) showed decreased serum levels of triglyceride, total cholesterol and low density lipoprotein cholesterol compared to the control group, and decreased triglycerides and total cholesterol levels in liver and breast muscle. The effect appeared to be linked to biliary cholesterol excretion being increased by 15%. Whether this is a common mechanism of product-cholesterol-lowering plants is not yet clear. The pattern of fatty acids in the abdominal fat of chicken was also altered by oregano oil (Wald, 2002), and dietary carvacrol lowered plasma triglycerides (Lee *et al.,* 2003*a*).

Yolk colour is also a quality trait that is influenced by plant additives. Green tea decreased the yellowness of the yolk (Uuganbayar *et al.,* 2005), as did mixed herbs (Poltowicz and Wezyk, 2001), while other dietary ingredients/additives, including alfalfa concentrate, tomato powder and marigold extract increased the colour intensity of yolk (Karadas *et al.,* 2006). These natural additives would be preferred over some synthetic pigments that have been fed to poultry for many years but which are now less acceptable to consumers (Karadas *et al.,* 2006).

BIOACTIVE COMPOUNDS AND THE GASTROINTESTINAL ENVIRONMENT

Normal flora

Most of the gut microbiological analysis of poultry used in food production has been done in the broiler chicken. The two main sites of microbial activity are the crop and the caecum (Smith, 1965), although microbe-host interactions elsewhere in the digestive tract may have important consequences for health (Lan *et al.,* 2005). Before the advent of molecular community profiling techniques, cultivation-based analysis indicated that the anterior part of the tract (crop, gizzard, small intestine) was dominated by facultative bacteria, principally *Lactobacillus* spp., while the caecum contained mainly strict anaerobes (Fuller, 1984). Numbers were high, up to 10^{11} per g of digesta. More recently, terminal restriction fragment length polymorphism (T-RFLP) analysis, also based on 16S gene sequence analysis, indicated that the bacterial communities at different parts of the gut were different, except when comparing jejunum and duodenum (Torok *et al.,* 2008). Lu *et al.* (2003) confirmed this difference when comparing 16S rRNA gene libraries from the ileum and caecum. The former contained nearly 70% *Lactobacillus* spp., while the latter had only 8% *Lactobacillus* and was dominated by *Clostridiaceae*-related species. Fuller (1984) estimated that there may be more than 200 species in the avian gut. Gong *et al.* (2002*a*) and Zhu *et al.* (2002) found that many of the 16S rRNA sequences from caecal clone libraries were dissimilar to known bacteria. The library sequences obtained by Zhu *et al.* contained 40% related to *Sporomusa* or enteric bacteria related to γ-proteobacteria, such as *E. coli*, a result that was not replicated in the other molecular studies. Smaller numbers of bacteria colonise the small intestine, yet they represent a surprisingly diverse community (Knarreborg *et al.*, 2002; van der Wielen *et al.*, 2002). A specific community colonises the caecal mucosa, different from that inhabiting the lumen (Gong *et al.,* 2002*b*). All sequence analysis studies report that there are large numbers of unknown bacteria present (Apajalahti *et al.,* 2001; Gong *et al.,* 2002*a*,*b*; Lu *et al.,* 2003; Apajalahti *et al.,* 2004). While some might assume that these bacteria might be unculturable (e.g. Apajalahti *et al.,* 2004), there is no reason to suppose that they will be functionally different to known gut species and that they may eventually be cultured.

Both diet and age have a major influence on the composition of the gut bacterial community.

The first report of the effect of diet on the community by molecular techniques was that of Apajalahti *et al.,* (2001), who used a G + C profiling method to demonstrate that diet had a major effect on gut composition. Even a relatively minor change in diet – the supplementation of a barley diet with glycosidases – resulted in 73% dissimilarity between bacterial communities in the ileum and 66% in the caecum (Torok *et al.,* 2008). The changes seemed to occur across many species, with no individual bacterial species contributing more than 1 to 5% of the total. Lu *et al.* (2008) made the point that the broiler should be thought of as a young animal whose mature flora has not yet been achieved. The caecal and ileal communities were similar up to 14 d, diverging thereafter.

Gut microbiota and productivity

How useful or harmful are the resident gut bacteria to health and productivity? Some would consider that the most telling observations about the role of gut microorganisms in the health and productivity of poultry are (i) that gnotobiotic and caecectomised chicken and quail chicks grow better than their conventional counterparts (Fuller and Coates, 1983; Furuse and Yokota, 1984, 1985) and (ii) that antibiotics enhance growth efficiency in broiler production (Graham *et al.,* 2007). Fuller (1984) concluded that, although bacterial glycosidases digested polysaccharides in the feed, there was no evidence of a net benefit to productivity from this activity. Indeed, he went so far as to say "In fact the net effect of the flora is harmful". Vispo and Karasov (1996), on the other hand, argue that the retention in evolutionary terms of a caecum must indicate that an advantage must be conferred by the retention of the structure. Torok *et al.* (2008) state "Gut microbiota positively influence the host's gastrointestinal development, biochemistry, immunology, physiology, and nonspecific resistance to infection", basing this assertion on the review by Gordon and Pesti (1971). The review covered mainly mammalian species, however, with much less reference to poultry. Nevertheless, Torok *et al.* (2008) managed, by sophisticated analysis of T-RFLP profiles, to link differences in the gut community composition with improved performance (apparent metabolisable energy), which has been a goal of researchers for many decades.

There are several challenges that pathogens, both acute and sub-acute, present to poultry production. *C. perfringens*, an anaerobic Gram-positive bacterium known to be a common pathogen in humans, domestic animals and in wildlife, is the primary cause of clostridial enteric disease in poultry production. *C. perfringens* associated necrotic enteritis and subclinical diseases are serious threats to poultry health, causing a spectrum of effects including subclinical infection, mild disease with focal intestinal necrosis, diarrhoeal illness and liver disease, as well as the classic form of acute fulminant necrotising enteritis (Wilson *et al.,* 2005). Necrotic enteritis is estimated to affect up to 40% of the commercial broiler flocks in the United States and it is believed to cost the US poultry industry about 5 USD cents per broiler (McDevitt *et al.*, 2006). Fuller *et al.* (1979) found that the poorer growth of conventional *vs.* germ-free birds was due, in part, to *Streptococcus faecium.* The mechanism appeared to involve adhesion to the duodenum (Fuller *et al.,* 1981) and the deconjugation of bile salts, leading to malabsorption of lipids (Cole *et al.,* 1981). The other major intestinal disease suffered by poultry is coccidiosis, caused mainly by *Eimeria* spp. (Kennedy, 2001). The disease is passed from bird to bird *via* droppings, which means that the problem is greatest in intensive units unless measures are taken to control oocyte numbers.

Immunity

The nutrient content of the diet has a major effect on immunity in poultry (Kidd, 2004), without reference to plant bioactives, but there is nonetheless growing published evidence for benefits to be obtained by incorporating plants rich in certain phytochemicals being beneficial for immune function in poultry (Swiatkiewicz and Koreleski, 2007). Chinese herbs in particular seem to be cited as positive for immune effects, though other plants and extracts have been reported to be positive. We have not been able to find a systematic account of the precise phytochemicals that might be beneficial, so what follows is inevitably rather disjointed: there does not appear to be a hypothesis linking the different plants. Immune function would be enhanced as a consequence of a more stable intestinal health favoured by feed additives, or by animals being less exposed to microbial toxins or other undesired metabolites, for example ammonia and biogenic amines. Consequently, additives like aromatic herbs or volatile oils may relieve the animals from immune defence stress during critical situations, raising the intestinal availability of essential nutrients for absorption and thus, assist the animal to grow better within its genetic potential.

Sometimes extracts of plants, not well characterised, have beneficial effect. Ethanol extracts of *Allium sativum* (garlic), *Glycyrrhiza glabra* (licorice), *Plantago major* (plantain) and *Hippophae rhamnoides* (sea buckthorn) all had some beneficial immunological effects on cellular immunity in laying hens (Dorhoi *et al.*, 2006). Polysavone, an extract of alfalfa, enhanced immunity in broilers (Dong *et al.*, 2007). The phagocytic activity of peripheral blood leucocytes in chickens orally administered sugar cane extracts or a polyphenol-rich fraction of the sugar cane extract (500 mg/kg/day) for 3 consecutive days increased significantly, when compared with that of saline-administered control chickens (Hikosaka *et al.*, 2007). Achyranthan, a low-molecular-weight Chinese herbal polysaccharide, showed immunostimulating effects in both growth assays and in vitro studies (Chen *et al.*, 2003).

Ligustrum lucidum and *Schisandra chinensis* improved antioxidative metabolism and immunity of laying strain male chicks (Ma *et al.,* 2007). Aniseed (*Pimpinella anisum*) used at up to 4% inclusion in laying quail diets provided beneficial effects on immune responses, although 5% caused negative effects on feed intake and feed conversion ratio (Bayram *et al.,* 2007). Similar effects were found in broiler chicks (Durrani *et al.,* 2007).

Coccidiosis

Coccidiosis is the most important disease in poultry production (Cox, 1998) causing annual costs of more than $3 billion to the worldwide industry as estimated by Dalloul and Lillehoj (2006). Mortality, malabsorption of nutrients, impaired growth rates and rapid and effective transmission between animals are characteristic of the disease, which is caused by several species of *Eimeria*. Secondary bacterial infections are frequently observed and may further increase the severity of the disease. In-feed medication by anticoccidial drugs has provided good protection of flocks for decades. Emerging problems with parasite resistances and concerns about drug residues however have stimulated the search for alternatives. Concomitantly with the ban of antibiotic growth promoters in animal production, the European Union (EU) has put to question the use of coccidiostats from the year 2012 onwards. The decision will have high impact on poultry production within the EU and is expected to influence also other regions.

Coccidiosis vaccines are mostly used for breeder and layer chickens, but hardly at all for broilers (EC, 2008), which is the most numerous type of chicken. Six *Eimeria* species are considered economically relevant (Holdsworth *et al.*, 2004), but immunity is highly species-specific and not all species and relevant strains are included in most commercial vaccines. To overcome these limitations, a lot of effort is put into new strategies for vaccine development (Dalloul and Lillehoj, 2006; Shirley *et al.*, 2007).

A summary of reported anticoccidial effects of plants and plant extracts in poultry is given in Table 2.

Prooxidants

Allen and Fetterer (2002) provided a comprehensive review on various feed ingredients and their influence on coccidiosis. Flaxseed, flaxseed oil and corn oil, which contain high amounts of polyunsaturated fatty acids (PUFA), reduced lesions caused by the chicken parasite *Eimeria tenella*, but not lesions caused by *Eimeria maxima* (Allen and Fetterer, 2002; Yang *et al.,* 2006). Artemisinin, a naturally occurring antimalarial compound, significantly lowered lesions (Allen and Fetterer 2002) and reduced oocyst output (Arab *et al.,* 2006) from *E. tenella* when given at low levels as a feed additive. The mechanisms of action of PUFA as well as artemisinin are assumed to involve induction of oxidative stress to the parasites. However, there might be practical difficulties in including sufficient amounts of PUFA for protection in the diets, also due to antioxidative ingredients which are usually included in feed, as studies by Allen *et al.* (2000) have shown. Furthermore, this mode of action seems to be effective against *E. tenella*, which is adapted to the specifically anaerobic conditions of the caeca, but not so much against other *Eimeria* species.

Antioxidants

Diets supplemented with γ-tocopherol, with the spice turmeric or curcumin, which all possess antioxidative properties, reduced small intestinal lesion scores and improved weight gains during *E. acervulina* and *E. maxima* infections (Allen and Fetterer, 2002). Antioxidative activity is also suggested as the mode of action of various South African plant species investigated by Naidoo *et al.* (2008). *Tulbaghia violacea* showed improved FCR and lowered oocyst output during a mixed *Eimeria* challenge. Green tea reduced oocyst shedding after an *E. maxima* infection, but no beneficial effects on weight gain were detected (Jang *et al.*, 2007). Wang *et al.* (2008) reported beneficial effects of grape seed proanthocyanidins by counteracting weight loss, mortality and lesion scores and lowering oxidative stress in intestinal tissues. All these compounds may exert their anticoccidial activity by protecting infected tissues from oxidative damage and therefore reducing the severity of coccidiosis. Similar to compounds causing oxidative stress, the effect of antioxidants seems to be restricted to certain *Eimeria* species only, especially *E. acervulina* and *E. ma*xima (Allen and Fetterer, 2002).

Table 2. *Plants and plant extracts with anticoccidial activity*

Plant species/extract	Scientific name	Activity/Mode of action	Literature
Flaxseed meal, flaxseed oil, n-3 fatty acids	*Linum usitatissimum*	*E. tenella* (not *E. maxima*) lesions, parasitation, development. Induction of oxidative stress	Allen *et al.* (1998, 2000), Allen and Fetterer (2002)
Corn oil	*Zea mays*	*E. tenella* body weight, higher IgA, lower plasma carotenoids,	Yang *et al.* (2006)
Artemisia annua dried herb, Artemisinin, 1,8-Cineol, Camphor, *A. sieberi* petroleum ether extract of aerial parts, *A. afra* acetone/water extract from aerial parts	*Artemisia annua, A. sieberi. A. afra*	*E. tenella* lesions, oocyst output, *E. acervulina* oocyst output. (not *E. maxima*) Induction of oxidative stress. *Eimeria* mix (Eten, Emax, Eace): FCR. 1, 8-cineol and camphor: weight gain, lesions	Allen *et al.* (1997,1998), Allen and Fetterer (2002), Arab *et al.* (2006), Naidoo *et al.* (2008)
Sophora flavescens root decoction	*Sophora flavescens*	*E. tenella* weight gain, mortality, bloody diarrhoea,	Allen and Fetterer (2002), Youn and Noh (2001)
Turmeric spice rhizome, Curcumin	*Curcuma longa*	*E. maxima* lesions, weight gain. *E. acervulina* (not *E. tenella*) Antioxidative	Allen *et al.* (1998), Allen and Fetterer (2002)
γ-Tocopherol	e.g. from *Linum usitatissimum,* various seed oils	*E. maxima* lesions, weight gain. *E. acervulina* (not *E. tenella*). Antioxidative	Allen *et al.* (2000), Allen and Fetterer (2002)
Betaine	e.g. from sugar beet (*Beta vulgaris* ssp. *vulgaris* var. *altissima*)	*E. acervulina* (and *E. tenella*, but less effective) invasion and development when used in combination with salinomycin. *E. maxima* weight gain (not *E. tenella*, *E. acervulina*)	Allen *et al.* (2000), Allen and Fetterer (2002), Fetterer *et al.* (2003), Klasing *et al.* (2002), Waldenstedt *et al.* (1999)
Oregano aerial parts and essential oil (containing carvacrol and thymol)	*Origanum vulgare* L. ssp. *hirtum*	Orego-Stim (Meriden): *Eimeria* mix (8 species, unknown ratio) lesions, oocyst output, feed efficiency. *E. tenella* weight gain, FCR, lesions, OPG (exact inclusion rate unclear!)	Batungbacal *et al.* (2007), Giannenis *et al.* (2003, 2004)
China bark tree extract, Quinine	*Cinchona succirubra*	*E. tenella*, *E. meleagrimitis* Sz invasion *in vitro*	Christaki *et al.* (2004), Fayer (1971)
Olympus tea	*Sideritis scardica*	*E. tenella* weight gain, diarrhoea, mortality, lesions, oocysts output	Florou-Paneri *et al.* (2004)
Grape seed proanthocyanidin extract, ethanol/ water extract from pomace	*Vitis vinifera*	*E. tenella* weight gain, mortality, lesion scores. *Eimeria* mix (Eten, Emax, Eace): FCR	Naidoo *et al.* (2008), Wang *et al.* (2008)
Sugar cane extract	*Saccharum officinarum* L.	*E. tenella*: body weight gain, oocyst output, lesions, antibody response. Small group sizes, no specification of extract.	El-Abasy *et al.* (2003)
Wild garlic acetone/water extract from whole plant	*Tulbaghia violacea*	*Eimeria* mix (Eten, Emax, Eace): FCR, OPG. Marasmine = S-(methylthiomethyl)cysteine sulfoxide), bis[(methylthio)-methyl] disulfide, and derivatives	Naidoo *et al.* (2008)
Green tea leaves	*Camellia sinensis*	*E. maxima* oocyst output	Jang *et al.* (2007)
Oriental plum, Japanese plum	*Prunus salicina*	*E. acervulina* body weight gain, OPG, IFN-g and IL-15 (mRNAs) of IEL, spleen cell proliferation. Phenolics, antioxidants,	Lee *et al.* (2008)

Essential oils

Amelioration of coccidiosis was observed when supplementing commercial feed additives containing oregano EO (Giannenas *et al.*, 2003; Batungbacal *et al.*, 2007) or ground aerial parts of oregano (Giannenas *et al.*, 2004). Both applications protected significantly from weight loss or improved feed efficiency and reduced oocyst output and lesion scores in a coccidiosis challenge. Improvement of the negative impact of coccidiosis was also reported for supplementation with Olympus tea (*Sideritis* *scardica*; Florou-Paneri *et al.*, 2004). The EO constituents 1,8-cineole and camphor, from *Artemisia annua* protected weight gain and reduced *E. tenella* as well as *E. acervulina* lesions (Allen and Fetterer, 2002).

Various other plants/products

Youn and Noh (2001) tested 15 therapeutic plants against an *E. tenella* challenge and found a root decoction of *Sophora flavescens* to be most active in protecting weight gain and reducing mortality and bloody diarrhoea. Moreover, a sugar cane extract had protective effects when inoculated in the crop of chicken simultaneously with a challenge. Body weight gain, haemorrhages, oocyst output, lesion scores and antibody response were improved (El-Abasy *et al.,* 2003). Betaine, an osmoprotectant ubiquitous among plants, enhanced the activity of anticoccidial drugs in some cases (Allen and Fetterer 2002; Fetterer *et al.,* 2003), but failed to do so in others (Waldenstedt *et al.,* 1999). Apparently, its effect is restricted to certain *Eimeria* species only.

Immunomodulation

Immunomodulatory effects are assumed to be responsible for protection by plum powder (*Prunus salicina*) against an *E. acervulina* challenge (Lee *et al.*, 2008). Body weight gain, oocyst shedding, IFN-γ and IL-15 levels were significantly improved. Furthermore, Guo *et al.* (2004*a*,*b*) found enhanced cellular and humoral immune responses of *E. tenella*-infected chickens when supplementing a polysaccharide extract from *Astragalus membranaceus*, which may become particularly interesting when used in conjunction with vaccination.

Future perspectives

In summary, plants and products derived thereof have clearly shown the potential to alleviate coccidiosis and reduce its severity in several studies. Moreover they might play a role in counteracting subclinical infections and secondary bacterial infections associated with the disease. Most of the active plant materials could improve some, but not all of the relevant parameters in coccidiosis and variable effectiveness against the different *Eimeria* species was observed in some cases. To date, no alternative to anticoccidial drugs is yet known with comparable efficacy and economy of use in broiler production, and a recently published EC report strongly recommends to maintain the actual status of so-called "coccidiostatic drugs" as feed additives within the EU (EC, 2008). Nevertheless, plant products may have increasing significance in organic farming, whenever antibiotic-free rearing of animals is desired, as supporting agent for vaccination (adjuvants), or in combination with conventional anticoccidial drugs, especially in the light of possible bans or reduction of approved drugs in large economies like the EU. This should be a great incentive for stimulating research in the field of alternatives to conventional anticoccidial drugs in general and especially on the role of plants and plant products.

Difficulties in comparing research data arise from the use of different experimental models and different strains of *Eimeria*. Parasite strains are known to possess variable virulence and may cause variable severity of challenge in different experiments. An important effort to harmonise techniques in coccidiosis research and models for evaluation of drug efficacy against coccidiosis was taken in the course of the COST 89/820 programme and by Holdsworth *et al.* (2004). Guidelines for efficacy testing are also published by regulative authorities, e.g. the recently published EFSA "guidance for the preparation of dossiers for coccidiostats and histomonostats" (EFSA, 2008). Such guidelines should also be taken into consideration when alternatives to anticoccidial drugs are investigated in order to provide sound and comparable scientific results.

Necrotic enteritis

Necrotic enteritis (NE) is a disease in poultry causing high economic costs and seriously impairs animal welfare. Due to the ban on subtherapeutic antibiotic usage, NE has become increasingly prevalent in the EU. Demands for safer food have put pressure on the development of alternative management or dietary strategies to control this disease. *C. perfringens*, a Gram-positive, anaerobic, spore-forming toxigenic bacterium is found in soil, dust, faeces, feed and poultry litter and has been identified as the main causative agent causing NE in poultry (Branton *et al.*, 1997; Annett *et al.*, 2002; Dahiya *et al.*, 2006; McDevitt *et al.*, 2006). *C. perfringens* is principally a normal inhabitant of the chicken intestine but under certain circumstances it can begin to proliferate rapidly, accompanied by increased toxin production causing the intestinal mucosal necrosis characteristic of NE (Branton *et al.*, 1997; Collier *et al.*, 2003; Dahiya *et al.*, 2005, 2006). However, even high doses of *C. perfringens* in the intestinal tract of broiler chickens do not always lead to the development of NE, as the gut flora of healthy birds can apparently prevent its pathogenicity (Fukata *et al.*, 1991). Various predisposing factors, among them dietary composition and incidence of coccidiosis (Williams, 2005), may lead to over-proliferation of

C. perfringens, and the subsequent progression to disease is still poorly understood. Host specific virulence factors like production of α-toxin are assumed to play a role, and recent findings point towards the importance of the *netB* gene, necessary for the production of the respective toxin (NetB) (Keyburn *et al.,* 2006; Chalmers *et al.,* 2008; Timbermont *et al.,* 2008). However, control of *C. perfringens* seems to be essential and dietary ingredients have a great influence on the incidence of NE in broiler chickens.

Reports of the effects of plants and their extracts on mainly avian *C. perfringens* are summarised in Table 3. Dahiya *et al.* (2006) reviewed the potential of plant-derived feed ingredients to control *C. perfringens* and NE. Numerous plants and plant products have been found to possess inherent antimicrobial activity against clostridia, although mostly their effects were only determined *in vitro*. Oregano, black pepper, cloves and the EO components carvacrol and eugenol possess antibacterial activity against clostridia as well as *E. coli*, *Staphylococcus aureus* and *Salmonella pullorum.* Furthermore, lemon myrtle (Wilkinson *et al.,* 2003), *Artemisia princeps* var. *orientalis* (Cho *et al.,* 2003), *Hypericum scabrum* (Sokmen *et al.,* 1999) and *Aristolochia paucinervis* (Gadhi *et al.,* 1999) displayed *in vitro* activity against *C. perfringens* and other bacteria.

Reports on *in vivo* investigations are scarce: according to Dahiya *et al.* (2006), supplementation of flaxseed may have benefits by modification of intestinal microbial colonisation. Linolenic acid, the main constituent of flaxseed fatty acids, may prevent the adhesion of bacteria to intestinal epithelial cells and mucus, whereas addition of pectin and guar gum to diets has reportedly eliminated NE from sick birds. Specific blends of EO components like thymol, carvacrol and eugenol (Mitsch *et al.,* 2004) as well as astaxanthin from the microalgae, *Haematococcus pluvalis*, were found to be effective in controlling *C. perfringens* colonisation and proliferation in the gut of broilers (Waldenstedt *et al.,* 2003). Finally, lupulone from hops, when administered in drinking water, inhibited proliferation of artificially inoculated *C. perfringens* in the chicken gastrointestinal tract (Siragusa *et al.,* 2008).

Escherichia coli

Escherichia coli is the most common bacterial pathogen of poultry and responsible for significant losses in the world's industry. Although our understanding of pathogenicity has increased in the past years, the virulence factors (genes) which lead to disease remain to be fully unravelled (La Ragione and Woodward 2002). For practical reasons, *E. coli* isolated from diseased chicken are termed avian pathogenic *E. coli* (APEC). Colisepticaemia or colibacillosis manifests itself most commonly as an infection of the respiratory tract, in rare cases also as enteritis. *E. coli* are common inhabitants of poultry intestinal microbiota, thus the gastrointestinal tract is seen as a possible reservoir for infection (Ewers *et al.,* 2009) and incidence of the disease might be reduced by keeping intestinal *E. coli* numbers low.

Reports of the effects of plants and their extracts on APEC are summarised in Table 3. There are numerous studies on *in vitro* effectiveness of plant-derived extracts and compounds, as well as EO against (avian) *E. coli* (Smith-Palmer *et al.,* 1998; Penalver *et al.,* 2005b,c,d; Fisher and Phillips 2006; Horosova *et al.,* 2006; Prakash, 2006; Geidam *et al.,* 2007). EO containing a high percentage of phenolic components (e.g. carvacrol and thymol) show higher inhibitory capacity compared to the oils containing, for example, the monoterpenic alcohol linalool (Penalver *et al.,* 2005a). However, literature supporting actual *in vivo* activity is scarce. An alternative strategy to suppress intestinal *E. coli* might involve preventing their adhesion to the intestinal mucosa. This may be achieved by feeding compounds which increase mucus production, thus reducing the possibility of bacterial adhesion to the intestinal epithelium. A mixture of cavracrol, cinnamaldehyde and capsaicin caused the release of large amounts of mucus on glandular stomach and wall of jejunum in chickens when incorporated into their diets (Jamroz *et al.,* 2006). Becker and Galletti (2008) exploited the ability of *E. coli* to adhere to mannose receptors and mannose-containing analogues to find food and feed components with gut health-promoting effects. Out of 18 dietary components tested, artichoke and sesame seed extracts performed well in binding various *E. coli* strains. Sesame seed extract was also most effective in binding chicken *Salmonella* isolates.

Antiviral effects

Although a vast range of plants possess antiviral activity (Jassim and Naji, 2003), they are probably underexplored and underutilised for this purpose in poultry farming. Actually, the most important viral diseases are sought to be controlled by vaccination. A polyphenolic extract from *Geranium sanguineum* aerial roots and an extract of the red marine alga *Ceramium rubrum* showed excellent *in vitro* inhibition of human and chicken influenza A viruses (Serkedjieva and Hay, 1998). The very scarce animal trials comprise sulfated *Astragalus* polysaccharides

Table 3. *Plants with activity against* Escherichia coli *and* Clostridium perfringens

Plant species/extract	Scientific name	Activity/Mode of action	Literature
Flaxseed, linolenic acid	*Linum usitatissimum*	Prevents adhesion of pathogenic bacteria	Dahiya *et al.* (2006)
Guar gum, pectin	*Cyamopsis tetragonolobus*	Unknown	Dahiya *et al.* (2006)
Thymol, carvacrol and eugenol		Inhibit *C. perfringens* colonisation and proliferation	Dahiya *et al.* (2006), Mitsch *et al.* (2004)
Turmeric, EO from rhizome	*Curcuma longa*	Inhibits *C. perfringens*	Dahiya *et al.* (2006)
Eugenol, EO from clove	*Syzygium aromaticum*	Inhibits *C. perfringens*	Dahiya *et al.* (2006)
Astaxanthin, from red algae	*Haematococcus pluvalis*	Inhibits C. perfringens caecal colonisation	Waldenstedt *et al.* (2003)
Lupulone, from hops	*Humulus lupulus*	Inhibits intestinal *C. perfringens*	Siragusa *et al.* (2008)
Lemon myrtle, leaf paste	*Backhousia citriodora*	Inhibits *C. perfringens* (*in vitro*)	Wilkinson *et al.* (2003)
Japanese mugwort, seco-tanapartholides	*Artemisia princeps* var. *orientalis*	Inhibits *C. perfringens* (*in vitro*)	Cho *et al.* (2003)
Hypericum, acetone extract from aerial parts	*Hypericum scabrum*	Inhibits *C. perfringens* (*in vitro*)	Sokmen *et al.* (1999)
Aristolochia paucinervis, defatted chloroform extract of rhizome	*Aristolochia paucinervis*	Inhibits *C. perfringens* (*in vitro*)	Gadhi *et al.* (1999)
Oregano, EO	*Origanum vulgare*	Bactericidial effect	Horosova *et al.* (2006)
Agave, extract	*Agave picta*	Inhibits *C. perfringens* (*in vitro*)	Verastegui *et al.* (1996)
Paper daisy, petroleum ether and ethanol extracts of flowers	*Helichrysum* sp.	Growth inhibition of various *Helicobacter* species	Aslan *et al.* (2007)
Plant extract + 5% carvacrol, 3% cinnamaldehyde, 2% capsicum oleoresin	*Origanum vulgare, Cinnamomum cassia, Capsicum annum*	Prevents adhesion of of *E. coli*	Jamroz *et al.* (2006)
Lemon, EO	*Citrus limon*	Growth inhibition by the disc diffusion method	Fisher and Phillips (2006)
Sweet orange, EO	*Citrus sinensis*	Growth inhibition by the disc diffusion method	Fisher and Phillips (2006)
Bergamont, EO	*Citrus bergamia*	Growth inhibition by the disc diffusion method	Fisher and Phillips (2006)
Shiitake, extract	*Lentinus edodes*	Increases the number of desirable bacteria in order to inhibit colonisation of invading pathogens	Guo *et al.* (2004*c*)
White jelly, herb polysaccharide extract	*Tremella fuciformes*	Increases the number of desirable bacteria in order to inhibit colonisation of invading pathogens	Guo *et al.* (2004*c*)
Huang Qi, herb polysaccharide extract	*Astragalus membranacea*	Increases the number of desirable bacteria in order to inhibit colonisation of invading pathogens	Guo *et al.* (2004*c*)
Spanish origanum, EO	*Coridothymus capitatus*	Antimicrobial activity	Penalver *et al.* (2005)
Thyme, EO	*Thymus mastichinia*	Antimicrobial activity	Penalver *et al.* (2005)
Geranium, EO from steam distillation	*Pelargonium* sp.	Partly greater efficacy against *E.coli* than commercial thyme oil	Penalver *et al.* (2005)
Guava, aqueous extract	*Psidium guajava*	Prevents adhesion of of *E. coli*	Geidam *et al.* (2005)
Artichoke	*Cynara cardunculus* var *scolymus*	Adhesion of *E.coli* (*in vitro*)	Becker and Galletti (2008)
Sesame seed extract	*Sesamum indicum*	Adhesion of *E.coli* (*in vitro*)	Becker and Galletti (2008)
Palm kernel, extract	*Aracaceae elais*	Adhesion of *E.coli* (*in vitro*)	Becker and Galletti (2008)
Tomato	*Solanum lycopersicum*	Adhesion of *E.coli* (*in vitro*)	Becker and Galletti (2008)
Betel pepper, aqueous extract	*Piper betel*	Inhibition zone in agar gel plates	Prakash (2006)
Senna, aqueous extract	*Cassia auriculata*	Inhibition zone in agar gel plates	Prakash (2006)

(Huang *et al.,* 2008), *Ocimum sanctum* and leaf galls of *Ficus racemosa* (Kolte *et al.,* 1999), which showed effects against infectious bursal disease (IBD) and *Aloe secundiflora*, which reduced mortality and severity of clinical signs during a Newcastle Disease infection (Waihenya *et al.,* 2002*b*).

Zoonotic infection

Of major concern to consumers are the hazards presented by zoonotic infection from contaminated poultry meat. *Campylobacter* spp. is the greatest hazard in terms of numbers of infections and days lost through illness (Friedman *et al.,* 2000). However, the infection is usually short-term and self-limiting. *Campylobacter jejuni* readily colonise the gastrointestinal tract (GIT) of poultry, without causing any disease in the host birds. The principal site of colonisation is the lower GIT, especially the caeca, large intestine and cloacae (Beery *et al.*, 1988; Stern *et al.*, 1988). *Campylobacters* do not adhere to the intestinal surface but are highly motile and rapidly track along intestinal mucus, preferentially within caecal and cloacal crypts (Beery *et al.,* 1988). Commercial broiler flocks rarely start shedding *Campylobacter* before 2 weeks of age however, when shedding occurs, *Campylobacter* is spread rapidly throughout the flock (Corry and Atabay, 2001; Mead, 2002; Newell and Fearnly, 2003). Until now, the main approaches evaluated for handling the *Campylobacter* problem in practice include hygienic barriers, diagnostics at the flock level, competitive exclusion, decontamination and intervention efforts targeting the lower GIT (Hariharan *et al.*, 2004). Despite major efforts, however, there are currently no really successful strategies for reduction or elimination of *C. jejuni* from the food chain. *Salmonella* infections in man that result from the consumption of poultry products are less numerous, but much more severe. Human infection by two common serovars, *S. enteritidis* and *S. typhimurium* usually occurs via food-borne transmission. Consumption of raw or undercooked contaminated eggs usually causes *S. enteritidis* infection, while *S. typhimurium* is transmitted by contaminated chicken meat (Babu and Raybourne, 2008). Dietary interventions, including fatty acid modifications, probiotic or prebiotic treatment have been investigated (Babu and Raybourne, 2008), but our understanding of conditions that lead to the proliferation of these zoonotic bacteria is patchy (Mead, 2004).

Antibiotic growth promoters, now banned in Europe but still permitted elsewhere, are so effective that their withdrawal has caused major difficulties in poultry production (Casewell *et al.,* 2003). But how do they achieve that growth promotion? Is it due to the suppression of major pathogens like *C. perfringens*? Or sub-pathogenic species like *S. faecium*? Or is it due simply to a decreased bacterial load (Windisch *et al.,* 2008), or perhaps due to the anti-inflammatory effects of AGP via the inhibition of production and excretion of catabolic mediators by intestinal inflammatory cells (Niewold, 2007)? Finding the answer to these questions is vital, because without that knowledge it will be difficult to select phytochemical replacements for AGP. Molecular ecological analysis described changes in the microbiota in response to bacitracin-virginiamycin (Lu *et al.,* 2008), but other than a decreased community diversity in birds receiving the GPA, it was difficult to explain why production benefits should occur. Indeed, the decreased numbers of *Lactobacillus* contradicted the usual perception of these being beneficial bacteria, the basis of their use as probiotics (Fuller, 1989).

SPECIFIC PLANT BIOACTIVES

Acamovic and Brooker (2005) estimated that plants produced around 5100 different secondary compounds (Figure). One of the most common problems of research performed using plant extracts in poultry nutrition has been the poor characterisation of the plant material or extracts and their standardisation. In many cases, the identity or concentration of active principle has been generally unknown. Due to the possible variation in plant material under different environmental conditions, harvest time, drying and storage conditions, repetition of the experiments is generally impossible to identify them. This is probably why different results are obtained in independent experiments even when using the same plant species. Thus, the development of analytical methods and the proper standardisation of the material used for feeding is crucial if the benefit of the knowledge is to be maximised. Moreover, feeding experiments have often been performed using negative controls only. To be able to compare data from different experiments, the commonly accepted GPA might be recommended as a positive control. The effects of some of the most common categories of plant bioactives and their physiological mode of action are described in following sections.

Essential oils

EO are steam-volatile or pressed-volatile (e.g. citrus extracts) extracts of plants, used traditionally by man for many centuries for the pleasant odour of their essence, their flavour, or their antiseptic and/or preservative properties. Although commonly thought of as being derived from herbs and spices, they are present to some degree in many plants for their protective role against bacterial, fungal or insect attack. They comprise mainly cyclic hydrocarbons (monoterpenes) and their alcohol, aldehyde or ester derivatives (Figure). EO appear as feed additives in the form of the EOs themselves, or as EO-rich plants, or as pure compounds, sometimes synthetic or ''nature-identical''.

The number of papers published on the use of EO and especially those containing the phenolic compounds carvacrol and thymol has increased dramatically over the last few years. The majority report, however, on production parameters (feed uptake, feed conversion, weight gain) only. Comparatively little information is given on their mode of action, metabolism or generally on science based functionality due to the fact that many reports deal with results of commercial products, avoiding statements on pharmacological effects or health claims.

EO used as feed additives for broilers were shown to enhance activities of trypsin, of amylase in tissue homogenates of pancreas, as well as the jejunal chyme content (Lee *et al.*, 2003*b*; Jang *et al.*, 2004). A mixture of carvacrol, cinnamaldehyde and capsaicin also stimulated the intestinal secretion of mucus. Jamroz *et al.* (2006) stated that the increased release of large amounts of mucus and the creation of a thick layer of mucus on glandular stomach and jejunum wall in chicks fed with the above mixture could be responsible for the reduced adherence of pathogens (*E. coli*, *C. perfringens* and others) to the gut epithelium. This confirms – as already known from human nutrition physiology (Teuscher, 2003) and phytopharmacology (Hänsel and Sticher, 2004) – the mode of action of spices and EO on gut function, namely that it involves at least partly an irritation of the exposed tissues and leading to higher secretion of mucus and enzymes.

In general, antimicrobial activity of EO and EO compounds, whether bacteriostatic or bactericidal, or against other microorganisms like fungi, protozoa or food-borne pathogens, is well documented (Smith-Palmer *et al.*, 1998, Dorman and Deans, 2000; Chao *et al.*, 2000; Burt, 2004; Si *et al.*, 2006). Most active in this respect are the phenolic compounds carvacrol, thymol and eugenol but also other substances, including phenylpropane, limonene, geraniol or citronellal, may be involved (Deans and Ritchie, 1987; Pauli, 1994).

The action of EO compounds as antimicrobials occurs via at least two separate mechanisms. The first is by rapidly depleting the intracellular ATP pool, through inhibiting ATP synthesis as a result of their effects on the transmembrane electrical potential. The leakage of ions such as potassium and phosphate out of the cell indicates clearly the membrane damage resulting in disturbances of the osmotic pressure of the cells (Ultee *et al.*, 1999; Lambert *et al.*, 2001; Veldhuizen *et al.* 2006). Furthermore, changes in the fatty acid composition of bacterial cell membranes have been observed at sublethal doses of several EO compounds (Di Pasqua *et al.*, 2006). A second growth-inhibitory mechanism is that substances like carvacrol prevent the synthesis of flagellin, causing bacterial/cells to be aflagellate and therefore nonmotile. Such cells are significantly less able to adhere to epithelial cells, which renders bacteria non-infective (Burt *et al.,* 2007), a mechanism similar to that known from acid galacturonides in the diet (Guggenbichler *et al.*, 2004). The anti-flagellate activity of EOs obtained from fresh leaves of *Cinnamomum aromaticum*, *Citrus limon* pericarps and *Allium sativum* bulbs was investigated *in vitro* on *Tetratrichomonas gallinarum* and *Histomonas meleagridis* with positive results (Zenner *et al.*, 2003).

As EOs comprise a large number of components, it is likely that their mode of action involves several targets in the bacterial cell. The hydrophobicity of EOs enables them to partition in the lipids of the cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents. Physical conditions that improve the action of EOs include low pH, low temperature and low oxygen levels. Synergism has been observed between carvacrol and its precursor p-cymene and between cinnamaldehyde and eugenol (Burt, 2004). Thus, extrapolating from the effects of single EO compounds to the effects of mixtures must be done with caution.

In vitro antimicrobial activities have been measured with a number of EOs and single compounds mainly against enteropathogenic strains of *E. coli*, *Salmonella* sp., *Cl. perfringens* and others. Using either the broth microdilution method or the agar diffusion test, EOs with a higher percentage of phenolic compounds showed the best inhibitory capacity in terms of MIC (minimum inhibitory concentration; Jugl-Chizzola *et al.*, 2005; Penalver *et al.*, 2005; Ben Arfa *et al.*, 2006). The combination of oregano EO with fluoroquinolones, doxycycline, lincomycin, and maquindox florfenicol to treat infections caused by ESBL-producing *E. coli* were reported to lower, to a great extent, the effective dose of these antibiotics and thus minimise the side effects of antibiotics (Si *et al.*, 2008). Differences have been observed, however, in the activities of plant species and plant parts on one side and the sensitivity of species and strains of the microorganisms on the other. This is due to the varying chemical composition of the used plant material (chemotype, morpho- and ontogenetic variation), a factor quite often neglected in microbiological or animal studies. The *in vitro* active concentrations exceeded furthermore in general the dietary doses accepted by the animals, which results in few studies being available so far demonstrating the efficacy of EO compounds against specific pathogens *in vivo*.

Some studies with poultry showed a clear reduction of *C. perfringens* in the jejunum and caecum of broilers fed with a mixture of EO components (Losa and Koehler, 2001; Mitsch *et al.*, 2004). The same blend of components as well as oregano oil or crude drug was effective against *Eimeria* ssp. infections in broilers, thus reducing the application of coccidiostats (Giannenas *et al.*, 2003, 2004; Oviedo-Rondon *et al.*, 2005, 2006). The components of *Artemisia annua*, camphor and 1,8-cineole, at 119 mg/kg, also protected weight gains, and reduced *E. tenella* lesion scores. Camphor decreased *E. acervulina* lesions (Allen *et al.*, 1997, 1998). Carvacrol, cinnamaldehyde, oregano oil and thymol also inhibit *C. perfringens* spore germination and outgrowth in ground turkey during chilling. Cinnamaldehyde was significantly more effective than the other compounds at a lower concentration (0.5%) at the most abusive chilling rate evaluated (Juneja and Friedman, 2007). A study to test the efficiency of carvacrol, thymol, *trans*-cinnamaldehyde and tetrasodium pyrophosphate on the radiosensitisation of *E. coli* and *Salmonella typhi* in chicken breast demonstrated that these active compounds helped reduce significantly the numbers of *E. coli* and *S. typhi* (Lacroix *et al.*, 2004). A combined administration of *Lactobacillus fermentum* and EOs of *Origanum vulgare* and *Thymus vulgaris* decreased the percentage of crop, caecum, liver and spleen colonisation by *Salmonella enterica* var. *dusseldorf* in chicks when compared to the control group without any treatment (Koscova *et al.*, 2006). In a study to test the effects of the antibiotic avilamycin and anise oil supplementation on broilers' body weight, including carcass characteristics and organoleptic analysis of meat, it was concluded that anise oil, at a dose of 400 mg/kg, can be used as an alternative to antibiotics for growth promotion in broiler diets (Simsek *et al.*, 2007).

It is sometimes assumed or implied that effects of the inclusion of aromatic herbs in the diet will be caused by the terpenes that comprise their EO. Cross *et al.* (2007) demonstrated that this need not always be true: thyme and yarrow had different effects on broiler performance to their corresponding EO. With oregano, marjoram and rosemary, the effects were similar.

Two further antimicrobial benefits can theoretically be achieved by adding EO to animal feed: the reduction of feed microbial load and the improvement of the microbial hygiene of the carcase (Aksit *et al.*, 2006). The number of reports in this area is, however, much too limited to draw conclusions.

Tannins

Tannins comprise a complex mixture of higher plant, water-soluble polyphenolic compounds of varying molecular masses that have the ability to react with proteins, polysaccharides and other macromolecules. They tend to be considered antinutritional, because they decrease the digestibility and metabolisable energy of feeds through direct interaction with proteins and carbohydrates from both exogenous and endogenous sources. In ruminants, tannins may be useful in limiting protein degradation in the rumen, thereby permitting more dietary amino acids to flow to the abomasum (McSweeney *et al.*, 2001). In poultry, contrastingly, growth is suppressed by vegetable tannins (Ahmed *et al.*, 1991). Amino acid absorption is compromised by tannins, especially of methionine, histidine and lysine (Mansoori and Acamovic, 2007). High tannin extracts did not alter the mortality of chickens, however they reduced the absorption of minerals such as calcium, magnesium, potassium, sodium and phosphorus from the feed (Hassan *et al.*, 2003). In another experiment tannins extracted from *Vicia faba* seeds increased mortality, reduced body weight, feed intake and poorer feed conversion ratio (Ortiz *et al.*, 1994) as well as decreased the digestibility of protein and the activity of digestive enzymes (Yuste *et al.*, 1992). There do not seem to be reports of beneficial effects of tannins in poultry.

Saponins

Some research has been performed on the application of plant saponins to poultry production, the compounds being recognised as natural detergents. Extracts from two saponins-rich plants, *Yucca schidigera* and *Quillaja saponaria* (Cheeke, 2000; Yeo and Kim, 1997; Preston *et al.*, 1999), had no clear effect on broiler chick performance. Some effects, like increased food intake, body weight gain and energy utilisation were reported in one experiment (Preston *et al.*, 1999), while in another the feed intake was not affected when a 0.2% concentration of the extract was incorporated (Yeo and Kim, 1997). It should, however, be made clear that the claim that it is the saponins present in *Yucca* or *Quillaja* extracts that are responsible for their positive effects has not been justified. These extracts are simply condensed juice pressed from the trunk, in which saponins are one of the dominant groups of compounds present in this matrix. Polysaccharides are also abundant, and their influence on nutritional parameters cannot be neglected. Structurally undefined saponins (75 mg/kg/day) showed positive effects on growth and carcase quality (Miah *et al.*, 2004), while *Balanites aegiptica* kernel saponins tended to reduce body weight in chicks (Nakhala *et al.*, 1992).

Betaine

Although betaine is a generic description of a type of zwitterionic chemical compound, the term used in poultry nutrition refers to a plant extract or a feed additive containing one particular betaine, namely trimethyl glycine. This review covers the effects of betaine only summarily, because a comprehensive review of betaine and poultry was published very recently (Metzler-Zebeli *et al.*, 2009). Betaine is an osmoprotectant present in all plants and particularly abundant in sugar beet and its byproducts. Benefits from its use as a feed additive include the prevention of heat stress and inhibition of *Eimeria* parasitic infection, both presumably due to the ionic nature of the molecule. Its methyl groups are thought also partly to substitute other methyl group donors such as methionine and choline. Both modes of action lead to improved nutrient digestibility and growth performance.

Other plant bioactives

A large group of phytochemicals that are widely distributed in plants is phenolics. Among these, the flavonoids (Figure) are the group which has been indicated as possibly most beneficial for poultry performance. The addition to feed of 300 mg/kg of flavonoids (rutin, hesperidine, quercetin and naringenin) together with mannanoligosaccharides (MOS) had a significant stimulatory effect on feed conversion ratio (Batista *et al.*, 2007). Additionally there was lower meat oxidation both after refrigeration and freezing when birds were fed flavonoids + MOS, which may be attributed to antioxidant effect of flavonoids; quercetine and hesperidine are considered the strongest antioxidants of the flavonoid family (Burda and Oleszek, 2001). Extracts containing isoflavones significantly increased serum testosterone levels in male chickens and decreased serum uric acids and abdominal fat (Zhengkang *et al.*, 2006). Supplementation with daidzein (3 mg/kg/day) significantly increased laying rate, average egg weight and egg cholesterol level in laying hens and ducks (Wang *et al.,* 1994).

Other phenolics of interest in poultry feed supplements are catechins and their complexes, proanthocyanidins, flavolignans, tannins and phenolic acids. Green tea (*Camellia sinensis*) extract containing catechins and their gallates minimised hyperlipidemia and oxidative stress induced by corticosterone treatment in broiler chickens (Eid *et al.*, 2003). Tea also acts as an antioxidant in meat storage. Silimarin, the polyphenolic extract from *Silybum marianum* and *Cynara cardunculus* containing flavolignans, which are strong antioxidants protecting the liver from toxins and pollutants by preventing free radical damage, was fed in broiler chickens at rates of 40 and 80 mg/kg. The treatment with silimarin had no effect on growth performance and had no specific haematoprotective effect, but slightly decreased slaughter yields. The lipid content of breast and thigh was decreased and the resistance of muscles to oxidative stress increased under this treatment (Schiavone *et al.*, 2007). Moreover it was shown that silimarin phytosome can provide protection against the negative effects of aflatoxin B_1 in broiler chicks (Tedesco *et al.*, 2004). Similarly, it protects against pollutants such as carbon monoxide, pesticides and herbicides, by breaking them down from potentially lethal substances into those that are less destructive to the human body. Polyphenol rich grape pomace (peels and seeds) added at the rate of 5–30 g/kg of diet reduced the lipid oxidation in meat during refrigerated storage and increased liver α-tocopherol concentration (Goni *et al.*, 2007). Grape seed extract in the rate of 2.59–5.28% of the feed reduced post mortem development of thiobarbituric acid reactive substances in dark poultry meat but had also detrimental effect on the body weight gain (Lau *et al.*, 2003).

Extracts from sage, thyme and rosemary (5000 mg/kg), rich in rosmarinic acid, another natural antioxidant, had no influence on feed intake and feed conversion, but from 14 to 21 d of age broilers grew faster and improved apparent whole tract and an improved ileal digestibility of nutrients was observed (Hernandez *et al.*, 2004).

An alcohol extract of Propolis (honey bee *glue*) containing polyphenols used at the rate of 50–250 mg/kg diet significantly increased average weight gain, feed consumption and feed efficiency in broiler chicks. The mortality rate determined after 21 d of growth was decreased as compared to the control diet (Khojasteh and Shivazad, 2006).

CURRENT RESEARCH PROJECTS

The global trend to move away from in-feed antibiotics and coccidiostats has strengthened since 2006, when the use of GPA was banned within the EU member states. Therefore research groups and poultry industries worldwide are searching to develop alternatives. Some have performed clinical trials with herbal feed additives.

At present different herbal preparations in poultry feeding are examined for their antibacterial, antiparasitic, antioxidative and/or other health and performance promoting properties like feed intake, feed conversion, body weight, weight gain, growth performance, feed conversion ratio, gizzard function, gut development, nutrient digestibility, digestibility of organic matter and crude protein, gut microflora or content on metabolisable energy of feed mixture.

For example at this time in Austria some companies are working in these fields: Richter Pharma AG www.richter-pharma.at, Delacon Phytogenic Feed Additives www.delacon.com, Indian Herbs GmbH www.indianherbs.at and Biomin AG www.biomin.at. They are conducting clinical trials in feeding herbal additives to different poultry, e g. laying hens, broilers and turkeys. In Australia, PRATU (Poultry Research and Teaching Unit, www.poultryhub.org) is involved in many aspects of poultry science including nutrition and physiology, health and welfare, disease, production and environment and they have funded research projects in all these sectors. There are also other research groups all over the world (both from universities, institutes and companies) as in Germany, United Kingdom, Poland, Finland, Spain, The Netherlands, Turkey, China, Taiwan, India, Pakistan, Ukraine, Lithuania, United States, Canada, Australia, and Brazil which are doing research in these fields of activity. Unfortunately details of these projects are for the most part not publicly available currently.

CONCLUSIONS

The banning of GPA was intended mainly to protect the human population from transmissible antibiotic resistance reaching human pathogens, rendering them refractory to treatment. The benefits of the ban extend to less stress on the environment in general, for example in terms of loss of microbial diversity in soils fertilised with manure from animals receiving GPA (Opalinski *et al.*, 1998). The main downside of the ban was the problems it presented to livestock producers, significantly poultry producers. The examples presented here demonstrate that there is a strong basis for looking to the plant kingdom for solutions to the problems, and indeed for new opportunities to benefit poultry production.

Although this is a lengthy review, it is by no means comprehensive. Plants or their bioactives have production or health benefits across a wide range of effects other than those described here, including fertility (Cerolini *et al.*, 2005), minimising lead concentrations in meat (Hanafy *et al.*, 1994), and relief of heat stress (Rajmane and Sonawane, 1997). We hope that the present review sets a framework for identifying some plant bioactives that hold particular promise for future research and application.

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