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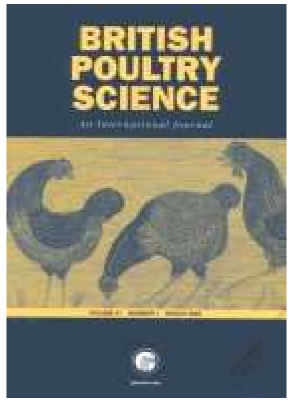

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The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 days to 28 days of age

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RUNNING TITLE: **PLANT EXTRACTS IN BROILER NUTRITION**

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Abstract 1. The effect of the dietary inclusion of 5 culinary herbs or their essential oils on the growth, digestibility and intestinal microflora status in female broiler chicks was assessed. From 7-28 d of age, either a basal control diet without supplement was given or one of 10 others, consisting of the basal diet with either 10g/kg herb (thyme, oregano, marjoram, rosemary or yarrow) or 1 g/kg of essential oil.

2. Body mass (BM) and feed consumption (AFC) were measured on a weekly basis and used to calculate chick performance. Total viable counts of lactic acid bacteria, coliforms, anaerobes and *Clostridium perfringens* were determined at 25 d. Apparent nutrient digestibilities were calculated from the measured values for gross energy, nitrogen (N), dry matter (DM) and organic matter, and sialic acid concentration was also measured.

3. Generally, dietary thyme oil or yarrow herb inclusion had the most positive effects on chick performance, while oregano herb and yarrow oil were the poorest supplements. Only thyme and yarrow in these diets had a different effect when used as an herb or oil on weight gain and BM.

4. Dietary treatment had no effect on the intestinal microflora populations, apparent metabolisable energy (AME) or the calculated coefficients of digestibility. Sialic acid concentration was greatest in the birds given dietary thyme oil, compared with all other treatments except those birds receiving marjoram oil, rosemary herb and the controls. However, less sialic acid was excreted in those birds given diets with oregano or rosemary oils, or oregano herb, than in the controls.

5. Plant extracts in diets may therefore affect chick performance, gut health and endogenous secretions, although the chemical composition of the extract appears to be important in obtaining the optimal effects.

INTRODUCTION

Plants contain an extensive variety of phytochemical compounds with antimicrobial activity (Cowan, 1999), which may have either beneficial or detrimental effects in animals (Panter *et al*., 2004; Wink, 2004; Acamovic and Brooker, 2004), depending on both the compound used and its concentration. The removal of antimicrobial growth promoters (AGP) from poultry diets has triggered a search for suitable natural alternatives, to combat the increased potential for bacterial disease development in growing flocks, especially under conditions of average management quality. The use of various plant materials as dietary supplements, including culinary herbs, may positively affect poultry health and productivity. Essential oils and herbs are used in human aromatherapy for their holistic mode of action. The large number of active components in these supplements may therefore present a more acceptable defence against bacterial attack than synthetic antimicrobials. The main constituents of the herbs and essential oils are terpenes, which are responsible for the bulk of the antimicrobial activity (Charai *et al*., 1996). The oils of *Thymus vulgaris* L. (thyme), *Rosmarinus officinalis* L. (rosemary), *Origanum majorana* L. (marjoram) (Hammer *et al*., 1999), *O. vulgare* subsp. *hirtum* (oregano) (Hammer *et al*., 1999; Dorman and Deans, 2000) and *Achillea millefolium* L. (yarrow) (Candan *et al*., 2003) have *in vitro* antimicrobial properties. Yarrow has also been reported to reduce the effects of viruses and helminths (Cowan, 1999). However, there is only limited evidence for a bioactive effect of herbal compounds in live poultry. It is unknown whether the inclusion of terpenes in different forms, *i.e.* as solid herb material or as the extracted oil, would have the same antimicrobial or growth promoting effects *in vivo*.

The present experiment aimed to assess the suitability of a number of culinary herbs; thyme, marjoram, rosemary, oregano and yarrow, and their extracted oils as dietary supplements for poultry. The effects of these herbs and oils on the growth, nutrient utilisation and intestinal microflora in growing broiler chickens were determined.

MATERIALS AND METHODS

Provision of plant supplements and extraction of essential oils

The oregano (*O. vulgare* subsp. *hirtum*), marjoram (*O. majorana* L.), rosemary (*R. officinalis* L.) and thyme (*T. vulgaris* L.) were purchased commercially as dried herb supplements (Green City, Glasgow, UK) in early 2001. Yarrow (*A. millefolium* L. var. Alba) seeds were selected in Slovakia and imported to the UK, where the yarrow plants were grown in experimental plots at SAC Ayr (Dr K. Svoboda). The flowering heads of the yarrow plants were collected in the summer of 2000 and dried on the day of harvest in an oven at 40˚C. Essential oil was distilled from the ground plant material using Clevenger distillation apparatus in accordance with BS 4585 (1985), following British Pharmacopoeia methods. The oil samples were stored in sealed glass vials below 4˚C until required.

Birds and housing environment

The experiment was subjected to an assessment for its ethical acceptability and approved by the Animal Experimental Committee (AEC) within SAC. The birds were housed in cages in an environmentally controlled room. Heating was provided by a single gas brooder, where the initial temperature (study d 0) was set at 32°C and decreased linearly by 0.5°C per d to a final temperature of 21°C at 21 d (d 21). Supplementary heating was provided as required by mobile butane gas heaters. The birds were provided with 1 h of darkness following a period of 23 h light, from the start to the finish of the study. Each cage was provided with a single feed trough and nipple line, with 3 nipples per cage, to provide *ad libitum* access to feed and water.

Experimental design

The chickens (165 Ross 308 female birds) were placed at d-old in a single colony in a floor pen containing fresh wood shavings to a depth of 10 cm, and all were fed on a starter ration. At 7 d old, 5 replicate cages containing 3 chicks selected at random were assigned to each of 11 dietary treatments using a randomised block design, giving a total of 55 experimental cages. The study was completed at 28 d of age. Body mass (BM) was measured on an individual basis when the birds were 7, 14, 21 and 28 d of age. The average feed consumption (AFC) was determined on a weekly basis from 7-28 d. The feed conversion efficiency (FCE) per cage was derived from the average weight gain (AWG) and AFC per bird on a weekly basis. The treatment diets contained titanium dioxide (TiO_2) as an indigestible marker and excreta samples from each cage were collected on d 25. These samples, along with samples of each diet, were stored at -20°C until required for laboratory analysis. Excreta samples were then freeze-dried prior to analysis. At the end of the study, a bird from each cage was randomly selected and killed using 1 ml/kg BM of sodium pentobarbitone (euthatal), to allow caecal contents collection.

Diet formulation

The basal starter ration was formulated to be marginal in energy and nutrients and also low in tocopherol (<50 mg/kg), in order to provide the birds with slightly sub-optimal conditions for growth. This diet contained mainly wheat and soybean meal, and was manufactured at SAC as a mash (Table 1). The dietary treatments therefore comprised the basal diet only (control), or, alternatively, the basal diet supplemented with one of the 5 herbs (10 g/kg) or one of the distilled essential oils from these herbs

(1 g/kg), resulting in 11 dietary treatments in total. Since the herbs were assumed to contain up to about 100 g oil/kg it was decided to include the oils in the diets at 1 g/kg and the herbs at 10g/kg thus ensuring the presence of adequate amounts of the terpenes in the diets. All the birds were given the control diet from d 0-6 of the experiment, and then either the control diet on its own or one of the 10 supplemented diets from d 7-29. During mixing, each plant herb was incorporated directly on a weight for weight basis into the dry mix of the diet. The essential oils were first mixed into the vegetable oil component of the ration, and then the oil mixture was added to the basal ration. No in-feed antimicrobials, anti-coccidial drugs or feed enzymes were included.

Table 1 near here

Analytical methodology

Enumeration of microbial populations in the caecal and faecal contents of the chickens was performed using various dehydrated media (Oxoid Ltd., UK), which were prepared and sterilised according to the manufacturer's instructions before being poured into sterile Petri dishes. The media used included MRS (DeMan, Rogosa, Sharpe), Wilkins Chalgren anaerobe agar (with 10% defibrinated horse blood), MacConkey agar No. 3 and Perfringens OPSP (Oleandomycin-polymixin-sulphadiazine-perfringens). Caecal and faecal samples were serially diluted in Maximum Recovery Diluent, and all plates were inoculated according to the method of Miles and Misra, (1938) except those containing Perfringens OPSP agar. The Perfringens OPSP agar plates were inoculated using 1ml bacterial suspensions by means of the pour plate method. The MRS and WCA plates (48 h) and perfringens OPSP plates (24 h) were incubated anaerobically at 37˚C, while MacConkey plates were incubated for 24 h aerobically at 37˚C.

The dry matter (DM) and organic matter (OM) contents of each diet and of the excreta samples collected from each cage were determined according to BS 5766, Parts 1 and 8 (1983). Gross energy was determined by adiabatic bomb calorimetry (Autobomb A. Gallenkamp and Co Ltd., England) in accordance with BS 1016, Part 105 (1992), using a benzoic acid standard (VWR Ltd., UK), with a known heat capacity. Nitrogen contents were determined using the Dumas combustion method (Leco FP-428/328, St. Louis, USA). The concentration of titanium dioxide in diet and excreta samples were determined according to the method of Short *et al*., (1996), and sialic acid concentrations in excreta samples according to Jourdian *et al*., (1971). The condensed tannin (CT) contents in the herb samples were measured by the butanol/HCl method, following Makkar, (2003).

Statistical analysis

This experiment was designed as a 5 X 2 factorial experiment, incorporating a non-supplemented control, to give a total of 11 treatments. The statistical analysis was carried out using a balanced randomised block ANOVA in General Linear Model (GLM), using Fishers Least Significant difference (LSD) test to separate means (Genstat, Release 7). A factorial analysis of the experiment was enabled when the term Cont/(herb_oil*type) was entered into Genstat, allowing the separation of effects due to plant species and due to the supplementation of herbs or essential oils.

RESULTS

Diet compositional analysis

The proximate analysis carried out on treatment diets showed that there was no difference in the nutritional composition of the dietary treatments. The dietary DM concentration ranged from 882-890 g/kg over all treatments and N content was between 198-214 g/kg. Ash content ranged from 64-75 g/kg DM in all treatment diets, and the GE of the treatment diets was within the range 18.06-18.63 MJ/kg DM. The OM content in each treatment diet varied from 925-936 g/kg DM. The content of extractable CT in the oregano herb supplement was 16 g/kg, while each of the other herb supplements contained <1 g/kg CT. The terpene compositions in the essential oils were determined by gas chromatography: marjoram oil contained (g/kg of oil) carvacrol (560), terpinene-4-ol (70) and *trans*-cinnamaldehyde (60), oregano oil contained γ-terpinene (170), 1,8-cineole (60) and an unidentified terpene, thyme oil contained α-terpineol (297), thymol (100) and carvacrol (70), rosemary oil contained primarily 1,8-cineole (470), camphor (180) and α-pinene (110), and the yarrow oil contained β-pinene (180), linalol (170), sabinene (100) and α-terpineol (60).

Effect of herbs and essential oils on broiler growth performance

In this experiment, 2 chicks died in the first week from different dietary treatments. Generally, those birds given diets including thyme oil and yarrow herb had the greatest overall body mass (BM), average gain, average feed consumption and FCE in the study, compared to the other dietary treatments (Table 2). The average weight of chicks at 7 d of age was 79.2 g (standard deviation, SD=0.982), which was low, but a large proportion of these birds weighed less than 30g on arrival at d of hatch and the diets were in mash form, which may have subsequently reduced overall weights achieved in the trial. No oil-herb interactions were observed between supplements at any stage of this experiment. In each case, the differences described are for individual plant supplements in comparison with each other or the control. Only the birds given diets with thyme oil had a greater BM than those fed on the control diet (d 21, $P<0.01$; d 28, $P=0.001$). There was also a differential effect in feeding essential oil and herb supplements from some plant species on BM. The birds given yarrow herb in their diet had a greater BM than those receiving yarrow oil (d 21, $P<0.01$; d 28, $P=0.001$), whereas those birds given thyme oil in their diet had a greater BM than those with thyme herb supplements (d 21, $P<0.01$; d 28, $P=0.001$). The birds fed on diets with thyme oil and yarrow herb also had the largest overall weight gain (AWG) over the study period, although only thyme oil was significantly greater than the control treatment (15-21 d, $P<0.01$; 7-28 d, $P\leq0.001$). Differences were again observed between oil and herb supplements from the same plant, with a higher AWG in birds receiving dietary thyme oil rather than thyme herb supplements (15-21 d, $P<0.01$; 22-28 d and 7-28 d, both $P\leq0.001$) and in those birds receiving yarrow herb rather than yarrow oil supplements (15-21 d, $P<0.01$; 22-28 d and 7-28 d, both $P\leq0.001$). Average feed consumption (AFC) was reduced for the birds given dietary oregano herb, when compared with those receiving the control treatment diet over the trial period ($P<0.05$). The birds fed on diets with thyme oil consumed more feed than the control birds, but this was not significant. Inclusion of rosemary oil in the diets reduced the AFC, when compared wit those birds given the control treatment (22-28 d, $P<0.01$; 7-28 d, $P<0.05$). The AFC was lower in the birds given diets containing thyme herb when compared with those containing thyme oil (15-21 d, $P\leq0.05$; 22-28 d, $P=0.006$, not shown). However, the birds given supplementary rosemary herb in the diet had higher AFC than those fed on diets with rosemary oil (22-28 d, $P<0.01$; 7-28 d, $P<0.05$), and those fed on diets with yarrow herb had a higher AFC than those given diets containing yarrow oil (22-28 d, $P<0.01$) in the study. During the study period overall (7-28 d), rosemary oil had a positive effect on chick FCE compared with rosemary herb, whilst yarrow herb also had a positive effect compared with yarrow oil (7-28 d, $P\leq0.05$).

Table 2 near here

Effect of dietary inclusion of herbs on intestinal microbial status

The bacteriological data required transformation before statistical analysis. There were no effects of treatment on the $\log_{10}$ population counts of the main intestinal microflora species, when the birds were sampled at 28 d of age (Table 3). The calculated lactic acid: coliform ratios of the original bacterial counts were transformed prior to statistical analyses and were not affected by dietary herbal supplementation; nor were the proportions of total lactic acid bacteria, total coliforms or *Clostridium perfringens* in relation to total anaerobes (Table 4).

Tables 3,4 near here

Effect of herbs and essential oil supplementation on nutrient digestibility

The apparent digestibility coefficients of DM (ADMD), OM (DOMD) and the apparent metabolisability of nitrogen (AMN) and the apparent metabolisable energy (AME) and also AME corrected to zero nitrogen retention (AME_N), contents were determined. The digestible OM content and also metabolisable nitrogen content in the treatment diets were calculated. The metabolisability of the dietary energy (AME:GE) was also determined. Concentrations of sialic acid in broiler excreta were used to calculate sialic acid excreted/kg food intake for the birds as a measure of endogenous losses.

No treatment effects were observed with any plant supplement within the diets in relation to the nutrient digestibility (Table 5). As a whole, the calculated nutrient digestibility content and coefficients were rather low in these diets when compared with commercial values. However, the low values for dry matter and organic matter digestibility are in accordance with the low values for AME and AME_N. One treatment replicate of the birds fed the diet with yarrow essential oil was removed as a statistical outlier (>3.5 standard deviations from mean) in the analysis of the coefficient of AMN and also the metabolisable N content in the diet.

This bird was measured with an abnormally low concentration of TiO_2 in the excreta and an error was assumed. In the adjusted dataset, there was a tendency (P=0.07) for the chicks to utilise nitrogen less effectively from the diet with rosemary herb when compared to the others, which resulted in a lower dietary metabolisable N content.

Sialic acid was measured to assess the effects of treatment on endogenous losses. Dietary treatment did not influence the excretion of sialic acid, whether this was expressed as concentration in the excreta, per kg of diet intake or related to metabolic liveweight.

Table 5 near here

DISCUSSION

These plant supplements are common dietary additives for humans, and were chosen for their non-toxic chemical composition, relatively low cost and easy availability. Phytochemical supplements are known to have considerable variability in their chemical composition. With the exception of oregano, the composition of each essential oil used in this experiment was consistent with those described in the literature. Oregano and thyme oils are usually composed of the monoterpenes thymol and carvacrol in varying proportions (Daferera *et al.*, 2000). Neither thymol nor carvacrol were present in the oregano oil used in this study, which they may have originated from senescent plant tissue since it had a similar composition (Russo *et al.*, 1998). Terpene compositions in peppermint plants are known to change over time (Burbott and Loomis, 1969), which may be important when selecting the best plant supplements for use in poultry feeding trials. Although the mechanisms behind terpene interactions are unknown, synergistic (Cox *et al.*, 2001) or antagonistic interactions in a plant extract may affect its antimicrobial potential. The diet with oregano herb also contained 0.16g of extractable CT/kg, which is very low, but the reduction in AWG by 10-13% in birds fed this diet over the experimental period compared with the control birds, suggests that there may have been bound tannin which may have had some influence as well as other components of the herb (Oduguwa *et al.*, 2007). This reduction in AWG is similar to that reported when tannic acid and sorghum were fed in chick diets (Dale *et al.*, 1980). The affinities of CT compounds for proteins and carbohydrates have been well characterised, and those in this oregano herb may have bound to other dietary substrates, thereby reducing nutrient availability (Bento *et al.*, 2005; Oduguwa *et al.*, 2007). The measurement of chemical composition in a plant supplement prior to its use may prevent reduced performance in broiler chicks.

Effects on bird performance

The different herbs and essential oils had variable effects on chick performance. Both the oregano herb and oil treatments were among the poorest dietary treatments, and were associated with reduced values for AFC and AWG in these chicks. Orego-stim is a commercial supplement based on oregano, and has been shown to improve AWG and FCR in pigs (Gill, 1999). However, no effect of Orego-Stim or oregano has been reported on performance in growing broilers (Botsoglou *et al.*, 2002; Lewis *et al.*, 2003), or turkeys between 12 and 16 weeks of age (Papageorgiou *et al.*, 2003). Orego-Stim has reduced the incidence of coccidiosis in chickens (Gill, 1999), which may indicate a protective effect of this supplement. In this study, the inclusion level of essential oil was higher than in previous experiments (Botsoglou *et al.,* 2002; Papageorgiou *et al.,* 2003), and was around 10 times that used in the diets fed to the pigs. Although oregano oil is a potent antimicrobial, these studies indicate that oregano in the diets of broiler chicks may not improve growth, and may even restrict it if the oil quality is less than optimal. Broiler AFC and BM has been increased in birds fed diets with Orego-stim without an effect on FCR, when fed in combination with a vaccination programme against coccidiosis (Waldenstedt, 2004), but the vaccination programme may have produced the bulk of the effect. The increased rates of intestinal transit in chickens may not allow for the same performance response as that observed in pigs, especially if microbial challenge is low.

In this experiment, the birds given diets with thyme oil performed best, although thyme oil in the diet did not affect performance in male Wistar rats (Youdim and Deans, 1999). Blends of essential oils have improved broiler performance when given as dietary supplements (Suk *et al.*, 2003; Hernandez *et al.*, 2004). In other studies, only very limited information is available concerning the composition of the blend. Blended supplements containing carvacrol, capsaicin and cinnamaldehyde have improved both BM and FCR in broilers at 21 d of age, to a greater extent than avilamycin (Jamroz *et al.*, 2003). Yarrow appears to be more effective when fed in these study diets as a herb, which may be due to the precursors of the sesquiterpene chamazulene, which is present in the herb but not the oil, or another phytochemical component. Lewis *et al.* (2003) observed an improved growth rate and AFC in broilers with yarrow herb inclusion in diets between 18 and 36 d of age. The dietary inclusion of yarrow at 30 g/kg improved AWG in broiler chicks (Fritz *et al.*, 1993). In diets diluted by 10% in energy provision, yarrow herb inclusion increased AFC and tended to increase AWG in broilers (Lewis *et al.*, 2004). Lewis *et al.* (2004) observed no positive effects of yarrow inclusion when the diets were not diluted, and concluded that the benefits of yarrow inclusion may be dependent on dietary quality. These experimental diets had a reduced compositional quality, which may have optimised the effects of yarrow herb, but these results suggest that both thyme oil and yarrow herb have potential as dietary supplements in growing poultry.

The form of herbal supplementation may also be influential in determining bioactivity. The present paper indicates that the terpene composition within essential oil and herb extracts from the same plant, or the presence of additional secondary plant compounds, may result in differential effects for some supplements on broiler performance. Only marjoram supplements produced an equivalent effect on AWG over the study, irrespective of whether the herb or the oil was included in the diet, which suggests that the chemical composition of both in this marjoram may be similar. The marjoram used in this study contained a high proportion of carvacrol. Carvacrol has reduced both AFC and AWG when included in broiler diets at 200 mg/kg (Lee *et al.*, 2003a). Thymol and carvacrol have been reported to differ in their effects on bird performance and lipid metabolism (Lee *et al.*, 2003a), which is surprising, as the two terpenes are isomers with identical chemical formulae. Complex interactions appear to exist between the terpenes in essential oils and herbs, and may also occur between terpenes and the remainder of the dietary ration. As the herbs used in this experiment were not composed of pure terpenes, the yarrow herb should contain compounds that are either absent in the oil or are damaged during extraction. It is possible that the reduced quality of this oil may have resulted in an overly negative perception of yarrow oil as a supplement. These compounds were purchased commercially, so any effects relating to the drying process are unknown.

Active terpenes in plants may be trapped within secretory gland structures, as suggested by Dorman and Deans (2000), which may favour the antimicrobial activity of essential oils rather than herbs. However, sage herb has shown a greater inhibitory effect against bacteria than sage oil in a meat broth medium (Shelef *et al.*, 1984). In future, milling the herb fraction may release the active terpenes more effectively into the gut where digestion and absorption are taking place. In this study, all herbs were fed as chopped leaf material, apart from yarrow, which came as complete flower heads and was then milled. The herbs were not milled as it was considered that any volatile components would be lost through vaporisation. In an *in vitro* study, extracted terpinene-4-ol has been reported to have a higher bioactivity separately than as part of an essential oil (Cox *et al.*, 2001), suggesting that separate terpenes may be the most bioactive when suitable compounds are identified.

Effect of essential oils on digestibility

In order to allow the effects of the herbs and oils to be more clearly observed, these experimental diets were formulated to be marginal in their energy and protein provision, which should at least partly explain the slow growth and poor digestibility values in these birds. The wheat used in this experiment was of a lower quality than expected, as the determined AME value in the excreta was about 3 MJ lower than the formulated dietary ME content. A reduced protein digestibility when diets with low AME were fed (Choct *et al.*, 1995), and an increased starch digestibility when AME is increased has been reported in poultry (Svihus and Hetland, 2001). Arabinoxylan and lectins can also be associated with poor quality wheat samples. The birds were also fed on mash instead of pelleted diets; mash is less dense in energy and have a greater bulk. This was done to allow easy incorporation of the plant supplements. No dietary enzymes were included, as some may have an independent antimicrobial effect (Fuglsang *et al.*, 1995), and the reduced vitamin E concentration will further have reduced dietary feeding value. Thymol and carvacrol have a known antioxidant activity *in vitro* (Deighton *et al.*, 1993). Previously, antioxidant effects have been demonstrated in poultry given dietary oregano, but this has not been associated with any effect on bird performance (Botsoglou *et al.,* 2002; Papageorgiou *et al*., 2003).

Exogenous xylanases in wheat diets have improved broiler performance by reducing arabinoxylan contents, improving AME and nutrient digestibility, reducing variation between birds and increasing the rate of dietary passage by an associated reduction in the digesta viscosity (Almirall and Esteve-Garcia, 1994; Choct *et al*., 1995). A reduced intestinal viscosity is associated with a reduction in the amount and water holding capacity of NSP (Choct *et al*., 1995), and enzymes are most efficacious in younger chicks (Almirall and Esteve-Garcia, 1994). A carbohydrase in future experimental diets may be beneficial to ameliorate any anti-nutrient effects.

Herbs and spices have traditionally been used to stimulate the production of endogenous secretions in the small intestinal mucosa, pancreas and liver, and thus aid digestion. Plant spices, when fed together with organic acids, have stimulated proteolysis, by lowering the pH to the optimal level for pepsin activity (Kamel, 2001). In rats, the dietary inclusion of spices has increased the endogenous secretion of enzymes, bile acids and pancreatic juice (Platel and Srinivasan, 2000, 2001 and 2003), and reduced the transit time for digesta passage (Platel and Srinivasan, 2001). Some spices were observed to reduce endogenous secretions, without an effect on food consumption (Platel and Srinivasan, 2000). An increased amylase activity in intestinal chyme was observed with dietary inclusion of Crina® Poultry, an essential oil blend, at 21 d of age in broilers, but the effect decreased with increasing age (Lee *et al*., 2003b). In the present experiment, the herb and oil supplement had no effect on sialic acid excretion suggesting that endogenous losses were not a major factor associated with the dietary supplements. It is possible that terpene and phenolic compounds are excreted as a conjugate with other components, which may increase the endogenous losses from the bird and reduce the utilisation of nutrients (Cowieson *et al*., 2006; Mansoori and Acamovic, 2007; Oduguwa *et al*., 2007).

Effects of herbal supplementation on the intestinal microflora

The results in the present study were disappointing, with no clear effects observed on the intestinal microflora populations, which may have been due to an insufficient degree of replication since only 3 of the 5 treatments could be sampled within the time frame of 24 h. Thyme, marjoram and rosemary inclusion in these diets reduced the numbers of caecal *Cl. perfringens* by >1 log, a trend which may potentially be significant under a different experimental methodology. It is possible that the birds should be fed on the test diets from the day of hatch, to influence the intestinal bacterial composition more completely. A decreased exposure time to the air may have influenced the bacterial population but unfortunately, the samples had to be transported before analysis.

Several studies have reported effects on intestinal microflora when herbs and essential oils have been included in broiler diets. The dietary supplementation of XTRACT, an encapsulated product containing capsaicin, carvacrol and cinnamaldehyde, reduced the numbers of *E. coli* and *Cl. perfringens* in broiler rectal contents to the same extent as avilamycin (Jamroz *et al.,* 2003). *Cl. perfringens* has been reduced in number when the blended essential oil supplement CRINA®poultry, was fed in poultry diets (Losa and Kohler, 2001; Mitsch *et al*., 2002). Selective inhibitions in the growth of the hyper-ammonia-producers *Cl. sticklandii* and *Peptostreptococcus anaerobius* were observed when a blend of dietary terpenes containing thymol, eugenol, vanillin and limonene were fed to ruminants (McIntosh *et al*., 2003). The growth of *E. coli* and *C. perfringens* was reduced in broilers, when blends of essential oils were fed in industry trials (Losa and Kohler, 2001; Tucker, 2002), while numbers of *Lactobacillus* spp. increased (Tucker, 2002). Thus, essential oils may act differently compared with synthetic antimicrobials, which tend to depress bacterial numbers across species.

In this study, the caecal coliform populations in the birds given any of the herbal treatments compared to those fed the control diet were higher, which may suggest that these dietary herbs may select more against Gram-positive rather than Gram-negative bacteria. Conventional antimicrobials used in broiler production have preferentially targeted Gram-positive bacteria, especially *C. perfringens* (Engberg and Petersen, 2001). If this is true, conditions suitable for the presence of *Campylobacter* spp. or *Salmonella* spp. may exist in birds fed on diets supplemented with herbs. If terpenes bind to dietary components rather than disable bacteria, and are then released as dietary components are absorbed, the pH in the distal tract may be decreased, thus preventing the growth of pathogenic bacteria. Each herb contains an extensive variety of phytochemicals, all with variable bioactivity, so the use of herbal combinations in poultry diets may be most effective when suitable terpenes have been identified. In broiler chicken production, the cage environment has been shown to be best in reducing populations of both helminth (Permin *et al.*, 1999) and red mite (Höglund *et al.*, 1995), whereas floor litter provides a substrate for pathogenic bacterial growth (Pope and Cherry, 2000). It is concluded that responses to phytochemicals may be greater in a more challenging environment, in agreement with Lee *et al.*, (2003b).

This study has demonstrated that various herbs and oils have different effects on broiler performance, which may be primarily related to differences in their terpene composition. The inclusion of a single herb or its extracted essential oil in diets may not always have a similar effect on broiler performance, therefore it is necessary to measure the chemical quality in a plant extract to identify optimal compositions of secondary plant compounds for future reference. Dietary quality and environmental conditions are likely to be important in the assessment of responses to terpene inclusion. In conclusion, herbs and essential oils may influence both the performance and the production of endogenous secretions in broilers.

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Table 1. *Diet formulation and calculated chemical composition of the basal ration (as fed)*

Feed Ingredient	*Amount in diet (g/kg)*	*Calculated chemical composition*	*(g/kg)*
Wheat	671.0	ME (MJ/kg)	13.4
Soya bean meal	251.0	Crude Protein	201.6
Soya oil	35.0	Ether Extract/Fat	52.8
Monodicalcium phosphate	15.0	Crude Fibre	27.8
Limestone	15.0	Calcium	9.6
Sodium chloride	3.0	Phosphorus	7.5
Lysine	2.0	Lysine	12.7
Methionine	3.0	Methionine + Cysteine	8.9
Vit/ Min premix[1]	5.0		

Diets were also mixed with TiO_2 (5 g/kg) as a dietary marker and with the plant supplements.

[1]Supplied per kg diet: vitamin A 4128 µg (retinyl acetate), vitamin D3 125 µg cholecalciferol, vitamin E (as α-tocopherol) 50 mg, vitamin K 3 mg, folic acid 1mg, nicotinicacid 50 mg, vitamin B1 (Thiamine) 2 mg, vitamin B2 (Riboflavin) 7 mg, vitamin B6 (Pyridoxine) 5 mg, vitamin B12 15 µg, biotin 200 µg, calcium pantothenate 15 mg, iodine 1mg, molybdenum 0.5 mg, selenium 200 µg, cobalt 0.5 mg, copper 10 mg, iron 80 mg, manganese 100 mg, zinc 80 mg, limestone 4.18 g.

Table 2. *Effect of herbs or their essential oils on growth performance, when included in broiler chick diets from 7-28 d of age*

	Chick growth performance data from 7-28 d			
Treatment	*BM at 28d*	*Average gain (g/bird/d*	*Average feed cons (g/bird/d)*	*FCE (gain/cons)*
Control	727^{bcde}	30.9^{bcdef}	53.1^{ab}	0.588^{abc}
Marjoram herb	754^{abcd}	32.1^{abcd}	53.5^{ab}	0.602^{ab}
Oregano herb	645^{e}	26.9^{ef}	45.1^{c}	0.604^{ab}
Rosemary herb	734^{abcde}	31.2^{abcdef}	53.9^{ab}	0.580^{bc}
Yarrow herb	789^{ab}	33.8^{ab}	55.5^{ab}	0.610^{ab}
Thyme herb	691^{cde}	29.2^{cdef}	52.0^{abc}	0.564^{bc}
Marjoram oil	764^{abc}	32.6^{abc}	55.2^{ab}	0.594^{ab}
Oregano oil	680^{cde}	28.6^{cdef}	47.9^{bc}	0.594^{abc}
Rosemary oil	664^{de}	27.9^{def}	44.6^{c}	0.634^{a}
Yarrow oil	641^{e}	26.8^{f}	49.0^{bc}	0.544^{c}
Thyme oil	821^{a}	35.3^{a}	58.7^{a}	0.600^{ab}
sed	45.14	2.2	3.9	0.0277
Plant species (herb+oil)	P=0.021	P=0.021	P=0.015	NS
Herb v Oil	P=0.001	P=0.001	P<0.05	P<0.05

Means were tested at *P*<0.05, but the largest treatment differences were significant to the level shown.

Means within a column without a common superscript differ at *P*<0.05 (NS: *P*>0.05).

Values are means for 5 replicates of each treatment.

695 **Table 3.** *Bacterial population counts in samples of faecal and caecal material taken*

696 *from broilers aged 28 d, when birds were fed diets with herbs from 7-28 d*

697

	Microfloral populations in samples from the intestinal contents of broilers (log_{10} c.f.u./g)							
	Coliforms		*Lactic Acid Bacteria*		*Total Anaerobes*		*C. perfringens*	
Treatment	Faecal	Caecal	Faecal	Caecal	Faecal	Caecal	Faecal	Caecal
Control	8.91	6.94	8.91	8.33	9.42	11.24	3.54	3.94
Marjoram	8.79	7.25	8.67	8.68	9.06	10.98	4.19	3.61
Oregano	8.83	7.50	8.21	8.27	9.13	11.36	3.77	3.57
Rosemary	8.62	7.24	8.51	8.15	9.21	11.12	2.41	2.80
Yarrow	8.60	8.40	8.76	8.63	8.62	10.99	2.57	2.00
Thyme	8.32	7.82	8.74	8.05	9.21	11.15	3.68	2.27
sed	0.70	0.78	0.49	0.69	0.35	0.33	0.76	0.75
	NS	NS	NS	NS	NS	NS	NS	NS

698 Data comparisons are done within each column and were statistically compared after transformation.

699 NS (P>0.05).

700 Values are means in colony forming units per g sample of the logarithms of 3 treatment replicates.

716

717 **Table 4.** *Proportions of bacterial species in relation to each other in broiler chicks*

718 *aged 28 d, when the birds were fed diets with plant herbs from 7-28 d of age*

719

Treatment	*LAB: Coliforms*		*Coliforms: Anaerobes*		*LAB: Anaerobes*		*C. perfringens: Anaerobes*	
	Faecal	*Caecal*	*Faecal*	*Caecal*	*Faecal*	*Caecal*	*Faecal*	*Caecal*
Control	1.000	1.217	0.947	0.618	0.947	0.742	0.373	0.350
Marjoram	0.987	1.206	0.970	0.661	0.957	0.794	0.462	0.332
Oregano	0.933	1.117	0.967	0.661	0.901	0.727	0.412	0.314
Rosemary	0.992	1.124	0.936	0.651	0.925	0.734	0.263	0.252
Thyme	1.077	1.061	0.901	0.700	0.949	0.722	0.403	0.203
Yarrow	1.020	1.028	1.001	0.765	1.020	0.785	0.296	0.185
s.e.d.	0.095	0.150	0.059	0.068	0.044	0.069	0.081	0.068
	NS	NS	NS	NS	NS	NS	NS	NS

Proportions of intestinal microflora species in broilers (spanning header above all data columns)

720 Significance comparisons are done within each column and statistically analysed after transformation.

721 NS (P>0.05).

722 LAB = Lactic acid bacteria.

723 Values are proportions of 3 replicate sample means for each count measured in $\log_{10}$ c.f.u./g.

724

Table 5. *Effect of dietary herb or essential oil supplementation from 7-28 d of age on nutrient digestibility in broiler chicks aged 25 d, measured as apparent metabolisable energy (AME) and the coefficient of energy metabolisability (AME:GE), with correction to zero nitrogen retention (AMEn and AMEn:GE), and also the coefficients of the digestibility of dry and organic matter (ADMD and DOMD) and nitrogen metabolisability (AMN) in the ration, presented with the digestible OM and metabolisable N content in each treatment diet*

	Energy and its metabolisability, dry and organic matter digestibility and the metabolisability of nitrogen in the treatment rations									
	AME	*AMEn*	*AME:GE*	*AMEn:GE*	*ADMD*	*DOMD*	*Digestible OM content*	*AMN*	*Metabolisable N content*
Treatment	*(MJ/kg DM)*		*(MJ/MJ)*	*(MJ/MJ)*	*(g/g)*	*(g/g)*	*(g/kg DM)*	*(g/g)*	*(g/kg DM)*
Control	10.46	9.96	0.567	0.579	0.534	0.574	531	0.405	13.61
Marjoram herb	10.39	9.89	0.568	0.583	0.539	0.578	536	0.399	13.64
Oregano herb	10.59	10.14	0.572	0.592	0.538	0.580	540	0.371	12.45
Rosemary herb	10.11	9.76	0.550	0.574	0.515	0.562	526	0.307	9.72
Yarrow herb	10.78	10.26	0.582	0.608	0.544	0.589	547	0.421	14.29
Thyme herb	10.34	9.94	0.558	0.588	0.516	0.558	520	0.331	10.89
Marjoram oil	10.05	9.56	0.556	0.570	0.526	0.568	527	0.382	13.25
Oregano oil	10.65	10.21	0.576	0.607	0.536	0.573	534	0.359	11.96
Rosemary oil	10.59	10.12	0.576	0.607	0.539	0.576	533	0.377	12.89
Yarrow oil	10.31	9.96	0.553	0.569	0.523	0.561	525	0.365	11.49
Thyme oil	10.63	10.15	0.572	0.593	0.539	0.579	540	0.386	13.12
sed 1	0.553	0.498	0.030	0.036	0.029	0.029	27.09	0.045	1.556
sed 2								0.048	1.651
Plant spp. (herb+oil)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Herb v Oil	NS	NS	NS	NS	NS	NS	NS	NS	P=0.07

Values are the means of 5 replicates for each dietary treatment.

Data comparisons are done within each column. NS (P>0.05)

For most treatment comparisons, sed 1 is used, but sed 2 should be used for nitrogen metabolisability, when comparing yarrow oil with all other treatments.

732