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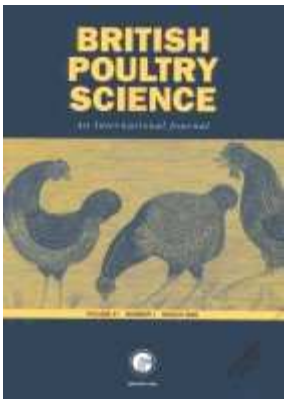

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Herbs, thyme essential oil and condensed tannin extracts as dietary supplements for broilers, and their effects on performance, digestibility, volatile fatty acids and organoleptic properties

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Abstract 1. Herbs, thyme essential oil (EO) and condensed tannin (CT) extracts were compared for their effects, as dietary supplements, on broiler growth performance, nutrient digestibility and volatile fatty acid (VFA) profiles in the gut. Cooked meat from the birds fed on diets with 4 herbs and an EO extract was compared by a taste panel against those fed on the control treatment, for organoleptic properties in the meat.

2. Female broiler chicks were fed on wheat-soybean meal diets from 0-42 d of age. These chicks were given either the basal diet (control), or the basal diet with one of rosemary, garlic or yarrow herbs, mimosa, cranberry or grapeseed CT's, or thyme EO supplements (8 treatments in total). Body weight (BW) and feed consumption (FC) were measured.

3. The garlic supplement tended to improve growth rate over the first 7 d, while mimosa CT and thyme EO supplements reduced weight gains. The mimosa supplement in diets significantly reduced FC to d 21. Meanwhile, the addition of a cranberry supplement reduced the digestibility of DM, OM and N, compared with the controls. Dietary thyme EO, yarrow, rosemary and garlic supplements significantly modified caecal isovaleric and isobutyric acid proportions, but not other VFA. Dietary herb supplements significantly affected the intensity of meat flavour, and the potential of observing both garlic and abnormal flavours. There were large differences between the consumption of red and white meat samples, while meat temperature affected several flavour attributes.

4. Broiler performance and digestibility for birds given dietary garlic and grapeseed CT supplements were similar to the controls, and these supplements appear suitable for dietary inclusion. Careful choices are necessary when selecting dietary plant extract supplements for broilers, but beneficial effects can be observed.

INTRODUCTION

Previous work (McEwan *et al*., 2002; Mitsch *et al*., 2004) suggested that plant extracts may be included in poultry diets as bioactive supplements. Plant extracts may have potential as

natural alternatives to antimicrobial growth promoters (AGP) in animal diets, but their effects on gut health and broiler performance have not been clearly established. Garlic (*Allium sativum* L.) is noted for its bioactive properties, which include antimicrobial (Benkeblia, 2004), anti-fungal (Pai and Platt, 1995) and antioxidant (Yin and Cheng, 1998) effects. Tannins have substantial effects on microbial populations and activity (Bento *et al*., 2005). Cultivars with varying amounts of tannins also have effects on nutrient digestibility (Elkin and Rogler, 1990), and different chemical structures in tannins may have varying effects on digestibility; sometimes negative (Mansoori and Acamovic, 2009). The present experiment used three purified condensed tannin (CT) extracts to assess the potential of different tannin types on nutrient digestibility. Various EO's distilled from culinary herbs, for example thyme EO, have antimicrobial properties (Viuda-Martos *et al*., 2008). Various antimicrobial (Candan *et al*., 2003) and bioactive properties (Chandler *et al*., 1982) have been described for yarrow. This experiment aimed to assess the effect of herbs, thyme EO and CT's as dietary supplements, on broiler performance, nutrient utilisation and gut microflora. The experiment also aimed to assess the effect of dietary herb and thyme EO supplements for broiler chickens on the organoleptic properties of the cooked meat.

MATERIALS AND METHODS

Dietary supplements

Yarrow (*Achillea millefolium* L. var. Alba) seeds were selected in Slovakia and the plants grown in experimental plots at SAC Ayr (Dr K. Svoboda). On the day of harvest, the flowering yarrow heads were collected and dried in an oven at 40˚C, and ground herb material was used. *Thymus vulgaris* L. (thyme) EO (Essentially Oils Ltd., UK) was chosen for its high thymol content. Dried *Rosmarinus officinalis* L. (rosemary) herb was selected for its camphor content, and an air-dried *Allium sativum* L. (garlic) powder was purchased (both Park Tonks Ltd., UK). Powdered CT extracts of *Vaccinium macrocarpon* Ait (cranberry) and *Vitis*

vinifera L. (grapeseed) were provided (BFI Innovations Ltd., UK), as well as *Acacia mollissima* (mimosa) CT extract (Roy Wilson Dickson Ltd., UK). An emulsifier with lecithin [E322] (Lysoforte™ Booster Dry, Kemin Ltd., UK) was included in the diet.

Housing and environment

The experiment was approved by SAC's Animal Experiment Committee, and was conducted in floor pens with a bedding substrate of wood shavings. A small quantity of used broiler litter material (1 kg per 30 kg of shavings) was added to present a potential microbial challenge. The birds were provided with feed and water on *ad libitum*. Heating in the experimental facility was provided by a single gas brooder, initially set at 32˚C on d 0 and decreased linearly by 0.5˚C per d to a temperature of 21˚C (d 21). Supplementary heat was provided using butane gas heaters. During the study, the birds received a lighting regimen of 23 h light:1 h darkness.

Experimental Design

At 1-d-old, 960 Ross 308 female birds were placed in the pens and reared to 42 d of age. Using a simple randomised experimental design, the pens were randomly arranged as 6 replicates of 8 treatments (48 pens in total), with groups of 20 birds bulk-weighed on arrival and randomly assigned to each pen. The birds were fed on a starter (0-7 d), grower (8-21 d) or finisher (22-42 d) basal dietary ration (Table 1). For each dietary treatment, either an herb supplement (rosemary, yarrow or garlic) was added at 10 g/kg or a plant extract (cranberry CT, mimosa CT, grapeseed CT or thyme EO) supplement was added at 1 g/kg. The basal ration without supplement was fed as the control treatment. For each pen, the mean body weight (BW) was measured at the start of the experiment (d 0), and again on d 7, 21 and 42, when feed consumption was also measured. On d 42, all birds from the control, thyme EO, yarrow, garlic and rosemary supplemented treatments were processed as whole birds. The

carcases were stored at -20˚C, and taken to CHARIS Innovative Food Services (Hannah Research Institute, Ayr), for organoleptic analysis.

Table 1 near here

Diet formulation and nutrition

The basal diets, as fed, were formulated to be balanced for energy and protein, and to match the requirements for growing birds of this age and genotype (Table 1). No synthetic antimicrobials or anti-coccidial drugs were included, and the diets were supplied as a mash. The wheat/soybean meal basal diet was formulated with a low α-tocopherol content (<50 mg/kg), and included an emulsifier and a xylanase/β-glucanase mixture (0.5 g/kg each). The enzyme mixture (Avizyme 1210) used contained guaranteed minimum activities of xylanase [EC3.2.1.8; 5000 units/g] and β-glucanase [EC3.2.1.6; 50 units/g] (Danisco Animal Nutrition, UK). The basal diet was divided into treatment diets and the plant extracts added. Each supplement was pre-mixed into about 6 kg of the basal ration for 5 min with an industrial food mixer (Hobart A200, Hobart Manufacturing Co. Ltd., London). This concentrated carrier supplement was then mixed into the treatment rations. Titanium dioxide (TiO_2) was included at 5 g/kg as a dietary marker for a period of 48 h before digesta samples were collected.

Sample preparation

Samples from each treatment diet were retained during mixing and stored until required at –20 ±2˚C. On d 21, 1 bird from each pen was randomly selected and euthanased using sodium pentobarbitone (Euthatal™) administered intravenously into the wing vein. Caecal contents were collected, and the jejunum and ileum removed to the point of Meckel's diverticulum at the ileo-caecal junction. Intestinal contents were flushed into petri dishes using 20 ml syringes filled with water. Both ileal and caecal contents were stored at -20˚C. All ileal digesta samples were freeze-dried prior to analysis. All samples (diets and digesta) were milled to pass through a 0.75 mm sieve aperture (Retsch, Hahn, Germany).

Laboratory analyses

The dry matter (DM) and organic matter (OM) contents in the diet and ileal digesta samples were determined according to BS 5766, Parts 1 and 8 (1983). Gross energy (GE) was determined by adiabatic bomb calorimetry (Autobomb A. Gallenkamp & Co Ltd., England) in accordance with BS 1016, Part 105 (1992), using a benzoic acid standard (VWR Ltd., UK) of known heat capacity. Nitrogen contents were determined by the Dumas combustion method (Leco FP-428/328, St. Louis, USA). The concentrations of TiO_2 in diet and ileal digesta samples (Short *et al*., 1996), and the CT concentrations in the plant extracts using the butanol/HCl method (Makkar, 2003) were determined. As no other reference standards were found for CT, the plant extracts were measured against a known sample of quebracho tannin. All ground plant material was distilled using Clevenger apparatus to obtain the EO's in accordance with BS 4585 (1985), following British Pharmacopoeia methods. The EO samples were stored in sealed glass vials below 4°C until required for terpene analysis by Gas Chromatography (GC) against known commercially obtained reference terpene standards. The alliin content of garlic was assessed according to a modification (Holder and Boyce, 1996) of the method of Nathan *et al*. (1978). In a well-diffusion assay, a volume of 0.1 ml aqueous suspension of freeze-dried garlic powder was placed in 6 mm wells cut in the surface of a Mueller-Hinton agar plate which had previously been uniformly inoculated with *Candida albicans* NCYC 1470 grown to the density of a 0.5 McFarland standard. Plates were incubated overnight at 35°C. The diameters of inhibition (clear agar) around the test wells were measured and compared against a range of alliin standard concentrations and an internal reference standard with an activity of 100% (Interprise Ltd, Wales UK).

Volatile fatty acids (VFA) were analysed with reference to internal and external standards by HPLC using a modified method from rumen contents (Rooke *et al*., 1990). A 1-ml aliquot was drawn from a suspension of caecal contents (0.5 ml shaken in 5 ml deionised water), and

0.2 ml propan-2-ol metaphosphoric acid solution (215 g in 1 l deionised water) and 0.1ml internal standard (1 ml 2-ethyl-n-butyric acid solution in 100 ml deionised water) were added. The tubes were mixed and left to stand overnight to allow maximum VFA separation. After centrifugation at 1740 *g* (Sorvall RT6600) for 15 min and then 10,000 *g* for 5 min to remove precipitated protein, samples of supernatant were analysed by HPLC. VFA's were separated isocratically, using 5mM sulphuric acid in the elution phase on a Supelco Supelcogel C-641H column (Supelco, Dorset, UK; 7.8X300 mm) packed with spherical 9 mm sulphonated polystyrene/divinyl benzene. The column had an injectable volume of 80 µl at a temperature of 57˚C, with 0.45 ml/min flow rate. Peaks were detected by refractive index (Shodex SE61 refractive index detector) and the data collected on a Dionex Chromeleon 6.20 system, Dionex Ltd., Sussex, UK. Under these conditions, the detection limit for an individual fatty acid was 1 µg/ml. Samples were analysed in one batch and the intra-assay coefficients of variation (%) for acetic (5 mg/ml), propionic (2.5 mg/ml), n-butyric (1.5 mg/ml) and valeric acids (0.5 mg/ml) were 3.2, 3.0, 3.0 and 5.4 respectively.

Effect of dietary herbal inclusion on organoleptic properties of cooked chicken

The organoleptic analysis was carried out following a modified method (Muir and Hunter, 1992). None of the birds fed on diets with CT supplements were included in this part of the experiment. The carcases from birds given control, rosemary, thyme EO, yarrow and garlic dietary supplements were thawed and cooked on a commercial rotisserie to an internal temperature of 80°C, cut into joints of white (breast) and red (leg) meat and served at a temperature of 100°C. Other portions were allowed to cool and stored overnight at 4°C, and then served cold. The sensory properties of the meat were rated by a panel of 14 highly trained assessors. During a 3-month period prior to the experiment, each assessor was familiarised with organoleptic assessment of chicken meat served hot and cold. During this time, an experimental vocabulary containing 23 attributes (variables) was constructed and

validated for its ability to discriminate between cooked meat samples, and used later in the experiment. Meat was presented to the panel in isolated booths and samples were coded in such a way that the assessors were unaware of the nature of the sample they were assessing. Samples were served in an order allowing estimation of the sample, order of presentation, session and assessor effects to be measured. On each of 5 separate occasions, samples drawn from the 5 dietary treatments, *i.e.* control, garlic, rosemary, thyme EO and yarrow, were assessed by the panel. On each occasion, repeated measurement of samples took place.

Statistical analysis

As the pen represented the experimental replicate, digestibility analysis was carried out at 2 time points (21 and 42 d) using birds sampled at each stage (42 d data not shown). The experimental data was analysed using ANOVA within the General Linear Model (GLM) in *Genstat 7th Edition* (Lawes Agricultural Trust, 2003). This experiment was not factorial in structure. For each experimental variable, Fishers Least Significant Difference test (LSD) was used to separate the treatment means. The statistical analysis of sensory ratings was carried out in 2 steps. Firstly, panel mean values together with the standard error of the difference of the means were estimated by Analysis of Variance using the REML routine in *Genstat 7th Edition*. The results were tabulated as panel mean attribute ratings for the 5 dietary treatments, for the white and the red meat served hot and cold. There were 30 values for each of the 23 attributes. Secondly, the treatment effects, diet, type of meat and serving temperature, were estimated using analysis of variance on the panel mean attribute ratings.

RESULTS

Chemical composition of plant extracts and diets

Terpene and CT concentrations were determined for the supplements (Table 2). The garlic used in the study was compared against a refrigerated reference sample (Interprise Ltd, Port Talbot, Wales, UK), and was considered to have average activity (69.9%) against *Candida*

albicans. All tannin supplements were obtained as purified CT extracts. In the finished feed, the level of carbohydrase was calculated at 2500 U/kg xylanase and 25 U/kg β-glucanase. Individual treatment diets were determined to be similar in composition to both the basal diet and calculated nutrient values. Treatment rations given to the birds at 21 d contained 892 g/kg DM, 235 g/kg DM crude protein (CP), 56 g/kg DM ash, 944 g/kg DM organic matter and 20.2 MJ/kg DM gross energy.

Tables 2 and 3 near here

Effect of dietary phytochemical inclusion on broiler performance

There were no mortalities during the 42-d experiment. Dietary garlic supplement significantly increased BW at 7 d, compared with all other treatments, but had no effect after 7 d (Table 3). Between 0-7 d, the birds fed on diets with mimosa CT and thyme EO supplements had significantly lower BW gain than all others except those fed on diets with rosemary supplement. After d 7, no treatment differences were observed in BW gain. During d 0-7, the diet supplemented with mimosa CT was consumed significantly less than the other treatments, apart from the diets with grapeseed CT and yarrow supplements. During d 8-21, diets supplemented with mimosa CT were consumed significantly less than the control diets. No treatment effects were observed after 21 d or over the study as a whole (0-42 d), with regards either feed consumption (FC) or feed conversion efficiency (FCE).

Effect of supplementary phytochemicals in chick diets on nutrient digestibility

At 21 d of age, digestibility analysis was measured using the ileal digesta. Coefficients of DM (DMD), digestibility of OM in the DM (DOMD), N (ND) digestibility and metabolisable energy (ME) were calculated, and ME was also corrected to nitrogen equilibrium (MEn). The metabolisability of energy (ME:GE) and the digestible OM and N contents within the diets were determined. Thyme EO and garlic powder were measured for GE content. There were no significant differences in ME or MEn at d 21 (Table 4), but there was a tendency (P = 0.067) for a lower ME:GE ratio in the birds receiving cranberry CT supplement, compared

with those given garlic. Samples with insufficient digesta mass were removed during the analysis; this was unrelated to treatment. At d 21, DMD, DOMD and the digestible OM content in the diet were all significantly reduced in those birds fed on diets with cranberry supplement compared with all other treatments except yarrow (Table 5). Both ND and the content of digestible N in the diet were significantly reduced when the birds were fed on diets with cranberry CT supplement.

Tables 4 and 5 near here

Effect of inclusion of dietary phytochemicals on VFA concentrations

When displayed as a proportion of total caecal VFA concentrations, the control treatment birds had significantly greater proportions of isovaleric and isobutyric acids than those given yarrow, thyme, rosemary or garlic supplements at 21 d (Table 6). Due to the relatively minor importance of these acids, they were grouped together and analysed as 'Other VFA', although the actual concentrations are shown in Table 6. Only n-butyric acid concentrations tended (P = 0.089) towards a treatment x age interaction (21 and 42 d; data not shown).

Tables 6 and 7 near here

Effect of dietary herbal inclusion on organoleptic assessment of chicken meat

For each flavour attribute, striking differences were observed between the white and dark meat samples (Table 7). The temperature of meat at assessment also significantly influenced sensory character perceptions for abnormal, sweet, synthetic, garlic and astringent flavour attributes. Temperature also significantly affected the likelihood that a sample was regarded as having an oily mouth feel. Dietary treatment significantly affected the flavour intensity, abnormal and garlic flavour attributes. Significant diet x temperature interactions were observed for garlic flavour, and significant meat type x temperature interactions for both chicken and rosemary flavours. There were relatively small differences in flavour intensity, which were significantly greater between the birds fed on diets with rosemary and garlic supplements (Table 8). Primarily, the significant differences in abnormal and garlic flavours were due to the garlic supplement, more so than any other including the thyme EO, and birds

given thyme EO supplements tended to have an abnormal flavour. The largest differences in the meat from garlic-fed birds, when compared with all other treatments, generally occurred when served cold rather than hot (Table 9). However, for the 'abnormal' flavour attribute, the birds given garlic supplements were rated more highly when served hot.

Tables 8 and 9 near here

DISCUSSION

Phytochemical composition

Chemical composition and activity in garlic depends on the processing, physical damage and the unstable nature of the disulphide compounds (Kamel and Saleh, 2000), so can be difficult to predict. Allicin, the major active component of garlic, is unstable and quickly converts to other compounds. Consequently, the garlic used in the current study was not analysed for chemical composition other than its energy content, but the well-diffusion assay indicated a fairly average alliin determination and activity against *Candida albicans* (Interprise Ltd, Wales). Oil distilled from the rosemary herb supplement contained 165 g/kg camphor, a high concentration in agreement with the oil used by Piccaglia *et al*. (1993). Camphor was the principal component in *Artemisia annua* oil noted for its strong anti-fungal and antimicrobial activity *in vitro* (Juteau *et al*., 2002). The high borneol and camphene in this rosemary oil also suggest its antibacterial nature. Low CT contents in the herb supplements suggest that terpenes or other compounds were responsible for the observed effects. In this experiment, three different commercial CT extracts were chosen, to explore a range of tannin structures for their effects on growth and nutrition in poultry. Cranberry CT extracts contain a large proportion of A-type condensed tannins, while mimosa is composed of 5-deoxy tannins and grapeseed contains variable numbers of gallic acid groups linked to the condensed tannins (Frazier *et al*., 2010; Mueller-Harvey, 2006). Surprisingly, the cranberry extract contained very little CT, at 24 g/kg DM. Cranberries have a high content of flavonoid compounds, including proanthocyanidins or CT, which may be mainly epicatechin units (Foo *et al*., 2000).

Cranberries were also reported to contain mainly quercetin and myrectin flavanols, and anthocyanidins (Wilson *et al*., 1998). The cranberry CT supplement used in this study could have contained a higher proportion of hydrolysable tannins (HT) or a phenolic compound mixture. Higher CT concentrations in the grapeseed and mimosa supplements were in agreement with Yilmaz and Toledo (2004). Different CT types are known to have varying abilities to precipitate proteins and affect nutrient digestibility (Vitti *et al*., 2004). It is clear from these results that CT structure can have a profound and differential effect in the poultry gut.

Effect of the phytochemical supplements on broiler performance

The largest response to the dietary garlic supplement occurred in the first week, with no improvement after d 21. Horton *et al*. (1991) also found that supplementary garlic increased BW gain during the first 21 d of growth in broiler chicks, with no effects after this time. However, Qureshi *et al*. (1983) reported that different dietary garlic extracts had no effect on either FC or BW gain in broilers. Any positive effect of garlic supplements may be limited to the first few weeks of growth in broiler chicks. Mechanisms describing the effects of allicin in garlic as inhibiting RNA synthesis have been reported (Feldberg *et al*., 1988). Other proposed mechanisms for either herbs or garlic include variations in enzyme activity (Gupta and Sandhu, 1998), such as cysteine proteinase inhibition by allicin (Ankri *et al*., 1997), increased availability at the intestinal brush border (Khajuria *et al*., 2002) or affected glucose metabolism (Roman-Ramos *et al*., 1995). The majority of the effects of supplementary terpenes or tannins on growth also appear to be most evident within the first few weeks (Schiavone *et al*., 2008). Increasingly negative and concentration-dependent effects on broiler performance were observed with dietary mimosa tannin supplementation (Iji *et al*., 2004). It is considered that the mimosa CT supplement used in this study may have contained a mixture of HT and CT compounds, and these may have been responsible for reducing

performance in the birds. Negative effects of dietary grapeseed extract supplements for broiler chick performance have been reported (Lau and King, 2003), despite there being no effect on performance in rats (Martin-Carrón *et al*., 1999). Terpene or tannin compositions in the final supplement blend were considered important for determining positive or negative broiler growth responses (Cross *et al*., 2007), and this experiment further supports a careful choice of any herb or tannin based on chemical composition. Thyme, cranberry and mimosa supplements should be used within a blend, and may require a dietary adaptation period. Such compounds may be better fed after d 7, when the gastrointestinal tract is more fully developed.

Effects of phytochemical supplements on nutrient digestibility

Compared with the control birds in this experiment, reduced nutrient digestibility parameters were observed in birds given cranberry CT supplements, and reduced digestible N content of the diet in the birds given mimosa CT supplements. Dietary mimosa supplements also reduced the ileal digestibility coefficients of energy, protein, alanine, arginine and lysine when fed at 20 g/kg and above (Iji *et al*., 2004), where considerably higher inclusion levels than in the present study were used. Mimosa may be suitable for use at low concentrations or within an ingredient blend. These results suggest that it is not suitable as the sole dietary supplement for broiler chickens. In this experiment, the decreased dry and organic matter, and N digestibility values, in birds fed on diets with cranberry supplements were surprising. Cranberries are known to bind to specific pathogenic bacteria and have acted to prevent uropathogenic phenotypes of P-fimbriated *E. coli* infecting the urinary tract (Howell *et al*., 1998). In elderly women, the daily consumption of cranberry juice reduced urinary tract bacterial concentrations, thus reducing infection from 28% to 15% (Avorn *et al*., 1994). In this experiment, the cranberry may have bound to other dietary components, such as sugars or proteins, which then negatively affected their digestion and absorption. Conversely, the

grapeseed supplement used, also with a high CT concentration, resulted in nutrient digestibility values that were on a par with the control birds. In broiler chicks, no negative effects on performance or crude protein digestibility were associated with the dietary inclusion of grape pomace concentrate at levels up to 60g/kg to 42 d of age (Brenes *et al*., 2008). Additionally, there were no negative effects of feeding chicks up to 30 g/kg grape pomace in diets on performance, protein or amino acid digestibility (Goni *et al*., 2007). Ideal dietary tannin concentrations should be investigated in further experiments. From these experimental results, grapeseed CT supplements or tannins with similar chemical composition may be more suitable for supplementation in broiler chicken diets.

Effect of phytochemicals on intestinal microflora populations

Herbal inclusion in the diets of these broilers tended to decrease the total caecal VFA concentrations, and thus may have suppressed either microfloral activity or numbers. In a commercial environment, much greater effects of dietary herbal inclusion are considered possible with the much larger microbial challenge faced by the birds. In this experiment, differences were limited to the minor VFA (isovaleric and isobutyric acids) concentrations. The antimicrobial nature of garlic is well established (O'Gara *et al*., 2000). Similarly, thyme oil, thymol and carvacrol have reduced *in vitro* VFA fermentation (Varel and Miller, 2004). These results indicate that herbs can have antimicrobial effects *in vivo*, although these appear to be minor and selective. In an *in vitro* microbial culture system using rumen fluid, yarrow has increased acetate production at the expense of butyrate (Broudiscou *et al*., 2000). Tannins have also inhibited microfloral activity and affected VFA concentration, and may be selective in their effects. Bacterial enzyme activity was reduced in rats fed on diets with grapeseed CT (Tebib *et al*., 1996). In calves, dietary green tea extracts have reduced scouring, which decreased concentrations of various bacterial strains in the excreta, but with no effect on *Bifidobacterium* spp. or *Lactobacillus* spp. (Ishihara *et al*., 2001).

Organoleptic assessment of the chicken meat

In this experiment, dietary garlic supplements significantly changed organoleptic properties in chicken meat, but the tests did not identify changes which were detrimental to meat flavour. When intestinal samples were removed in the growth study, the smell of garlic permeated throughout the carcase. Meat colours, taste and smell were all improved when broiler chickens were fed on diets with XTRACT®, containing capsaicin, carvacrol and cinnamaldehyde (Jamroz *et al*., 2003). Dietary yarrow herb supplements fed either singly or in combination with St. John's wort have enhanced organoleptic perception in broiler chicken meat (Fritz *et al*., 1993). These results suggest that dietary herb and oil inclusion may influence chicken meat flavour. Further tests could determine whether these flavour changes would positively enhance the market value of meat, or indicate negative sensory changes.

While dietary garlic and grapeseed CT supplements maintained bird performance and dietary digestibility was similar to the birds fed on the control diet, cranberry CT supplements reduced nutrient digestibility. Furthermore, both cranberry and mimosa supplements may bind to nitrogenous components in the digesta. Thus, CT components require careful selection, and could be used either within supplement blends, or fed after 7 d in a more developed gut. Optimal dietary CT concentrations in tannin supplements should be assessed, and CT chemistry has an effect on the ability of a tannin to dissociate from nutritional fractions. Assessment of dietary plant supplements on organoleptic properties in meat represents an interesting area for further study, as subtle flavour modifications can be discerned. In summary, this study indicated that careful choices are necessary in preparing dietary phytochemical supplements for broilers, but beneficial effects of their inclusion can be observed.

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Table 1. *Formulation and chemical composition of the basal diet*

Ingredient (g/kg)	Diet Formulation		
	(0-7 d)	(8-21 d)	(22-42 d)
Wheat	489.4	478.3	530.3
Barley	120.0	120.0	120.0
Soya bean meal (Hi-pro)	302.5	242.2	187.0
Soya bean meal (Full fat)	0.0	57.9	64.1
Soya oil	45.0	60.0	60.0
Monodicalcium phosphate	12.6	11.1	12.6
Limestone	15.5	15.5	12.5
Sodium chloride	3.0	3.0	3.0
Lysine	3.0	3.0	2.0
Methionine	4.0	4.0	3.5
Vit/Min premix[1]	5.0	5.0	5.0
Calculated composition (g/kg)			
ME (MJ/kg)	12.3	12.9	13.1
Crude Protein (CP)	223.5	213.5	194.0
Ether Extract/Fat	56.8	81.4	82.7
Crude Fibre	33.7	34.2	33.9
Calcium	9.5	9.2	8.3
Phosphorus	7.1	6.7	6.9
Lysine	14.5	13.9	11.6
Methionine + Cysteine	9.5	9.5	8.6

Plant supplements (see Materials and methods) and the TiO_2 dietary marker (5 g/kg) were added to the basal diets as required.

[1]Supplied per kg diet: retinyl acetate, 4128 µg; cholecalciferol, 125 µg; α-tocopherol acetate, 50 mg; menadione, K 3 mg; folic acid, 1 mg; nicotinic acid, 50 mg; thiamine, 2 mg; riboflavin, 7 mg; pyridoxine, 5 mg; cobalamin, 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. *Determined chemical composition of plant extracts or their derivations*

Extract	Major Terpene and Tannin Concentrations (g/kg)
Thyme EO	Thymol (440.8), *p*-cymene (320.4), α-terpineol (95.9), linalol (46.2); Negligible CT (<1)
Yarrow EO	Chamazulene (279.4), linalol (151.3), camphene (100.9), β-pinene (50.6); Negligible CT (<1)
Rosemary EO	Borneol (219.6), camphor (165), Unidentified terpenes (49.6 and 49.4), linalyl acetate (27.0); Negligible CT (<1)
Garlic herb[1]	Not determined. Gross energy content of garlic powder = 16.51 MJ/kg
Grapeseed CT	373 g/kg DM
Mimosa CT	258 g/kg DM
Cranberry CT	24 g/kg DM

Essential oils (EO) and condensed tannins (CT) were determined for chemical composition.

[1]Garlic was not analysed due to the unstable nature of its constituent compounds.

Table 3. *Effect of the dietary inclusion of various phytochemicals on broiler growth performance from 0-42 d of age*

Treatment	Average BM (g)			Average weight gain (g/bird/d)			Average feed consumption (g/bird/d)			Feed conversion efficiency (FCE) (gain/unit feed)		
	7d	21d	42d	0-7 d	8-21 d	22-42 d	0-7 d	8-21 d	22-42 d	0-7 d	8-21d	22-42 d
Control	113^{b}	593	1944	11.2^{ab}	34.6	64.1	17.3^{abc}	50.2^{ab}	119.0	0.645^{ab}	0.688^{ab}	0.541
Yarrow	111^{b}	583	1931	10.5^{bc}	34.2	64.2	16.7^{bcd}	50.0^{ab}	118.0	0.630^{ab}	0.684^{ab}	0.541
Thyme	107^{b}	576	1880	10.2^{d}	33.5	62.1	17.4^{ab}	47.9^{bc}	115.0	0.585^{b}	0.699^{b}	0.541
Rosemary	111^{b}	564	1958	10.7^{bcd}	32.4	66.4	17.8^{ab}	48.4^{abc}	121.0	0.601^{b}	0.668^{a}	0.541
Garlic	120^{a}	601	1960	11.9^{a}	34.4	64.7	18.0^{a}	50.3^{a}	119.0	0.660^{a}	0.684^{ab}	0.541
Mimosa	107^{b}	571	1900	10.0^{d}	33.1	64.2	15.8^{d}	46.7^{c}	117.0	0.629^{ab}	0.711^{b}	0.546
Grapeseed	112^{b}	573	1923	10.8^{bc}	33.7	64.0	16.7^{bcd}	48.0^{abc}	117.0	0.646^{ab}	0.700^{b}	0.546
Cranberry	113^{b}	587	1952	10.9^{bc}	33.9	65.0	17.4^{abc}	49.1^{ab}	119.0	0.630^{ab}	0.690^{b}	0.549
SED	2.58	16.35	67.2	0.36	1.02	2.67	0.55	1.20	3.55	0.023	0.011	0.068
P value	<0.001	NS	NS	<0.001	NS	NS	0.008	<0.05	NS	<0.05	0.016	NS

Within a column, values not sharing a common superscript are significantly different at $P < 0.05$.

NS = $P > 0.05$. Data are means of 6 treatment replicates.

Table 4. *Effect of dietary inclusion of phytochemicals on the metabolisability of energy, both with and without a correction for nitrogen equilibrium (ME and MEn), and the ratio of ME:GE within the diet in broilers at 21 d of age*

Treatment	Dietary energy utilisation at 21 d			
	ME (MJ/kg DM)	MEn (MJ/kg DM)	ME:GE	Replicates analysed
Control	11.66	10.92	0.587	6
Yarrow	11.47	10.78	0.572	5
Thyme	11.74	11.08	0.587	4
Rosemary	11.56	10.99	0.567	6
Garlic	12.20	11.48	0.619	5
Mimosa	11.43	10.88	0.569	4
Grapeseed	11.89	11.24	0.581	6
Cranberry	10.57	10.08	0.498	5
Pooled SED	0.809	0.749	0.039	
P value	NS	NS	0.067	

NS = $P > 0.05$.

Data are means of 6 replicates, adjusted for missing values unrelated to treatment.

Table 5. *Effect of inclusion of dietary phytochemicals on the coefficients of dry and organic matter in the dry matter (DMD and DOMD) and N digestibility (ND), along with digestible OM and N contents in the diets, as fed to broiler chicks at 21 d*

Treatment	Coefficients of DM, OM and Nitrogen digestibility, with the dietary contents of digestible OM and Nitrogen				
	DMD	DOMD[1]	Dig OM content of the diet (g/kg DM)	ND	Digestible N content of diet (g/kg DM)
Control	0.580^{a}	0.591^{a}	559^{ab}	0.508^{ab}	19.39^{ab}
Yarrow	0.543^{ab}	0.553^{ab}	522^{bc}	0.477^{abc}	17.86^{abc}
Thyme	0.594^{a}	0.609^{a}	575^{ab}	0.453^{abc}	16.94^{abc}
Rosemary	0.556^{a}	0.569^{a}	537^{ab}	0.414^{bc}	15.52^{bcd}
Garlic	0.625^{a}	0.636^{a}	600^{a}	0.528^{a}	19.86^{a}
Mimosa	0.590^{a}	0.605^{a}	570^{ab}	0.405^{bc}	14.94^{cd}
Grapeseed	0.584^{a}	0.596^{a}	562^{ab}	0.475^{abc}	17.87^{abc}
Cranberry	0.465^{b}	0.478^{b}	450^{c}	0.325^{c}	12.32^{d}
SED	0.040	0.041	38.4	0.056	2.114
P value	0.018	<0.05	0.019	<0.05	0.021

Within a column, values not sharing a common superscript are significantly different at $P < 0.05$.

Data are means of 6 treatment replicates.

[1]DOMD refers to the digestibility of organic matter in the dry matter

Table 6. *Effect of dietary phytochemical inclusion on concentrations of caecal VFA in broilers at 21 d of age, where each concentration is a proportion of total VFA*

Treatment	Proportions (%) of individual VFA in relation to total VFA concentration						Total VFA conc (g/kg)
	Acetic	Lactic	Propionic	n-Butyric	Valeric	Other (Isobutyric + Isovaleric)	
Control	42.18	13.75	6.08	34.73	3.23	1.03^{ab} (0.55+9.64)	10.54
Yarrow	38.92	10.65	5.35	41.10	3.68	0.30^{c} (0.32+2.81)	12.53
Thyme	41.28	11.38	8.78	34.85	3.47	0.22^{c} (0.00+2.11)	10.71
Rosemary	41.90	9.03	7.27	37.78	5.20	0.18^{c} (0.00+1.87)	10.36
Garlic	38.92	12.77	5.35	39.87	3.03	0.03^{c} (0.00+0.26)	10.21
Mimosa	41.67	11.35	6.67	35.40	4.28	0.60^{bc} (0.43+5.72)	11.76
Grapeseed	41.03	10.17	7.12	34.85	5.43	1.45^{a} (4.92+9.43)	12.10
Cranberry	41.57	11.87	7.50	36.03	2.99	0.47^{bc} (1.87+2.78)	11.65
SED	3.001	2.075	1.588	2.835	1.598	0.337	0.866
P value	NS	NS	NS	NS	NS	0.002	0.073

Within a column, values not sharing a common superscript are significantly different at $P<0.05$.

NS = $P>0.05$.

[1]Values in parentheses are the % proportions isobutyric and isovaleric acids as used in 'Other VFA'.

Data are means of 6 treatment replicates.

Table 7. *Summary of discriminant effects on the various organoleptic attributes measured in cooked meat from the dietary treatments, the type of meat samples assessed and temperature of test samples*

Attribute	Discriminant	Main effects			1st Order interactions		
		Diet (D)	Meat type (M)	Temperature (T)	D*M	D*T	M*T
Appearance	Y		***				
Hue	Y		***				
Aroma intensity	Y		***				
Flavour							
Intensity	Y	*	***				
Abnormal	Y	***		***			
Sweet	Y			*			
Chicken	Y		***				**
Synthetic	Y			**			
Salty	N						
Garlic	Y	***		**		*	
Rosemary	Y		*				*
Bitter	Y		**				
Astringent	Y			***			
Thyme	N						
Other	Y		*				
Aftertaste							
Intensity	Y		***				
Mouth feel							
Juicy	Y		***				
Tender	Y		***				
Oily	Y		***	*			
Fibrous	Y		***				
Melt-in-mouth	Y		***				
Moist	Y		***				
Slimy	Y		***				

The significance of effects are: * P <0.05, ** P <0.01 and *** P <0.001.
Meat type refers to the difference between dark and white meat samples of chicken.
Temperature describes samples given to the assessment panel at either 100ºC or 4ºC.

Table 8. *Effects of diet on sensory character of cooked poultry meat*

Attribute	Discriminant	Diet	Least Square Mean Values					
			Control	Garlic	Rosemary	Thyme	Yarrow	SED
Intensity	Y	*	49.9	51	47.8	50	50.6	1.02
Abnormal	Y	***	2.8	6.4	2.5	4	2.7	0.77
Garlic	Y	***	2.1	4.5	1.4	1.8	2.2	0.73

Significance of effects are: * (P <0.05), *** (P <0.001)

549 **Table 9.** *Interactive effects on garlic and abnormal flavours in meat samples, in relation to*
550 *the dietary herb supplement administered to chickens during growth*

Meat type	Temperature	Garlic flavour Rating	Abnormal flavour Rating
Control	Hot[1]	1.6	2.7
Garlic	Hot	2.3	7.6
Rosemary	Hot	1.2	4.2
Thyme	Hot	2.1	6.0
Yarrow	Hot	1.3	2.8
Control	Cold[2]	2.6	0.8
Garlic	Cold	**6.7**	**3.4**
Rosemary	Cold	1.6	2.0
Thyme	Cold	1.6	1.9
Yarrow	Cold	3.2	2.8
SED		0.77	0.77

551 [1]100°C, [2]4°C.
552 Means in bold type indicate significant difference from controls at $P < 0.05$.