Chapter 7

Plant Extracts, Energy, and Immune Modulation in Broilers

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Additional information is available at the end of the chapter

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Abstract

This chapter presents information obtained from experiments involving male Ross 308 broiler chickens on the effects of a standardised combination of plant extracts (PE) including carvacrol, cinnamaldehyde and capsicum oleoresin, on bird performance, hepatic antioxidant concentration and immunomodulation. Birds were reared under industry-recognised environments and were fed one of four diets. There were two control diets based on either wheat or maize, formulated to be iso-energetic and iso-nitrogenic. The other two diets were the control diets supplemented with 100 g per tonne of PE, respectively. Feeding PE improved dietary feed efficiency, dietary net energy and hepatic antioxidant contents of the birds, but did not change dietary metabolisable energy (ME). Overall, feeding PE reduced the mRNA transcript levels of three cytokines (IL-12B, IFN-G, and IL-6) and the marker CD 40 LG in caecal tonsils. Dietary PE may maximise the nutritional value of feed through improving gut health by reducing intestinal inflammation. Their mode of action is associated with improved dietary energy availability, immune status and hepatic antioxidant contents of the birds. However, studies that have focused solely on the effect of PE on ME alone may not have detected their full benefit to improve the efficiency of broiler meat production.

Keywords: plant extracts, essential oils, broiler chickens, immunity, available energy

1. Introduction

Phytogenics, also referred to as plant secondary metabolites, phytochemicals, phytobiotics, or botanicals, are plant-derived products/extracts (PE) and include a wide range of substances. These include those derived from herbs and spices such as essential oils and oleoresins, reported

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to exhibit growth promoting and/or therapeutic properties [1, 2]. Initially, PE were extensively studied because of the adverse effects they have when ingested by animals [3]. However, the use of PE as an alternative to in-feed antibiotics to prevent the risk of developing pathogens resistant to antibiotics and to satisfy consumer demand for a poultry food chain free of drug residues has gained recent interest [4]. Antibiotics have been added to poultry diets to maintain health and production efficiency in the last few decades [5]. The withdrawal of in-feed antibiotics as growth promoters have increased the risk of bacterial disease, especially in growing poultry [6]. The ability of PE to contribute to the health of the host is well documented [1]; however, the exact mechanisms by which PE exerts its effects remain speculative. As documented, PE are composed of a diverse group of natural products [7]. However, while some are nutritionally valuable, others have no nutritional value or even possess antinutritional properties. This is likely due to PE varying widely in their chemical structures [3]. Since the effects of PE depend to a great extent on the chemistry of the compounds, it is impossible to have a uniform explanation of their mode of action. This chapter provides a brief overview of the main benefits of adding selective PE to poultry diets. Specifically, it describes the mode of action of carvacrol, cinnamaldehyde and capsaicin when fed to broiler chickens as a standardised commercial mix.

2. Effects of dietary plant extracts when fed to poultry

2.1. Bird growth performance and dietary available energy

It has been hypothesised that PE additives may stabilise overall digestive functions in the gastrointestinal tract of poultry, however, the available literature does not provide a consistent picture. Numerous feeding trials involving dietary supplementation with various PE have been reviewed [1, 6]. Regarding growth performance (assessed primarily as feed efficiency) and nutrient digestibility, the effect of added PE was beneficial in 11 of the studies; no effects were observed in 17 and there was a detrimental effect in 18 of the reviewed studies.

The information on the effect of PE on dietary metabolisable energy (ME) is also inconsistent. Some authors found an increase in dietary ME in response to PE [8, 9], others [10, 11] did not. There is also a discrepancy in the published data in the differences in ME and growth performance of birds fed dietary PE. Recent studies [10, 11, 8] found an improvement in bird growth performance but not in dietary ME when various PE were fed to poultry. It has been reported [9] that there is a parallel improvement in dietary ME and feed efficiency when feeding a standardised commercial mix of carvacrol, cinnamaldehyde and capsaicin to broiler chickens.

2.2. Antioxidant status

Due to the general consumer rejection of synthetic food additives there is a growing interest in studies of natural additives as potential antioxidants. Research on the antioxidative properties of herbs and spices showed that they are effective in retarding the process of lipid peroxidation in oils and fatty foods (summarised by [12]). Herbal phenolic compounds also improved the oxidative stability of animal derived products such as poultry meat, pork, rabbit meat and

eggs (reviewed by [1]). Furthermore, research with rats [13] and poultry [14] suggested that dietary phytogenic supplements may improve the antioxidative status of the animals, reducing intestinal cell damage and sustaining the integrity of the intestinal mucosal layer. These supplements acted as effective free radical scavengers and also influenced the *in vivo* antioxidant defence systems in the animal. In addition, diets supplemented with turmeric, curcumin, green tea, grape seed proanthocyanidins and society garlic, which all possess antioxidative properties, reduced small intestinal lesion scores, lowered oxidative stress and improved weight gains during coccidial infection (summarised by [6]). All these compounds may exert their anticoccidial activity by protecting infected tissues from oxidative damage and therefore reducing the severity of coccidiosis.

2.3. Immune status

Immunomodulation is described as a change of the indicators of cellular and humoral immunity and nonspecific defence factors [15]. Immunomodulation can present as immunosuppression (substances that inhibit the immune system) or immunostimulation (substances that activate or induce the mediators or components of the immune system), thus regulating or altering the scope, type, duration or competency of the immune response [16, 17].

It has been speculated that the benefit of using PE in animal diets is associated with reduced intestinal inflammation in part from a reduction of proinflammatory cytokines. One study [18] reported that cinnamaldehyde suppressed the lipopolysaccharide-induced production of tumour necrosis factor (TNF), interleukin 6 (IL-6) and IL-1, thus suggesting that the inclusion of cinnamaldehyde could show suppressive effects on the production of various types of inflammatory cytokines. Similarly, [19] also found that a mixture of capsicum and turmeric oleoresins was an effective phytonutrient against clinical signs of experimental avian necrotic enteritis when supplied in dietary form. Research by Lee et al. [20] suggested that immunomodulatory effects are responsible for improved weight gain, oocyst shedding, increased interferon gamma (IFN-γ) and IL-15, when powder from oriental plum (a plant rich in phenolic compounds) was fed to coccidia challenged birds. Furthermore, supplementation with Chinese mushroom and herb extracts resulted in enhancement of both cellular and humoral immune responses in *Eimeria tenella* infected chickens [21].

There is strong evidence that PE have antimicrobial properties, being able to reduce the proliferation of pathogenic organisms at minimal inhibitory concentrations of 0.05–5 microliters per millilitre *in vitro*, and at higher concentrations (0.5–20 microliters per gram) in food [22]. While these levels are unlikely to be met in animal feedstuffs, and therefore not the primary use of PE in feed, there is evidence that PE have effective antimicrobial action against pathogens common in poultry production. These include *Escherichia coli* and *Clostridium perfringens* [23–25]. Pathogenic microorganisms in the gut are able to trigger immune responses in the gastrointestinal tract. This results in inflammation of the intestine contributing to poor gut health. In addition to reducing pathogenic challenge, PE may also possess direct immunomodulation properties. For example, it is known that cinnamaldehyde in particular is involved in gene regulation, including antigen presentation, humoral immune response and inflammatory disease [26].

2.4. Chemical structure and properties of carvacrol, cinnamaldehyde and capsaicin

Carvacrol ($C_{10}H_{14}O$) is a chemical (see **Figure 1**) found in several plants including: wild bergamot, thyme and pepperwort, but it is most abundant in oregano (*Origanum vulgare*) oil [27]. Carvacrol gives oregano a slightly spicy flavour, is colourless, and has a distinct warm odour. Overall, carvacrol has a promising antibacterial, antiviral, antioxidant, and antifungal impact. Carvacrol also demonstrated significant anti-cancer effects when tested against breast cancer, prostate, lung and mouth cancer cells [28].

Cinnamaldehyde (C_9H_8O) is a chemical (see **Figure 1**) that naturally occurs in the inner bark of several tree species from the genus Cinnamomum. Cinnamaldehyde, the principal component of the essential oil of cinnamon bark, gives the cinnamish odour responsible for the characteristic taste and odour of cinnamon spice. Cinnamaldehyde has strong antimicrobial, antifungal and anticorrosion properties [29].

Capsaicinoids ($C_{18}H_{27}NO_3$) are produced by peppers as a protection against certain mammals and fungi (see **Figure 1**). They have no flavour or odour, but act directly on the pain receptors in the mouth and throat. Capsaicin, the most common capsaicinoid, is an irritant for most mammals, including humans, and produces a sensation of burning in any tissue with which it comes into contact [30]. However, birds are not sensitive to the capsaicin [31] and can benefit from the nutritional value of the chilli peppers. Capsicum oleoresin (active ingredient capsaicin) is found in pepper fruits and has antifungal and antibacterial activity [32].

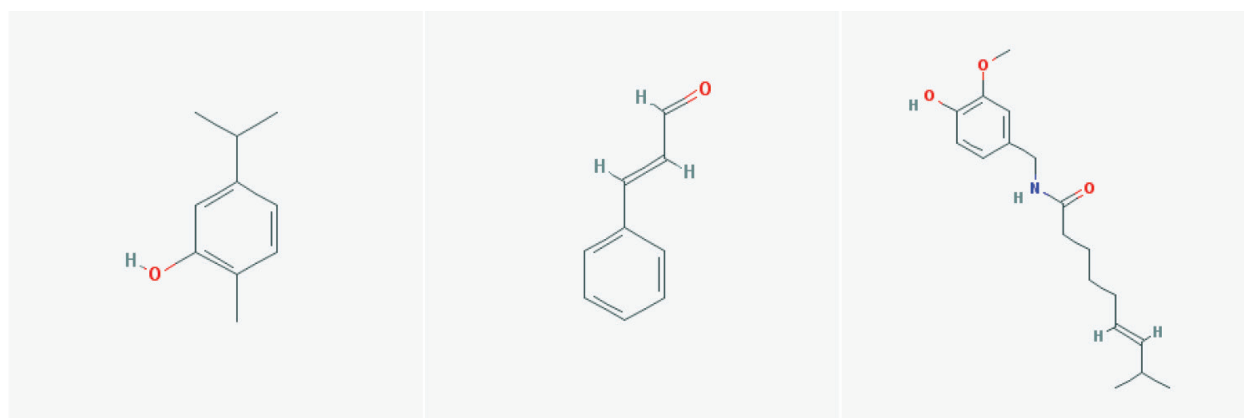

Figure 1. Chemical structure (2-D) of (left to right) carvacrol, cinnamaldehyde and capsaicin.

3. Poultry experiments (methodology)

The experiments described in this chapter followed internationally recognised guidelines for work with poultry. All birds were cared for according to laws and regulations detailed in UK guidelines. Data from four experiments performed under similar environmental and dietary conditions were used in this chapter.

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3.1. Dietary formulation, husbandry and sample collection

The four experiments employed the same dietary formulations (**Table 1**). Birds were fed one of four diets. There were two control diets based on either wheat (WC) or maize (MC) which were formulated to be iso-energetic (12.13 MJ/kg AME) and iso-nitrogenic (215 g/kg CP).

Dietary ingredients	Wheat-based diet	Maize-based diet
	kg/100 kg	kg/100 kg
Maize	—	52.86
Wheat	54.68	—
Soybean meal (48)	27.49	31.30
Vegetable oil	3.50	1.00
Barley	5.84	6.33
Rye	5.00	5.00
Monodicalcium phosphate	1.43	1.43
Limestone	1.15	1.15
NaCl	0.27	0.33
Lysine	0.15	0.15
Methionine	0.39	0.35
Vitamin mineral premix[1]	0.10	0.10
	100	100
Calculated analysis (as fed)		
Crude Protein, g/kg	215	215
ME, MJ/kg	12.12	12.13
Crude Fat, g/kg	47	34
Ca, g/kg	8.4	8.3
Available P, g/kg	4.5	4.4
Lysine, g/kg	12.3	12.3
Methionine + Cysteine, g/kg	9.5	9.5

ME = metabolisable energy.

[1]The Vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by the National research Council (1994). The premix provided (units/kg diet): retinol, 12,000 IU; cholecalciferol, 5000 IU; α-tocopherol, 34 mg; menadione, 3 mg; thiamine, 2 mg; riboflavin, 7 mg; pyridoxine, 5 mg; cobalamin, 15 μg; nicotinic acid, 50 mg; pantothenic acid, 15 mg; folic acid, 1 mg; biotin, 200 μg; 80 mg iron as iron sulphate (30%); 10 mg copper as a copper sulphate (25%); 100 mg manganese as manganous oxide (62%); 80 mg zinc as zinc oxide (72%); 1 mg iodine as calcium iodate (52%); 0.2 mg selenium as sodium selenite (4.5%); 0.5 mg molybdenum as sodium molybdate (40%).

Table 1. Composition of the control diets.

The other two diets were the control diets supplemented with a standardised combination of PE (XTRACT 6930; Pancosma S.A., Geneva, Switzerland) including 5% carvacrol, 3% cinnamaldehyde and 2% capsicum oleoresin (100 grams per tonne, respectively, i.e. WC + PE; MC + PE). The PE was added in powder form to the diets and all diets were fed as mash. The diets did not contain any coccidiostat or antimicrobial growth promoters, prophylactic or other similar additives.

Day old male Ross 308 broiler chickens were purchased from a commercial hatchery and reared in floor pens littered with wood shavings. The temperatures were kept at 32°C during the first 2 days on birds arrival and were gradually reduced to 20°C by 21 days of age. A standard lighting programme following breeder's recommendations (Aviagen Ltd., Edinburgh, UK) for broilers was used. Access to the feed and the water was *ad libitum*. At 17 days of age, two birds from each pen were transferred to a pen with wire mesh floor and excreta samples were collected for four consecutive days from each pen, immediately dried at 60°C and then milled for further analyses. The birds were weighed on a per-pen basis and the average bird feed intake (FI), weight gain (WG) and gain: feed ratio (GF) were determined.

In the experiments where dietary net energy (NE) was determined, at the end of the study, all chickens were killed by cervical dislocation and the carcass of the birds, including intestine, blood and feather, from each pen were frozen and then minced, thoroughly mixed and sampled, and used for following analysis and calculations.

In the experiments where the hepatic antioxidant content was determined, at 21 days of age, one bird from each pen was randomly selected, stunned/killed and the liver was collected and stored at –20°C prior to analysis of antioxidant contents.

In the experiments where the gene expression was measured, at 21 days of age one bird from each pen was randomly selected, stunned/killed and the left caecal tonsil was collected and stored in RNAlater® (Sigma-Aldrich, USA) at −70°C prior to analysis of the relative expression of selected genes.

3.2. Chemical analysis of diets and excreta

The experimental diets and the excreta were milled (0.75 mm mesh) and analysed further. Dry matter (DM) was determined by drying samples in a forced draft oven at 105°C to a constant weight. Crude protein (6.25 × N) in samples was determined by dry combustion method [33] using a LECO (FP-528 N, Leco Corp., St. Joseph, MI). Oil (as ether extract) was extracted with diethyl ether by the ether extraction method [33], using a Soxtec system (Foss UK Ltd.). The GE value of the samples was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL), using benzoic acid as the standard.

3.3. Dietary available energy determination

The N-corrected apparent metabolisable energy (AMEn) of the diets was calculated as described by [34].

A comparative slaughter technique [35] was used to determine the energy retained in the carcase of birds. In brief, dietary NE was calculated using the following equation:

$$NE\ (MJ/kg) = (REc + FHP)/FI.$$

Where: FI is the dry matter (kg) consumed for the experimental period. REc is the total energy retained in the carcass (see [50]).

The fasting heat production (FHP MJ/bird) was estimated to be 0.450 MJ/d per kg of metabolic body weight $(BW)^{0.70}$ per day, which correspond to the asymptotic heat production at zero activity (as proposed by [36]).

3.4. Determination of hepatic antioxidant concentration

Concentration of antioxidants in liver was determined by high-performance liquid chromatography (HPLC) [37, 38]. In brief: approximately 300 mg of liver samples were mixed in 0.7 ml 5% sodium chloride solution, then 1 ml ethanol was added and samples homogenised. During homogenisation, 2 ml hexane was added. Then samples were centrifuged and the hexane phase, containing the vitamin E and coenzyme Q_{10} were collected. Extraction with hexane was performed twice, and the combined phase was evaporated under nitrogen and re-dissolved in a mixture of dichloromethane–methanol (1:1, v/v).

Vitamin E (α-, γ- and ϭ-tocopherols) was determined as previously described [39] using an HPLC system (Shimadzu Liquid Chromatograph, LC-10 AD, Japan Spectroscopic Co Ltd., with a Jasco Intelligent Spectrofluorometer 821-FP) fitted with a Spherisorb, type S30DS2, 3 mm C18 reverse phase HPLC column, 15 cm × 4.6 mm (Phase Separations Limited, UK). Chromatography was performed using a mobile phase of methanol–water (97:3, v/v) at a flow rate of 1.05 ml/min. Fluorescence detection of tocopherols and tocotrienols involved excitation and emission wavelengths of 295 and 330 nm, respectively. Standard solutions of tocopherols in methanol were used for instrument calibration and tocol was used as an internal standard.

Coenzyme Q_{10} was analysed in the same extract by injecting 50 ml into the same HPLC system, but using a Vidac 201TP54 column (5 μm, 25 cm × 4.6 mm) and mobile phase ethanol–methanol–2-propanol (70:15:15, v/v) and flow rate of 1.5 ml/min with a diode array detection at 275 nm [40]. Coenzyme Q_{10} (Sigma, Poole, UK) standard was used for calibration.

3.5. Gene expression analysis

The analyses of relative expression of genes of interest (GOI) in the caecal tonsils were performed by qStandard (Middlesex, UK).

3.5.1. Total RNA extraction and reverse transcription

Approximately 30 mg of macro-dissected caecal tonsil tissue per sample (previously submerged in RNAlater® (Sigma-Aldrich, USA) and stored frozen at -80°C) was homogenised in 500 uL

QIAzol lysis reagent for 10 min at 30 Hz in a Tissuelyzer LT (Qiagen, UK). Lysates were mixed with 100 μL chloroform, transferred to pegGold PhaseTrap tubes (PeqLab, UK) and centrifuged for 5 mins at room temperature. The aqueous phase was poured into fresh tubes, mixed with 1.5 volumes of ethanol and applied to Qiagen RNeasy columns (Qiagen, UK). RNA was purified according to the manufacturer's instructions (Qiagen, UK). RNA integrity was assessed using an Agilent Bioanalyzer and RIN was >8 for all samples. Purity and quantity were measured using a NanoDrop spectrophotometer; for all samples the absorbance peak was at 260 nm, A260/280 > 2 and A260/230 > 1. About 800 ng of RNA were reverse transcribed using a Quantitect reverse transcription kit (Qiagen, UK) in a 10 μL reaction according to the manufacturer's instructions. This RT kit includes a mandatory gDNA wipe out step. The completed reaction was diluted 10-fold with 5 μg/mL tRNA in water.

3.5.2. Quantitative real-time PCR

Two microlitres of cDNA were amplified in a 10 μL reaction using Agilent Brilliant III SYBR Ultra-Fast SYBR Green mix with each primer at a final concentration of 500 nmol/L. The no-template control reaction contained 2 μL of tRNA (0.5 μg/mL). DNA standards (10^7–10^1 copies/rxn) for each gene were included in each run. Reactions were pipetted robotically using a Qiagility (Qiagen, UK). Amplification parameters were: 95°C for 3 min followed by 40 cycles of 95°C for 5 sec, 57°C for 1 sec in a Rotor-Gene 6000. Melt curves were checked

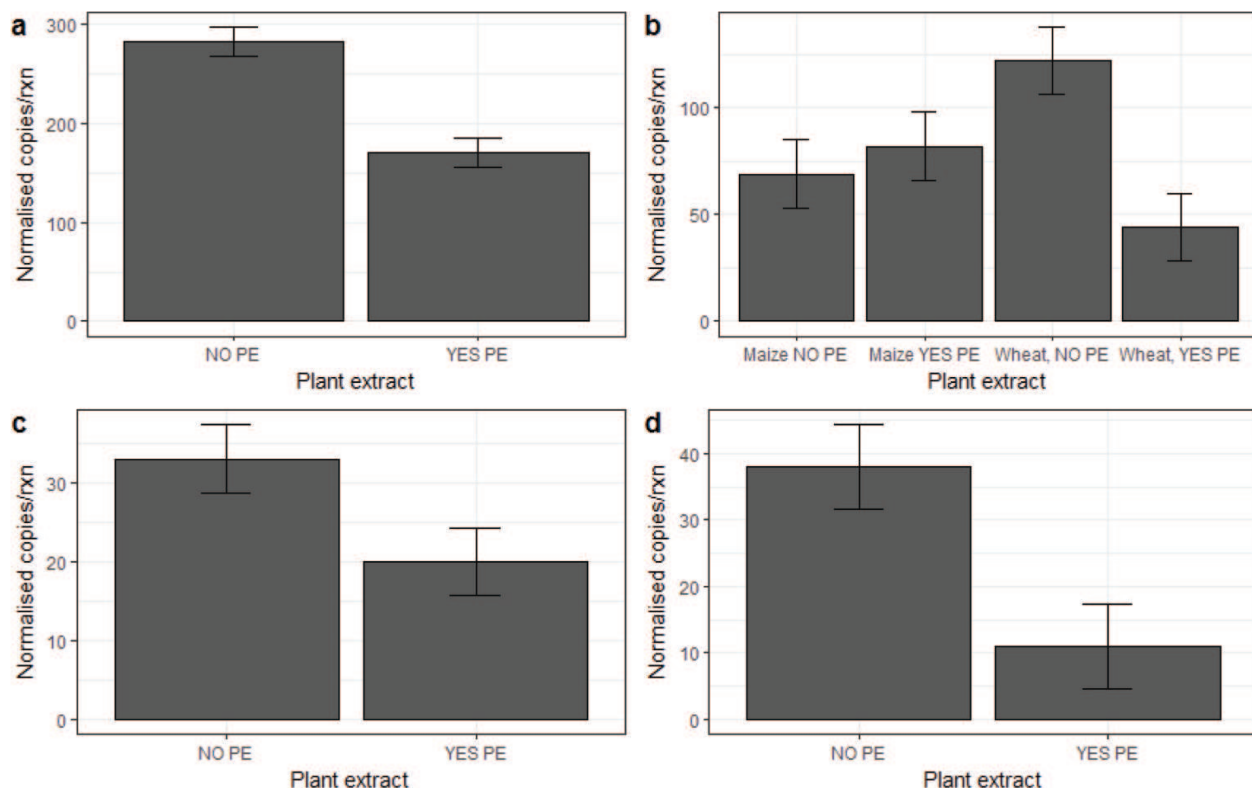

Figure 2. The effect of dietary plant extracts (PE) on the normalised mRNA copy number (per reaction) of (a) CD40 LG, (b) IL-12B, (c) INFG, (d) IL-6 in chicken caecal tonsils. Error bars represent ±1 pooled SEM.

for product specificity (single peak) and the presence of primer dimers. All primers were designed to be intron-spanning so that any residual gDNA present could not be detected and avoided known SNP and secondary structure. Assays were designed by qStandard (www.qstandard.co.uk) and were tested for specificity by electrophoresis, efficiency >95%, sensitivity to 10 copies/rxn, and linearity over 7 log by qPCR. Copy numbers/reaction were derived from the standard curves using the Rotor-Gene software. The four reference genes identified as the most stable using geNorm software were B2M, GAPDH, PPIA and YWHAZ. The normalisation factor for each sample was used to normalise GOI copy numbers per reaction.

3.6. Statistical analysis of data

Data were statistically analysed by two way analysis of variance (ANOVA) using a 2 × 2 factorial arrangement of treatments, blocked by experiment. The main effects were the cereals (maize and wheat) and additives (with and without PE). All data were processed using the procedure of Genstat (18th Edition) statistical software (IACR, Rothamstead, Hertfordshire, UK). In all instances, differences were reported as significant at $P < 0.05$. Graphics (**Figure 2**) were produced in "ggplot2" package version 2.2.1. [41] using R version 3.4.1. [42].

4. Effect of PE on bird growth performance and dietary available energy

Dietary PE supplementation significantly improved ($P < 0.05$) gain to feed (G:F) ratio by 2 points and dietary NE by 0.34 MJ (**Table 2**). No changes ($P > 0.05$) were observed in dietary ME due to PE supplementation. The increase in feed efficiency is in agreement with the ability of spices and mixtures of spices to increase bile secretion, activity of the pancreatic, and brush border enzymes [43, 44]. Maize based diets produced higher ($P < 0.05$) daily FI and ME, although wheat based diets had higher NE ($P < 0.001$). The values of ME and NE were in similar to previous reports [9, 45]. In agreement with [24], there were dietary type x PE interactions ($P < 0.05$) observed in bird growth, as birds fed wheat diets did not respond ($P > 0.05$) to PE supplementation. Similar tendency ($P = 0.074$) was observed for daily feed intake. Compared to maize, wheat contains more water-soluble non-starch polysaccharide (NSP), a carbohydrate complex possessing antinutrient activity, which may reduce dietary nutrient availability [46], thus explaining the reduced performance of birds fed wheat based diets. The observed interaction may also be due to the relatively high fat content of the wheat compared to maize based diets, and not to the cereals alone. Widening the dietary ME to protein ratio is likely to affect body fat retention more than bird growth performance, suggesting an explanation for the inconsistency between weight gain and NE of birds fed wheat based diets. However, the impact of dietary formulation (cereals, protein sources, fat content etc.) on the effectiveness of supplementary PE in poultry nutrition warrants further investigation.

Although there is a lack of consistency between growth performance and dietary ME, this is in agreement with many studies [8, 10, 11] but is in disagreement with others [9]. The

Items treatment factor	FI[1] (g DM/b/d)	WG[1] (g/b/d)	G:F[1] (g/g)	ME[1] (MJ/kg DM)	NE[1] (MJ/kg DM)	Vit E[2] (μg/g)	CoQ_{10}[2] (μg/g)
Cereals							
W	42.1	31.7	0.753	14.05	10.00	82.4	91.4
M	43.6	32.6	0.747	14.23	9.47	86.7	79.4
PE							
no	42.1	31.1	0.739	14.08	9.56	72.5	74.7
yes	43.6	33.2	0.762	14.05	9.90	96.7	96.1
SEM	0.496	0.460	0.0064	0.061	0.092	7.03	9.80
Cereals & PE							
W + 0	42.0	31.4	0.746	13.93	9.76	63.7	77.1
W + PE	42.2	32.0	0.760	14.16	10.23	101.2	105.8
M + 0	42.3	30.8	0.732	14.22	9.37	81.3	72.3
M + PE	45.0	34.4	0.763	14.24	9.57	92.2	86.4
SEM	0.702	0.651	0.0091	0.087	0.127	9.95	13.85
Probabilities of statistical differences							
Cereals	0.030	0.166	0.499	0.037	<0.001	0.542	0.221
PE	0.037	0.002	0.015	0.159	0.008	<0.001	0.032
Cereals x PE	0.074	0.030	0.347	0.211	0.309	0.062	0.458

Source: adapted from [47–51].

W, wheat-based diet; M, maize-based diet; PE, supplemental plant extracts (100 g PE/t).

[1]There were 38 observations per treatment (three experiments involving male Ross 308 broilers).

[2]There were 24 observations per treatment (two experiments involving male Ross 308 broilers).

Table 2. The effect of supplemental plant extracts in wheat and maize based diets on broilers daily feed intake (FI), daily weight gain (WG), gain to feed (G:F) ratio, dietary apparent metaboilisable energy (ME), dietary net energy (NE), concentration of hepatic vitamin E and coenzyme Q_{10}.

results show an improvement in feed efficiency in association with improved NE but not with ME. The beneficial effects of supplementary PE to poultry diets may therefore be mediated via a decrease in the energy required for maintenance, thereby providing more energy for growth. The improvement in feed efficiency is likely explained by increases in dietary NE, suggesting that PE may be improving the metabolic efficiency of the conversion of energy into tissue. Usually NE is described as the ME of the diet corrected for the energy losses that result from the heat released during absorption of the dietary nutrients and accretion of body mass [35]. Changes in maintenance energy are more likely to be detected by determination of NE but not ME. Thus confirming that dietary ME may not be the most sensitive method to

evaluate the feeding quality of supplementary PE. This is in agreement with previous reports suggesting that NE is a more meaningful measure of energy utilisation with regard to prediction of the nutritive value of poultry diets [35].

5. Effect of PE on hepatic antioxidant content

Dietary PE supplementation significantly improved hepatic vitamin E ($P < 0.001$) by 33.4% and coenzyme Q_{10} ($P < 0.05$) by 28.6% (**Table 2**). No changes ($P > 0.05$) were observed in hepatic antioxidants content due to dietary type ($P > 0.05$), although the response of wheat based diets tended ($P = 0.062$) to be higher compared to maize based diets. More importantly, the increase in G:F and NE is coupled with the increase in the hepatic concentrations of vitamin E and coenzyme Q_{10} [48].

Infectious diseases have been demonstrated to reduce tissue antioxidants [52], suggesting that higher concentrations of vitamin E and coenzyme Q_{10} in liver may decrease the challenge provoked by infectious diseases. In addition, feeding a combination of PE in the current study resulted in not only improved feed efficiency, but also increased hepatic antioxidants retention compared with the non-supplemented diet. When birds are reared under commercial farm conditions, where the potential for challenge is greater, and fed diets supplemented with PE, then there may be improvements to their overall nutrition, antioxidant and health status, and resistance to diseases [48]. The improvements observed may indicate that vitamin E and coenzyme Q_{10} may be effective at reducing production and effects of free radicals [53]. Coenzyme Q_{10} is provided by the diet, however significant levels are also produced in the body. Increased concentration of coenzyme Q_{10} in the liver of the growing chickens is therefore likely the result of dietary PE supplementation and dietary sources should thus be considered beneficial at improving the antioxidant status. It has also been reported [13] that PE, increased the activity of the antioxidant enzymes of the mucosal cells of rats, thus reducing the intestinal cell damage and cell turnover and sustaining the integrity of the intestinal mucosal layer.

6. Effect of PE on the immune status of birds

As shown in **Figure 2** the expression of CD 40 LG, IFN-G, and IL-6 was reduced ($P < 0.001$ and $P < 0.05$, respectively) in birds fed PE compared to the control fed chickens in accordance with other reports [18, 19]. There was a cereal X PE interaction for IL12B, showing that dietary PE reduced IL-12B expression in a wheat but not in a maize based diet ($P < 0.05$). Both, IL-6 and IFN-G, are major pro-inflammatory cytokines, so if the levels of these cytokines are decreased this would indicate that there are lower levels of inflammation than in the other groups, presumably due to plant extracts. Birds fed with diets supplemented with the same PE mixture also reduced the expression of CD40LG and IL-12B genes. The IFN-G cytokine belongs to the T helper (Th) type 1 response and is driven by IL-12 production. Th1 type response drives the cell mediated inflammatory responses largely to intracellular pathogens [54] but chronically

high levels of these cytokines in the intestine may have a damaging effect on the gut integrity, compromising nutrient absorption and overall gut health. The results of this study suggest that feeding PE may dampen chronic gut inflammation that may be partially attributed to the improved feed efficiency and dietary net energy.

7. Conclusions

Plant extracts can be used as growth promoters in poultry production independent of enzyme supplementation. Dietary PE may maximise the nutritional value of feed through improving gut health by reducing intestinal inflammation. Their mode of action may therefore be associated with improved immune status of the birds. This immune modulating effect of PE may explain improvements in growth performance and dietary NE seen in the present study. However, dietary supplementation with PE may improve bird growth performance without corresponding improvements in dietary ME. Studies that have focused solely on the effect of PE on ME alone, may not have detected their full benefit to improve the efficiency of broiler meat production. More research is needed to study the effect of supplementary PE on immune status of birds in relation to dietary available energy and growth performance of birds in commercial conditions using different practical feed formulations.

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Conflict of interest

No conflicts of interest.

Notes

Chemical structure (2-D) images (**Figure 1**) reproduced from PubChem (see [55 – 57]).

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