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Dietary essential oils improve feed efficiency and hepatic antioxidant content of broiler chickens

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Short title: Feeding essential oils to broilers

Abstract

The aim of this study was to test the hypothesis of an improved growth, dietary nutrient availability, and overall health of broiler chickens reared on recycled litter when fed a standardised combination of essential oils (EO; carvacrol, cinnamaldehyde and capsicum oleoresin). To assess the effect of dietary treatments, feed intake, weight gain, feed efficiency, availability of dietary nutrients and energy, villus morphometry,

excreta sialic acid concentration, hepatic antioxidants, and serum amyloid A (SAA) when fed to broiler chickens were evaluated. Counts of *Eimeria* spp. oocysts were also determined in excreta samples. Four experimental diets were offered, including two basal control diets based on either wheat or maize that contained 215 g crude protein/kg and 12.13 MJ/kg metabolisable energy and another two diets using the basal control diets supplemented with the EO combination at 100 mg/kg diet. Each diet was fed to eight floor pens, containing two birds each, following randomisation. Birds fed the EO supplemented diets had an improved ($P < 0.05$) feed conversion ratio (FCR). Birds fed maize-based diet had an improved daily weight gain and FCR (P<0.05) compared to wheat-fed birds. Wheat-based diet tended ($P = 0.056$) to have higher N-corrected apparent metabolisable energy (AMEn) and had higher fat retention coefficient (FR) ($P < 0.05$) compared to maize-based diets. No differences ($P > 0.05$) were observed in villus morphometry, sialic acid secretion, number of oocysts and SAA. Feeding the EO improved ($P < 0.05$) the retention of dietary Ca and Na. Compared with maize, feeding wheat-based diets improved the retention coefficients for Ca, P and Na ($P < 0.05$). Feeding dietary EO improved ($P < 0.05$) the concentrations of the hepatic antioxidants, including carotene, coenzyme Q_{10} and total vitamin E. The hepatic concentration of carotene of the maize-fed birds was 55.6% higher ($P < 0.05$) compared to the wheat-fed birds. These results demonstrated that the addition of a standardised combination of EO in wheat and maize based diets provided benefits in terms of feed efficiency, mineral retention and antioxidant status of the birds when reared on recycled litter.

Keywords: essential oils, broilers, growth performance, hepatic antioxidants, rearing hygiene status

Implications

Experimental comparisons of the nutritional value of essential oils is often performed under relatively high hygiene status, even though the large-scale broiler producers rear birds in houses with relatively high stocking density and lower hygiene status. Essential oils can influence intestinal microflora, immune responses, and animal health, thus their impact may differ between rearing conditions. This information helps to inform the poultry industry of the benefit of using standardised essential oil combinations for inclusion in broiler chicken feeds, reared under relatively low hygiene status provided by recycled litter.

Introduction

Phytogenic feed additives are plant-derived products (also referred to as essential oils, phytogenics, phytobiotics) used in animal feeding to improve the performance of agricultural livestock (Windisch *et al*., 2008). Although the number of scientific publications on phytogenic feed additives significantly increased over the last two decades, the knowledge regarding their modes of action and aspects of application is still rather limited. Most experiments involving plant extracts in poultry have studied separately their impact on production performance (Iskender *et al.*, 2017), dietary nutrient and energy availability (Bravo *et al*., 2011 and 2015), intestinal microflora (Altop *et al*., 2017), immune responses (Lee *et al.*, 2011), animal health (Uyar *et al.*, 2016), however, very few of them studied the impact of the rearing conditions on the aforementioned variables.

77 Research by Pirgozliev *et al*. (2014) suggested that the efficiency of dietary essential
78 oils (EO) may be influenced by the hygiene-status of poultry houses. Thus, suggesting
79 that the increased levels of normal flora and opportunistic pathogens from the litter
80 flooring may have an impact on the studied variables. Previous studies indicated that
81 certain EO might have beneficial effects on animal performance and health status
82 because of other properties besides their respective functional characteristics
83 (Windisch *et al.,* 2008). Report by Burt (2004) have shown that EO, including
84 carvacrol, cinnamaldehyde and capsicum oleoresin, *in vitro* exhibit antibacterial and
85 antimicrobial effects. EO have also been reported to improve animal performance
86 because of their stimulating effect on pancreatic and intestinal enzyme activity, on bile
87 flow and bile acid secretion, or by a direct bactericidal effect on potential pathogen
88 microorganisms of the gut microflora (Hardy, 2002). Moreover, mixtures of spices
89 exhibited an additive effect regarding their pancreatic enzyme stimulation compared
90 with the spices taken individually (Platel *et al*., 2002). In addition, EO supplementation
91 would affect some components of gut health and intestinal barrier, including intestine
92 structure, bacteria populations and microbial metabolites released in the gut lumen
93 (Lee *et al*., 2011; Salami *et al.*, 2016; Altop *et al*., 2017). Based on this we
94 hypothesised that beneficial effects of EO are more pronounced under less hygienic
95 housing conditions, e.g. microbial loading in the litter.
96 Therefore, the objectives of the current study were to investigate the effect of a
97 commercial mixture of EO on the performance, available energy, mineral and nutrient
98 utilisation, digestive tract variables, antioxidative status and inflammation when fed to
99 broilers reared on recycled litter.
100
101 **Material and methods**

Diet formulation

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) (Table 1). Barley and rye were included in the diet formulation to enhance the detection of differences between treatments, due to their non-starch polysaccharide (NSP) content. The other two diets were the control diets supplemented with a standardized combination of essential oils (XTRACT 6930; Pancosma S.A., Geneva, Switzerland; thereafter EO) including 5% carvacrol, 3% cinnamaldehyde and 2% capsicum oleoresin (100 grams per tonne, respectively, i.e. WC+EO; MC+EO). The EO were 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.

Husbandry and sample collection

Sixty four male Ross 308 day-old chickens were used in the study. The study was approved by the Harper Adams University Research Ethics Committee. From day old to 7d age all birds were reared in a single floor pen and fed a proprietary chicken starter feed that did not contain any coccidiostat or antimicrobial growth promoters, prophylactic or other similar additives. The birds were vaccinated for Infectious Bronchitis at the hatchery.

On the first day of the experiment (7 d of age), all chicks were weighed and allocated to one of 32 pens, two birds in a pen. Each of the thirty two pens has a solid floor with an area of 0.16 m^2 that was covered with recycled wood shavings. The recycled litter

material was from a previous flock reared for 42 days in the National Institute of Poultry Husbandry, Harper Adams University, which had no obvious health problems, although some sub-clinical necrotic enteritis, coccidiosis or presence of some other pathogens was possible. It has been assumed that the use of recycled litter may impose some additional stress on the birds and may emphasise the effect of the fed mixture of essential oils. Each diet was offered to birds housed in one of eight pens in a randomised complete block design. The temperature was kept at 29°C at 7 d of age and was gradually reduced to 21°C at the end of the 14 d feeding period (21 d of age). The light regimen was 18 h light and 6 h dark. At 17 d of age, the solid floor of each pen was replaced with a wire mesh 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 at the beginning, at 7 day old, and at the end of the study, at 21 d old, and the average bird feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were determined. Although the feeding period was 14 days, the birds were in contact with the litter for 10 days only, from 7 to 17 days of age. At the end of the study, at 21 day old, one bird from each pen was stunned and then killed, and blood and ileal intestinal samples from one birds per pen were collected for analysing serum amyloid A (SAA), an acute phase protein, and ileal villus morphometry, respectively. The liver from the same birds was collected and stored at –20°C for further analysis on antioxidants content.

Chemical analysis

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 x N) in samples was determined by dry combustion method (AOAC 2000) using a LECO (FP-528N, Leco Corp., St. Joseph, MI). Oil (as ether extract) was extracted with diethyl ether by the ether extraction method (AOAC 2000), 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), and benzoic acid was used as the standard. Minerals in the samples were determined by inductively coupled plasma emission spectrometry, ICP (Optima 4300 DV Dual View ICP-OE spectrometer, Perkin Elmer, Beaconsfield, UK) (Tanner et al., 2002). The N-corrected apparent metabolisable energy (AMEn) of the diets was calculated as described by Hill and Anderson (1958). The coefficients of total tract fat (FR) and mineral retention, dry matter retention (DMR), and nitrogen retention (NR) were determined as the difference between intake and excretion of the nutrient divided by its respective intake.

The concentration of sialic acid in excreta was determined by the periodate - resorcinol method as described by Jourdian *et al*. (1971).

Concentration of total carotenoids in diets and liver, hepatic coenzyme Q_{10} and vitamin E (α-, γ- and ϭ-tocopherols) were determined as previously described (Karadas *et al*., 2006, 2014).

The Serum Amyloid A (SAA) in blood collected post mortem was determined by a solid phase sandwich enzyme linked immuno sorbent assay (ELISA) using the Tridelta Phase™ (Tridelta Development Limited, Co. Kildare, Ireland) range SAA kit, according to manufacturer recommendations.

Ileal villus morphometry

Approximately 4 cm of the middle part of the ileum, between the Meckel's diverticulum and the ileoceacl junction, of one of the birds was sampled and stored in 10% formalin-buffered saline. The samples then were embedded in paraffin wax, sectioned at approximately 5 µm, and 3 gut segments were fixed in each slide. Morphometric measurements were determined on 20 intact well-oriented villus–crypt units for each slide (microscope Microtec, TEC Microscopes LTD, Axbridge, UK; CCD camera Infinity 2, Lumenera Corporation, Ottawa, Canada; Image analysis software, Infinity Analyse – Infinity 2-2 for Windows version 6.5.2, Lumenera Corporation, Ottawa, Canada) as previously described (Abdulla *et al*., 2017).

Oocyst counts

Counts of *Eimeria* spp. oocysts were determined in excreta samples taken from each pen at 16 days of age, 9 days after beginning of the experiment. Sampling was carried out by collecting about 20 g samples of excreta, two times per day from each pen. Samples collected from each pen were placed in separate tub, homogenized thoroughly by a mixer, and kept refrigerated for two days, until assessed for total oocyst counts. Homogenized samples were ten-fold diluted with tap water to be further diluted with saturated NaCl solution at a ratio of 1 : 10. Oocyst counts were determined using McMaster chambers and presented as the number of oocysts per g of excreta (Hodgson, 1970).

Statistical procedures

202 Statistical analyses were performed using the Genstat statistical software package
203 (Genstat 18th release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The
204 AMEn content of the experimental diets, broiler growth performance and nutrient
205 digestibility were compared statistically by ANOVA using a 2 × 2 factorial arrangement
206 of treatments. The main effects were the cereal type (wheat and maize) and the EO
207 supplementation (with and without). The comparison between the experimental results
208 was performed by ANOVA. In all instances, differences were reported as significant at
209 $P < 0.05$. When a significant F test was detected, means were separated using the
210 Fisher's protected LSD. Tendencies towards significance ($0.05 < P < 0.1$) were also
211 reported.
212
213 **Results**
214 The analysed chemical composition of the basal diet is shown in Table 1. The analysed
215 dietary protein content was lower than expected, and the analysed Ca content was
216 lower than expected in the wheat-based diets in particular.
217 Birds remained healthy throughout the study period and there was no mortality. The
218 weight of the birds fed maize-based diets was 0.690 kg, or 12% heavier ($P < 0.05$)
219 than the weight of the birds fed wheat-based diet, i.e. 0.616 kg (data not included in
220 tables). The overall liver weight was 15.6 g and was not influenced by dietary
221 treatments ($P > 0.05$), although when expressed as per cent of the body weight, the
222 liver of the birds fed maize was 2.32% and of those fed wheat was 2.46% ($P < 0.05$;
223 data not included in tables).
224 Table 2 shows the data on growth performance, metabolisable energy and
225 nutrient utilisation coefficients. Birds fed maize-based diet had higher daily weight gain
226 and reduced FCR ($P < 0.05$) compared to wheat fed birds. However, wheat-based diet

tended (P = 0.056) to have higher AMEn and had higher FR (P < 0.05) compared to maize-based diets. Birds fed the EO supplemented diets had a lower FCR, using 56 g less feed to produce a kilogram of growth (P < 0.05). Daily FI, DMR and NR were not influenced (P > 0.05) by dietary treatments.

Data on morphological variables of the ileum and excreta sialic acid concentration are presented in Table 3. There were no differences (P > 0.05) in villus height, crypt depth and the ratio between them due to EO supplementation or cereal inclusion. Sialic acid concentrations were not affected (P > 0.05) by dietary treatments. The overall oocysts count in excreta was relatively low, i.e. 5119 eggs per gram fresh excreta, and not affected (P > 0.05) by cereal type or EO supplementation.

Results on dietary mineral retention coefficients are presented in Table 4. Feeding EO improved the coefficients of Ca and Na retention (P < 0.05). Feeding wheat-based diets improved the retention coefficients of Ca (P < 0.05), P (P < 0.05) and Na (P < 0.001).

Data on hepatic antioxidants and SAA in blood are presented in Table 5. Feeding dietary EO improved (P < 0.05) the concentrations of hepatic vitamin E by 53.2%, carotene by 34.3% and coenzyme Q_{10} by 19.2%, respectively. The hepatic concentration of carotene of the maize-fed birds was 55.6% higher (P < 0.05) compared to the birds fed wheat-based diets. No dietary impact (P > 0.05) on SAA in blood samples was observed.

Discussion

The analysed dietary protein and Ca contents differed from the calculated values. This was probably due to differences between the composition of the actual ingredients

that were used in the present study and the values in the database used by the software for the dietary formulations.

Essential oils, such as carvacrol, cinnamaldehyde and capsicum oleoresin, when supplemented to diets, are known to exert positive effects on the performance and nutrient utilisation in broilers reared in houses with poor hygienic status (Pirgozliev *et al*., 2014). These effects are likely mediated by a gastrointestinal microbial modification, promoted local protective immunity against *Eimeria* infection, improved hepatic antioxidative status, dietary energy and nutrient utilisation (Lee *et al*., 2011; Karadas *et al*., 2014). The major findings of the current study are the improved FCR and hepatic antioxidant status of the birds fed the EO in both wheat and maize based diets. However, Jamroz *et al*. (2006) using the same blend of EO, reported EO x cereal type interaction on FCR from 0 to 21d age. The EO reduced the FCR in maize fed birds, although there was no effect in wheat fed birds, as overall wheat produced lower FCR than maize. This coupled with reducing the crypt depth in maize fed, but not in wheat fed birds (Jamroz *et al*., 2006). The intestinal villus morphometry may be linked to the gut health of the birds. Changes in intestinal morphometry such as reduced villous height to crypt depth ratio, involving shorter villi and/or deeper crypts, have been associated with the presence of toxins or higher tissue turnover and poor growth performance (Xu *et al*., 2003). The length of the villus in the present study was in the expected range (Abdulla *et al*., 2017), but there were no significant differences in villus morphometry.

Comparing mash *vs* pelleted diets, Pirgozliev et al. (2016) found over 20% lower weight in birds fed mash diets, thus partially explaining the relatively low bird weight. The low dietary protein, inclusion of rye and barley, and the rearing conditions (i.e. recycled litter) probably also contributed to the lower than expected bird performance

observed in this study. In the present study, the birds fed maize based diets had an improved daily weight gain and FCR but had lower AMEn and FR coefficient. The improved growth performance of birds fed maize may partially be explained by the fact that compared to wheat, maize contains less water-soluble non-starch polysaccharides - a carbohydrate complex possessing antinutrient activity (Annison et al., 1996). Research by Bozkurt et al. (2012) further supports our findings and addresses the impact of NSP on efficacy of the EO. The opposite of those findings, Jamroz *et al*. (2006) reported no difference in the bird weight gain when maize or wheat based diets are fed. The use of different dietary formulations, rearing conditions and strains of birds may be a reason for the observed differences in response to EO in different studies.

It has been demonstrated that an increase of unsaturated fats in diets improves digestibility of fat and dietary metabolizable energy (Danicke *et al*., 1999). Indeed, the wheat based diet contained more vegetable oil, compared to maize based diet, thus explaining the response. However, the total fat content in the diet is relatively low (less than 5%), therefore the difference in fat retention observed is unlikely to cause major differences in growth performance. The ME and nutrient retention response to supplementary EO varies between different reports (Bravo *et al*., 2011 and 2014). The inconsistence may be due to different dietary compositions and experimental conditions (Amad *et al.*, 2011). In addition, relatively small differences in dietary metabolisable energy are not always directly consistent with growth performance of birds (Abdulla *et al*., 2017), thus a lack of correlation in the responses to growth and dietary AMEn might be expected.

The number of oocyst output per gram excreta in the reported study is relatively low compared to *Eimeria* infected birds (Chapman *et al*., 2002) and this suggests that

301 there was no major disease challenge to the birds. This is also indicated by the lack
302 of response of SAA to dietary treatments in this study. The SAA is a major acute-
303 phase protein of the chicken, and is produced predominantly by the liver as a systemic
304 manifestation of the body's response to inflammation (Eckersall, 2000). The very low
305 concentration of SAA was in agreement with Chamanza *et al*. (1999) and showed that
306 no acute inflammation occurs in the chicks. This very low SAA concentration in blood
307 may also be a reason for the lack of impact of the EO blend fed to the birds in this
308 study.

309 Recent research (Amad *et al*., 2011) demonstrated that feeding phytogenics
310 improves the digestibility of dietary minerals, including Ca, P and crude ash in poultry.
311 Hosseini *et al*. (2013) also reported an increase in blood Ca and P concentrations in
312 broilers fed phytogenics. The results on mineral digestibility of the present report are
313 in the expected range (Scholey *et al*., 2017). The improved digestibility of Ca, K and
314 Na in EO fed birds coincided with higher hepatic antioxidant content of the birds. It can
315 be hypothesised that dietary EO, in combination with the relatively low dietary levels
316 of these minerals, are likely reasons for the better absorption and digestion reported.
317 The improved hepatic antioxidant content suggests reduced oxidative stresses of the
318 birds (Karadas *et al*., 2014). This favours gut health and overall animal health and can
319 at least partially explain the observed results. The improved digestibility of Ca, P and
320 Na in wheat-based diets however may also be attributed to the relatively low feed
321 intake of the birds, and the positive impact of the additional dietary fat (Danicke *et al*.,
322 1999). Birds fed XT had an increase in Na retention coefficient by 12.7%. Improved
323 Na retention (reduced exertion) was also reported in phytase fed broilers due to
324 phytate hydrolysis and reduced irritation of the gastrointestinal tract (Pirgozliev *et al*.,
325 2009). As the primary cation in extracellular fluids in animals and humans, sodium is

physiologically important and involved in maintaining the fluid and electrolyte balance in the body (Amad *et al*., 2011; Hosseini *et al*., 2013), thus further research clarifying the impact of EO on Na and mineral bioavailability in general is warranted.

In agreement with previous research (Karadas *et al*., 2014), feeding the experimental combination of EO improved the hepatic concentration of antioxidants, including carotene, coenzyme Q_{10} and total vitamin E. It is assumed that the diets are the main determinant of the carotenoid composition in liver tissue (Karadas *et al*., 2006). The improved carotenoid concentration in the liver of EO-fed birds suggests that the supplement either increases carotenoid absorption, or for some reason reduces oxidative stresses, thereby preventing carotenoid reserves from depletion, or perhaps a combination of the two. Overall feed intake did not differ between treatments, suggesting that efficiency of absorption and/or deposition was higher or reduced metabolism occurred. In agreement with previous research (Karadas *et al*., 2006 and 2014) there was an increase in the concentrations of the carotenoids, coupled with increased concentrations of coenzyme Q_{10} and vitamin E (in this study). This suggests that the involvement of the carotenoid in antioxidant interactions within the liver of growing chickens cannot be excluded. The improvements seen may indicate that the antioxidants may be effective at reducing production and effects of free radicals (Salami *et al.*, 2016). Coenzyme Q_{10} can be obtained from the diet but, more importantly, it is synthesised in the body. Therefore, an increased concentration of coenzyme Q_{10} in the liver of the growing chickens as a result of dietary EO supplementation could be considered beneficial. Indeed, this is coupled with an improved feed efficiency. In addition, Dhuley (1999) also reported that carvacrol and cinnamaldehyde, components of the EO mixture used in this study, increased the activity of the antioxidant enzymes of the mucosal cells, thus reducing the intestinal

cell damage and cell turnover and sustaining the integrity of the intestinal mucosal layer. Greater concentrations of antioxidants in body tissues, e.g. liver, may also improve health status of the birds and decrease the challenge provoked by infectious diseases (Salami *et al*., 2016). The improved hepatic carotenoid concentration in maize fed birds may be explained with the higher carotenoid content in maize compared to wheat (Panfili *et al*., 2004).

In conclusion, data from this study indicate that a standardised dietary combination of EO, including carvacrol, cinnamaldehyde, and capsicum oleoresin, improved the nutritional value of wheat and maize based diets, when fed to broiler chickens. Although there was no effect of the EO inclusion on the oocysts exertion from the birds, there was no evidence of coccidial challenge in the birds rearing environment, i.e. recycled litter. Differences in variables between maize and wheat diets are associated with differences in chemical composition between cereals and different amounts of dietary oil. These results demonstrated that the addition of EO in wheat and maize based diets provided benefits in terms of feed efficiency, mineral availability and antioxidant status of the birds.

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535 **Table 1.** *Composition of the control diets*
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Dietary ingredients	Wheat-based diet kg/100kg	Maize-based diet kg/100kg
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
Determined analysis (air dry)[2]		
Dry matter g/kg	883	884
Crude protein g/kg	188.2	195.3
Crude fat g/kg	46.2	33.2
Gross energy MJ/kg	16.69	16.27
Ca g/kg	6.5	8.0
P g/kg	5.6	5.7
Mg g/kg	1.6	1.4
K g/kg	9.4	8.7
Na g/kg	1.1	1.1

537
538 ME = metabolisable energy
539 [1] The Vitamin and mineral premix contained vitamins and trace elements to meet the requirements
540 specified by the National research Council (1994). The premix provided (units/kg diet): retinol, 12 000
541 IU; cholecalciferol, 5000 IU; α-tocopherol, 34 mg; menadione, 3 mg; thiamine, 2 mg; riboflavin, 7 mg;
542 pyridoxine, 5 mg; cobalamin, 15 μg; nicotinic acid, 50 mg; pantothenic acid, 15 mg; folic acid, 1 mg;
543 biotin, 200 μg; 80 mg iron as iron sulphate (30%); 10 mg copper as a copper sulphate (25%); 100 mg
544 manganese as manganous oxide (62%); 80 mg zinc as zinc oxide (72%); 1 mg iodine as calcium iodate
545 (52%); 0.2 mg selenium as sodium selenite (4.5%); 0.5 mg molybdenum as sodium molybdate (40%).
546 [2]Analyses were performed in duplicate.
547

Table 2. *The effect of diet formulation and EO on feed intake (FI), weight gain (WG), feed conversion ratio (FCR), total tract dry matter retention (DMR), nitrogen retention (NR) and fat retention (FR) coefficients when fed to broiler chickens from 7 to 21d age*[1]

Treatment factor	FI (g DM/b/d)	WG (g/b/d)	FCR (g/g)	AMEn (MJ/kg DM)	DMR	NR	FR
Cereals							
Wheat	46	33	1.365	11.86	0.772	0.679	0.819
Maize	49	38	1.297	11.62	0.769	0.690	0.758
EO							
No	47	35	1.359	11.71	0.771	0.691	0.794
Yes	48	37	1.303	11.77	0.770	0.679	0.784
SEM[2]	1.3	1.1	0.0161	0.086	0.0049	0.0064	0.0150
Probabilities of statistical differences							
Cereals	0.087	0.009	0.007	0.056	0.667	0.251	0.009
EO	0.718	0.228	0.023	0.592	0.939	0.183	0.625
Cereals x EO	0.076	0.079	0.687	0.193	0.064	0.086	0.824

EO = a commercial bled of essential oils.
[1]Each value represents the mean of eight replicates.
[2]Pooled standard error of mean.

558

559 **Table 3.** *The effect of diet formulation and EO on morphological parameters of the*
560 *ileum in 21 d old broiler chickens*[1]

Treatment factor	Villus height (µm)	Crypt depth (µm)	Height/ Depth	SA c DM	SA tot	Oocysts excretion (g excreta)
Cereals						
Wheat	557	62	9.2	0.887	58.2	5029
Maize	544	63	8.9	0.862	59.9	5208
EO						
No	529	60	9.0	0.880	57.6	5190
Yes	573	65	9.1	0.869	60.5	5047
SEM[2]	17.6	1.8	0.32	0.0352	3.24	1508.3
Probabilities of statistical differences						
Cereals	0.604	0.878	0.613	0.624	0.726	0.934
EO	0.098	0.095	0.676	0.826	0.542	0.947
Cereals x EO	0.345	0.904	0.330	0.841	0.109	0.983

561
562 EO = a commercial bled of essential oils.
563 [1]Each value represents the mean of eight replicates.
564 [2]Pooled standard error of mean.
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568 **Table 4.** *The effect of diet formulation and EO on dietary mineral retention coefficients*
569 *(data based on excreta collection from 17 to 21d age)*[1]

Treatment factor	Ca	Mg	P	K	Na
Cereals					
Wheat	0.619	0.311	0.589	0.368	0.828
Maize	0.516	0.298	0.533	0.364	0.696
EO					
No	0.533	0.322	0.555	0.384	0.716
Yes	0.601	0.287	0.567	0.348	0.807
SEM[2]	0.0205	0.0161	0.0130	0.0136	0.0208
Probabilities of statistical differences					
Cereals	0.002	0.554	0.006	0.850	<0.001
EO	0.029	0.146	0.515	0.078	0.006
Cereals x EO	0.126	0.397	0.251	0.440	0.243

570
571 EO = a commercial bled of essential oils.
572 [1]Each value represents the mean of eight replicates.
573 [2]Pooled standard error of mean.
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Table 5. *The effect of diet formulation and EO on the concentration of hepatic carotene, coenzyme Q_{10}, vitamin E and serum amyloid A (SAA) in blood, determined on 21 d old broiler chickens*[1]

Treatment factor	Carotene (µg/g)	Q_{10} (µg/g)	Vit E (µg/g)	SAA (µg/ml)
Cereals				
Wheat	0.243	69.7	69.6	2.669
Maize	0.378	68.4	65.7	2.932
EO				
No	0.265	63.0	53.4	2.846
Yes	0.356	75.1	81.8	2.755
SEM[2]	0.0272	3.30	5.64	0.1403
Probabilities of statistical differences				
Cereals	0.002	0.788	0.631	0.200
EO	0.029	0.018	0.002	0.653
Cereals x EO	0.096	0.394	0.668	0.281

EO = a commercial bled of essential oils.
[1]Each value represents the mean of eight replicates.
[2]Pooled standard error of mean.