A mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin improves energy utilization and growth performance of broiler chickens fed maize-based diet

D. Bravo,* V. Pirgozliev,†‡[1] and S. P. Rose‡

*Pancosma S. A., Geneva, Switzerland CH-1218; †Avian Science Research Centre, Scottish Agricultural College, Auchincruive, Ayr KA6 5HW, UK; and ‡The National Institute of Poultry Husbandry, Harper Adams University, Shropshire TF10 8NB, UK

ABSTRACT: A total of 210, 1-d-old Ross 308 male broiler chickens were used in an experiment to investigate the effects of a supplementary mixture containing 5% carvacrol, 3% cinnamaldehyde, and 2% capsicum on dietary energy utilization and growth performance. The 2 diets were offered ad libitum to the chickens from 0 to 21 d of age. These included a maize-based control diet and the control diet with 100 g/t of supplementary plant extracts. Dietary apparent ME, N retention (NR), and fat digestibility (FD) coefficients were determined in the follow-up metabolism study between 21 and 24 d of age. Feeding the mixture of carvacrol, cinnamaldehyde, and capsicum increased weight gain by 14.5% (P = 0.009), improved feed efficiency by 9.8% (P = 0.055), and tended to increase (P = 0.062) carcass energy retention and reduce (P = 0.062) total heat loss compared with feeding the control diet. There was a 16.1% increase (P = 0.015) in carcass protein retention but no difference in carcass fat retention. Feeding plant extracts improved dietary FD by 2.1% (P = 0.013) but did not influence dietary NR. Supplementation of plant extract resulted in a 12.5% increase (P = 0.021) in dietary NE for production (NEp), while no changes in dietary ME were observed. The experiment showed that although dietary essential oils did not affect dietary ME, they caused an improvement in the utilization of energy for growth. Plant extracts may affect metabolic utilization of absorbed nutrients. Studies that have focused solely on the effect of plant extracts on ME alone may well have not detected their full nutritional value.

Key words: chickens, energy metabolism, essential oils, net energy, plant extracts

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INTRODUCTON

To prevent the risk of developing pathogens resistant to antibiotics and also to satisfy consumer demand for a food chain free of drugs, the use of in-feed antibiotics in the European Union was banned in January 2006 (European Union, 2003), and this policy is being considered in other parts of the world. Consequently, the poultry industry seeks an alternative for antibiotics as growth promoters; one such alternative is the addition of plant extracts (essential oils) to poultry diets (Applegate et al., 2010; Wallace et al., 2010).

Most experiments involving plant extracts in poultry have studied their impact on growth performance, intestinal microflora, immune responses, and animal health, and very few of them evaluated the effect on dietary available energy (reviewed by Windisch et al., 2008; Wallace et al., 2010). Up to date, the experiments investigating the effect of plant extracts on available energy were performed using the ME system (i.e., dietary apparent ME). Although dietary ME is widely used to describe the available energy concentration in poultry feedstuffs, diets with the same ME are not necessarily used with equal efficiency when fed to poultry (Pirgozliev and Rose, 1999; Pirgozliev and Bedford, 2013). Work with plant extracts has shown that the improvement in performance is closely associated with immune alteration, intestinal microflora changes, and improvement in digestion and absorption of nutrients, although their influence on dietary ME per se has been inconsistent. Some authors found an increase in dietary ME in response to plant extracts (Mountzouris et al.,

[1]Corresponding author: vpirgozliev@harper-adams.ac.uk
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2010; Bravo et al., 2011), others (Juin et al., 2003; Cross et al., 2007) did not.

Dietary NE is the ME of the feed corrected for losses that result from the assimilation of the dietary ingredients, frequently termed the heat increment of digestion. The remaining NE is available for both maintenance and production. Net energy is a more meaningful measure of energy utilization with regard to prediction of the nutritive value of poultry diets (Pirgozliev and Rose, 1999; Pirgozliev and Bedford, 2013). However, to date, no data have been published on the effect of supplemental plant extracts on dietary NE for production (**NEp**).

Therefore, the objective of the present study was to quantify the responses of chickens fed plant extracts and reared in floor pens in dietary NE, determined by comparative slaughter technique. Growth performance variables, dietary N retention (**NR**), and fat digestibility (**FD**) coefficients and energy and nutrient metabolism were also determined.

MATERIALS AND METHODS

All procedures were approved by The Animal Experimental Committee of the Scottish Agricultural College.

Diet Formulation

A standardized combination of plant extracts (**XT**; XTRACT 6930; Pancosma S.A., Geneva, Switzerland), including 5% carvacrol, 3% cinnamaldehyde, and 2% capsicum oleoresin, was used in this study. A maize-based control diet (**CON**) was formulated to contain 215 g CP/kg and 12.13 MJ ME/kg (Table 1). The basal diet was then split into 2 equal batches and 1 of them was supplemented with 100 g of XT/t; **CON+XT**). The product was added to the diet in powder form and both diets were fed as mash. The diets did not contain any coccidiostat, antimicrobial growth promoters, prophylactic, or other similar additives.

Table 1. Ingredient composition of the control diet (as-fed)

Item	Content
Ingredient, g/kg	
Maize	528.6
Soybean meal (48% CP)	313.0
Vegetable oil	10.0
Barley	63.3
Rye	50.0
Monodicalcium phosphate	14.3
Limestone	11.5
NaCl	3.3
Lys HCl	1.5
Met	3.5
Vitamin mineral premix[1]	1.0
Total	1,000
Calculated analysis	
ME, MJ/kg	12.13
CP, g/kg	215
Crude fat, g/kg	34
Ca, g/kg	8.3
Nonphytate P, g/kg	4.4
Lys, g/kg	12.3
Met + Cys, g/kg	9.5
Analyzed values	
DM, g/kg	864
CP, g/kg	197
Crude fat, g/kg	35

[1]The vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by the NRC (1994). The premix provided (units/kg diet): retinol, 12,000 IU; cholecalciferol, 5,000 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 Fe as iron sulfate (30%); 10 mg Cu as a copper sulfate (25%); 100 mg Mn as manganous oxide (62%); 80 mg Zn as zinc oxide (72%); 1 mg I as calcium iodate (52%); 0.2 mg Se as sodium selenite (4.5%); and 0.5 mg Mo as sodium molybdate (40%).

Husbandry and Sample Collection

The present experiment used 210, 1-d-old Ross 308 male broiler chickens. At the beginning of the study, 10 chickens from the general group were selected at random and were killed by cervical dislocation and stored in a freezer at –20°C for analysis. During the first part of the study, all chickens were allocated to 20 floor pens with 10 chickens in each pen from 1 to 21 d of age. Each diet was offered ad libitum to chickens housed in 1 of 10 pens in a randomized complete block design. The room was kept at a temperature of approximately 31°C at d 0 and this was gradually reduced to approximately 20°C at the end of the 21-d feeding period. Relative humidity was maintained at about 50%. A standard lighting program for broilers was used, decreasing from 23:1 h (light:dark) from 1 d to 18:6 h at 7 d of age, which was maintained until the end of the study. The floor phase of the study ended when the chickens were 21 d of age. The chickens were group-weighed on a per-pen basis at the beginning and at the end of the study, and the average chick weight gain and G:F were determined.

At the end of the floor pen phase at 21 d of age, 4 chickens from each pen with a BW nearest to the pen average were selected. Two of the chickens were transferred to 1 of 20 metabolism cages following the same randomization and dietary treatments as in the floor pen phase. To maintain the effect of the floor pen rearing conditions, no adaptation period for cage housing was allowed. Feed and water were offered ad libitum. The chickens selected were kept in the cages for approximately 72 h until 24 d of age, and total excreta output were collected twice (following 36-h periods) in the trays beneath. Feed intake for

the same period was recorded for the determination of dietary NR and FD coefficients and ME.

The rest of the selected chickens, 2 from each pen, were provided with ad libitum access to water but feed was withdrawn for approximately 6 h before slaughter to minimize the contribution of undigested feed on the estimate of carcass energy retention. A comparative slaughter technique was used to determine retention of energy and nutrients. The chickens were killed by cervical dislocation and the carcass of the chickens, including intestines, blood, and feather, from each pen were frozen and then minced (Hobart A 200; The Hobart Mfg Co Ltd, London, UK). The minced carcass of chickens of each pen were pooled, thoroughly mixed and sampled, and used for the calculations. The carcass samples were freeze-dried, and carcass combustion energy content was determined and used for the calculations based on average pen bird weight. The same procedure was used for the carcass of 10 chickens taken at the start of the experiment, and the data were used to determine the carcass DM, protein, and fat and energy retention for the experimental period.

Chemical Analysis

The experimental diets and the excreta were analyzed for combustion energy content to determine dietary ME. Combustion energy was determined using a bomb calorimeter (Parr 6200; Parr Instruments Co., Moline, IL). The N content of feed and freeze-dried excreta and carcass samples was analyzed using the Kjeldahl method (Kjeltec 1035 Autoanalyser; Perstorp Analytical, Hoganas, Sweden; method 984.13, AOAC, 1994). The CP values were obtained as N × 6.25. The ether extract (**EE**) in the feed and the freeze-dried excreta and carcass samples was determined (Soxtec System; Foss UK Ltd., Warrington, UK; method 920.39, AOAC, 1994).

Calculations

Dietary ME (MJ/kg DM) was calculated as follows:

$$ME = (E\ int - E\ out)/feed\ intake,$$

in which E int is the GE (MJ/kg) intake of the chickens during the cage phase of the study, E out is the energy (MJ/kg) output of the chickens during the cage phase of the study, and feed intake is feed intake (kg DM) of the chickens during the cage phase. The coefficients of dietary NR and FD were obtained using the following equation:

$$\text{Retention or digestibility coefficient} = (\text{intake} - \text{excreted})/\text{intake},$$

in which intake is the intake of N or fat by the chickens during the cage phase and excreted is the N or fat excreted by the bird during the cage phase of the study.

To obtain data on energy metabolism of chickens, results from both pen and cage phases were used. The total energy retained in the carcass (REc; MJ) was calculated as follows:

$$REc = (E_{21} - E_0),$$

in which E_{21} is the total energy (MJ) of chicken carcass at 21 d old and E_0 is the total energy (MJ) of chicken carcass at the beginning of the experiment at day old. Dietary NE for production (NEp MJ/kg DM feed intake) was calculated using the following equation:

$$NEp = REc/FI,$$

in which FI is DM (kg) consumed from d 0 to 21. The efficiency of ME used for energy retention (Kre) was calculated as

$$Kre = REc/ME\ intake,$$

in which ME intake was the feed intake (kg DM) from 0 to 21 d old multiplied by determined ME (MJ/kg DM) of the diets.

Heat Production

The total heat production of the chickens from 0 to 21d old (HPt; MJ), which consists of the energy loss for tissue retention, maintenance, and the heat increment of production, was calculated as difference between dietary ME intake and REc:

$$HPt = ME\ intake - REc.$$

The NEp:HPt ratio was used as criteria for the use of heat production for body energy retention, assuming that a higher ratio indicates that most of the released heat was used for carcass energy retention and maintenance, instead for heat increment.

Statistical Analyses

For dietary ME, the experimental unit was the metabolism cage. For the rest of the observed variables, the experimental unit was the floor pen. Statistical analyses were performed with GenStat (11th ed.; Lawes Agricultural Trust, VSN International Ltd., Oxford, UK). The data were analyzed with ANOVA. The ME intake was used as a covariate in the analysis of energy utilization data because of the possible influence of variation in ME

Table 2. Effect of supplemental plant extracts on broiler chickens[1,2,3]

Item[3]	CON	CON+XT	SEM	*P*-value
FI, g DM/d	43.9	45.7	1.0	0.222
WG, g/d	28.2	32.3	0.9	0.009
G:F, g/g	0.644	0.707	0.020	0.055
NR	0.617	0.628	0.015	0.610
FD	0.844	0.862	0.004	0.013
ME, MJ/kg DM	14.51	15.03	0.21	0.122
ME intake, MJ/(chicken·d)	0.638	0.687	0.019	0.098

[1]CON = maize-based control diet; CON+XT = CON with supplemental essential oils (100 g XT/t; Pancosma S.A., Geneva, Switzerland).

[2]Based on feeding period from 0 to 21 d age for growth performance and from 21 to 24 d age for digestibility and energy metabolism and 10 observations per treatment.

[3]FI = feed intake; WG = weight gain; NR = N retention; FD = fat digestibility.

intake on the energy utilization response criteria. In all instances, differences were reported as significant at $P < 0.05$ and trends were noted when the *P*-value was less than 0.10.

RESULTS

The analyzed chemical composition of the basal diet is shown in Table 1. The analyzed protein content was less than the calculated values. The content of dietary EE was close to the calculated values.

All chickens remained healthy throughout the study period. Table 2 shows the data on growth performance of the chickens, dietary NR, FD, ME, and daily ME intake. Chickens fed the CON+XT diet had a 14.5 ($P = 0.009$) and 9.8% ($P = 0.055$) greater daily weight gain and G:F, respectively, than chickens fed CON diet. Plant extracts supplementation improved dietary FD by 2.1% ($P = 0.013$) but did not have an impact on NR. Feeding CON+XT did not influence daily DM intake and dietary ME but tended ($P = 0.098$) to increase dietary ME intake.

Table 3 shows the data on the variables describing the energy and nutrient metabolism of the experimental chickens. The retained total carcass CP and dietary NEp of the CON+XT diet were greater by 16.1 ($P < 0.05$) and 12.5% ($P < 0.05$), respectively, than those of CON. In agreement with these results, chickens fed CON+XT diet tended to have greater REc ($P = 0.062$) and lesser HPt ($P = 0.062$) than the chickens fed CON. However, dietary plant extracts did not have an impact on retained carcass fat, Kre, and NEp:HTp ratio.

The data of the determined body composition (as percent) is presented in Table 4. Feeding essential oils did not have any effect on the composition of the water, DM, protein, or fat in the carcass.

Table 3. Effect of supplemental plant extracts on energy and nutrient metabolism in broiler chickens[1,2,3]

Item[3]	CON	CON+XT	SEM	*P*-value
CPr, g/chicken	95.7	111.1	3.6	0.015
CFr, g/chicken	40.5	42.6	2.4	0.551
REc, MJ	3.84	4.29	0.15	0.062
Kre	0.279	0.310	0.012	0.112
NEp, MJ/kg DM	4.07	4.58	0.13	0.021
HPt, MJ	10.07	9.62	0.15	0.062
NE:HPt	0.420	0.484	0.027	0.129

[1]CON = maize-based control diet; CON+XT = CON with supplemental essential oils (100 g XT/t; Pancosma S.A., Geneva, Switzerland).

[2]Based on feeding period from 0 to 21 d age and 10 observations per treatment.

[3]CPr = retained carcass protein; CFr = retained carcass fat; REc = total energy retained in the carcass (in a bird from 0 to 21 d of age); Kre = efficiency of ME used for energy retention; NEp = dietary NE for production (carcass energy retained per kilogram feed intake); HPt = total heat production from 0 to 21 d of age.

DISCUSSION

The results obtained in the present study confirmed the stimulating growth and efficiency effect of the mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin (Jamroz et al. (2003; Bravo et al. 2011) . The positive change, that is, 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 (Platel and Srinivasan, 2001; Platel et al., 2002). The utilization of dietary lipids depends on adequate secretion of bile salts, which are essential for emulsification of fats and activation of lipase (Smits et al., 1998). The total fat content in the diet was only 3.4%; therefore, any treatment effects on fat availability were unlikely to cause major differences in growth performance.

Further partitioning of the chicken carcass into composition of gain showed that protein was responsible for the larger proportion of the carcass gain than fat, which is in agreement with previous reports (Jamroz et al., 2005). Lee-

Table 4. The effect of supplemental plant extracts on the composition (%) of the weight gain of broiler chickens[1,2]

Item	CON	CON+XT	SEM	*P*-value
Water	70.2	70.6	0.4	0.591
DM	29.8	29.4	0.4	0.591
Protein	16.2	16.4	0.2	0.557
Fat	6.9	6.2	0.4	0.292
Ash and carbohydrates[3]	6.7	6.8	0.2	0.513

[1]CON = maize-based control diet; CON+XT = CON with supplemental essential oils (100 g XT/t Pancosma S.A., Geneva, Switzerland).

[2]Based on the feeding period from 0 to 21 d of age and 10 observations per treatment.

[3]The content of ash and carbohydrates was calculated as difference between DM and carcass protein and fat contents.

son and Summers (1997) also showed that at an early stage of growth, broiler chickens are depositing proportionally more carcass protein than fat, further supporting the results.

Although dietary essential oil supplementation increased both ME and NEp with approximately 0.5 MJ, it was significant only for the NEp. The improvement in dietary NEp, coupled with reduced heat losses, and improved dietary fat digestibility, feed efficiency, and chicken growth were observed. The beneficial effects of supplementary plant extracts to poultry diets seems to be mediated through decrease in the energy required for maintenance, thereby allowing chickens to divert relatively more energy toward growth rather than maintenance. The improvement in growth performance with dietary essential oils could be explained by the increase in dietary NEp, indicating that they influence growth performance through improving the metabolic efficiency of converting absorbed dietary energy into tissue. Net energy is 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. Changes in maintenance energy are more likely to be detected by determination of NEp but not ME.

De Groote (1974) proposed that the efficiency of utilization of digestible carbohydrates, fats, and protein for growing poultry are 0.7, 0.9, and 0.6, respectively. Dietary plant extracts supplementation enhanced FD; therefore, it could be expected that this diet would have a greater efficiency of energy utilization. Diets with a lower efficiency of energy utilization could be a reason for more unabsorbed nutrients in the lumen that encourage the proliferation of microflora in the small intestine. The activity of the intestinal microbiota in the host is an important factor that may impact gut function. Intensified microbial proliferation in the gastrointestinal tract will result in impaired nutrient absorption (Partanen et al., 2001) and increased energy requirements for maintenance (Furuse et al., 1985). This may be a possible mechanism for the action of the combination of carvacrol, cinnamaldehyde, and capsicum that may also lessen the proliferation of potentially harmful gram-negative microbiota and promote the growth of beneficial microflora (Jamroz et al., 2005), thus reducing the production of endotoxins and inflammation and improving the functioning of the gut (Ulevitch and Tobias, 1999). In addition, Karadas et al. (2013) found an increased hepatic concentration of vitamin E in chickens fed the same mixture of plant extracts compared with those fed unsupplemented diet, indicating that feeding essential oils enhances the antioxidant status of chickens and helps to alleviate nutritional stress when reared under commercial conditions.

In summary, the present results indicate that dietary combination of plant extracts, including carvacrol, cinnamaldehyde, and capsicum oleoresin, improved the nutritional value of a low ME maize-based diet when fed to young broiler chickens. The increase of dietary NEp indicates that they influence growth performance through improving the metabolic efficiency of converting absorbed dietary energy into tissue. The experiment showed that although dietary essential oils did not affect dietary ME, they caused an improvement in the utilization of energy for growth, most probably affecting metabolic utilization of absorbed nutrients. Therefore, studies that have focused solely on the effect of plant extracts on ME alone, may well have not detected their full nutritional value.

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