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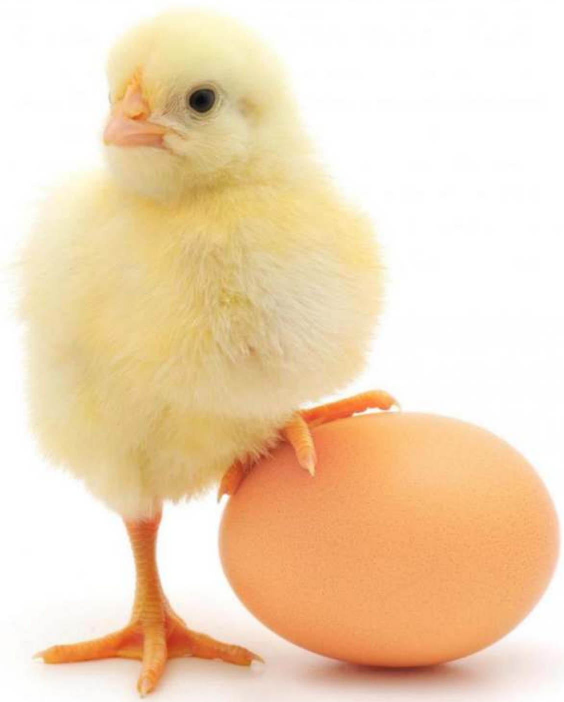

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Growth Performance of Broiler Chickens Fed a Carboxymethyl Cellulose Containing Diet with Supplemental Carvacrol and/or Cinnamaldehyde

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Abstract: The question addressed was whether the essential oil components, carvacrol and cinnamaldehyde, would have an interactive effect with regard to growth performance. One-day old female broiler chickens were subjected to one of 5 dietary treatments for 21 days: a base diet as a negative control, the base diet with 1% Carboxymethyl cellulose (CMC), the CMC diet with 200 ppm carvacrol, the CMC diet with 200 ppm cinnamaldehyde and the CMC diet with 100 ppm carvacrol plus 100 ppm cinnamaldehyde. Group mean daily weight gain was 10% less in birds fed on the CMC diet when compared with those fed the CMC-free diet. Birds fed the CMC-containing diet with the blend of carvacrol and cinnamaldehyde gained significantly less weight when compared with those fed on the CMC-free diet, the decrease in weight gain being 24%. The feeding of either carvacrol or cinnamaldehyde alone with the CMC-containing diet did not influence weight gain as opposed to the CMC control diet, but the combination of the two principles reduced group mean weight gain by 16%. The feeding of CMC caused hypertrophy of the small intestine, but the essential oil components had no further effect. No significant treatment differences were observed as to plasma lipid concentrations. The present data indicate that essential oil components can have interactive effects with respect to growth performance.

Key words: Broilers, growth, Carboxymethyl cellulose, carvacrol, cinnamaldehyde

Introduction

In the course of our research on growth promoters as alternatives to antibiotics, we have investigated the effects of essential oil components on growth performance of broiler chickens (Lee *et al.*, 2003a, 2003b, 2003c, 2004). The addition of 200 ppm carvacrol to a corn-soybean meal based diet lowered weight gain from 1-28 days of age by 3%. Carvacrol in the diet also lowered feed intake, but significantly improved the feed:gain ratio. Enrichment of a corn-soybean based diet containing Carboxymethyl cellulose (CMC) with 100 ppm cinnamaldehyde increased group mean weight gain from 1-21 days of age by 14%. CMC is a non-fermentable fiber that raises the viscosity of intestinal digesta and impairs growth performance of broiler chickens (Van der Klis *et al.*, 1993; Smits *et al.*, 1997, 1998). Although the effects not consistently reached statistical significance, it appears that essential oil components can affect nutrient utilization and counteract the antinutritional effect of CMC.

Cinnamaldehyde is a component of cinnamon essential oils. Carvacrol is found in thyme and oregano essential oils. The two principles each exhibit a wide range of antimicrobial activity *in vitro* (Helander *et al.*, 1998) and act synergistically when combined (Didry *et al.*, 1994; Montes-Belmont and Carvajal, 1998). Thus, it can be suggested that a blend of essential components may be more effective in improving growth performance than an identical amount of an individual component alone. In this communication we describe the influence of the combination of carvacrol and cinnamaldehyde on growth performance of female broiler chickens fed a diet containing CMC. Because dietary antibiotics have been shown to lower intestinal weight (Jukes *et al.*, 1956; Hill *et al.*, 1957), we also determined this variable. Essential oil components have hypocholesterolemic effects in chickens (Case *et al.*, 1995), which prompted us to measure plasma cholesterol as well.

Materials and Methods

The experiment protocol was approved by the animal experiments committee of the Utrecht Faculty of Veterinary Medicine.

Animals, diets and experimental design: 75 one-day old, feather-sexed female broiler chickens (Cobb) were obtained from a local hatchery. They were wing-banded, weighed on arrival and randomly allocated to one of 5 treatments. Each treatment had 3 pens with 5 chicks each so that there were 15 chicks per treatment. The 5 dietary treatments were as follows: a base diet as a negative control, the base diet with 1% CMC (AF 2805, Akzo Nobel, Arnhem, The Netherlands), the CMC diet with 200 ppm carvacrol, the CMC diet with 200 ppm cinnamaldehyde and the CMC diet with 100 ppm carvacrol plus 100 ppm cinnamaldehyde. The CMC preparation used has high viscosity and is not fermented (Smits *et al.*, 1997). Beef tallow and corn oil were used as fat sources, and included at the level of 5 and 1%, respectively (Table 1). Carvacrol (97% purity,

Table 1: Ingredient composition of base diet

Ingredients	Base	CMC[3]
Corn, yellow	322	322
Corn Starch	210	210
Soybean meal, 48% CP	260	260
Soybean protein isolate	85	85
Beef tallow	50	50
Corn oil	10	10
Sodium chloride	5	5
Calcium carbonate	17	17
Mono calcium phosphate	17	17
DL-Methionine	4	4
Premix[1]	10	10
Cellulose[2]	10	0
CMC[3]	0	10
Total	1000	1000

[1]The 10 g premix consisted of 24.0 mg vitamin A (500000 IU/g); 6.0 mg vitamin D_3 (100000 IU/g); 60.0 mg vitamin E (500 IU/g); 6.6 mg vitamin K_3 (purity, 22.7%); 100.0 mg vitamin B_{12} (purity, 0.1%); 2000.0 mg biotin (purity, 0.01%); 1100.0 mg choline chloride (purity, 50%); 1.1 mg folic acid (purity, 90%); 65.2 mg nicotinic acid (purity, 100%); 16.3 mg d-pantothenate (purity, 92%); 4.5 mg vitamin B_6 (purity, 100%); 12.5 mg riboflavin (purity, 80%); 2.5 mg vitamin B_1 (purity, 100%); 32.00 mg $CuSO_4.5H_2O$; 333.20 mg $FeSO_4.H_2O$; 166.80 mg MnO; 1.0 mg $Na_2SeO_3.5H_2O$; 220.00 mg $ZnSO_4.H_2O$; 4.80 mg $CoSO_4.7H_2O$; 0.56 mg KI, 100.00 mg ethoxyquin and 5742.94 mg corn meal as carrier. [2]Arbocel (Akzo Nobel, Arnhem, The Netherlands). [3]Carboxymethyl cellulose (AF 2805, Akzo Chemicals, Arnhem, The Netherlands).

Fluka Chemie, Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands) and cinnamaldehyde (99% purity, Acros Organics, Geel, Belgium) were dissolved in corn oil and then gently mixed with the diets to arrive at 1% corn oil and 200 ppm of additives in the diet. The diets were prepared freshly each day. The base diet and the CMC control diet were mixed with corn oil only. Feed and water were provided for *ad libitum* consumption. The temperature of the room in which the pens were located was 34°C on arrival of the chickens and was gradually decreased to reach 25°C when they were aged 3 weeks. Lighting was on continuously. Individual body weights and feed and water intakes per pen were monitored weekly. Feed and water intake were calculated as g/day/bird and used to calculate the feed:gain ratio.

On day 21, blood was collected by heart puncture. Plasma was obtained by centrifugation at 1700 × g for 15 min and stored at -70°C prior to analysis. Plasma total cholesterol, phospholipids, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured as described (Beynen *et al.*, 2000). Immediately after blood sampling, the birds were killed by cervical dislocation. Liver was removed and weighed. Small intestine, which was defined as the segment between gizzard and ileocecal junction was removed, extended on a table and measured for its length. The pancreas was removed. The intestine was slit longitudinally and the intestinal contents were removed and the intestinal tissue was then weighed.

Statistical analysis: The data were analyzed for the main effect of treatments using the general linear model (SPSS for Windows, Version 9.0.0; SPSS Inc. Chicago, IL). Treatment means were evaluated for statistically significant differences using Tukey's test. A P-value < 0.05 was preset as criterion of statistical significance.

Results

For the age interval of 1-21 days, daily weight gain, feed intake, feed:gain ratio and water intake were not significantly different between the treatments (Table 2). The feeding of CMC did cause a reduction in group mean weight gain by 10% and a decrease in feed intake by 9%. Enrichment of the CMC diet with a blend of carvacrol and cinnamaldehyde tended to further diet depress weight gain and feed intake. CMC feeding generally raised the water:feed ratio.

Birds fed on the CMC diet showed a significantly increased relative intestinal weight (g/100g of body weight) when compared to those fed on the base diet (Table 2). The CMC-induced hypertrophy of small intestine was not modified by the addition of essential oil components to the CMC-containing diet. The relative length of the small intestine (cm/100g of body weight) was increased in birds fed on the diet with CMC diet when compared to their counterparts fed the base diet. Addition of essential oil components to the CMC-containing diet did not affect length of the small intestine. No significant treatment differences were detected for relative liver weight (Table 2). Plasma lipid concentrations were not modulated by any of dietary treatments (Table 3).

Discussion

In a previous study with female broiler chickens (Lee *et al.*, 2004), CMC significantly depressed daily weight gain by 16% between 1-21 days of age, and by 9% between 1-40 days. In this study, CMC feeding induced a 10% growth depression between 1-21 days of age, but the effect did not reach statistical significance. Soluble fibers such as CMC raise the viscosity of gut contents, which may decrease digestion by diminishing the diffusion of digestive enzymes and nutrients (Choct *et al.*, 1996). Langhout *et al.* (2000) reported that macro nutrient digestion was impaired by gut viscosity in birds raised in a conventional environment, but not in germ-free chicks. It follows that the intestinal condition can determine the magnitude of the effect of dietary soluble fiber on growth performance. Possibly, in this study the conditions did not allow a marked effect of CMC on weight gain to become apparent. On the other hand, CMC clearly caused hypertrophy of the small intestine. This observation corroborates earlier research both in

Table 2: Effects of dietary essential oil components on growth performance and weights of small intestine and liver in female broiler chickens fed on CMC[1]

Measure	Diet					SEM[2]	P value
	Base	CMC[3]	CMC+ Carvacrol	CMC+ Cinnam-aldehyde	CMC+ Blend		
Growth performance							
Weight gain, g/d/bird	35.9[a]	32.3[a]	34.3[a]	32.7[a]	27.2[b]	1.817	0.060
Feed intake, g/d/bird	46.7	42.7	46.2	45.6	37.3	2.123	0.053
Water intake, g/d/bird	91.8	90.6	97.6	106.8	87.2	5.032	0.125
Feed:gain, g:g	1.30	1.33	1.35	1.40	1.37	0.029	0.252
Water:feed, g:g	1.97[b]	2.13[ab]	2.12[ab]	2.34[a]	2.34[a]	0.075	0.023
Small intestine							
Weight, g/100g BW	3.45[b]	3.95[a]	3.72[ab]	3.74[ab]	4.09[a]	0.100	0.011
Length, cm/100g BW	17.14[b]	21.04[ab]	20.09[ab]	21.15[ab]	23.69[a]	1.009	0.013
Liver, g/100g BW	2.40	2.41	2.18	2.22	2.28	0.109	0.511

[1]Results are expressed as means for three replicates per dietary group. [2]SEM = pooled standard error of mean.
[3]Carboxymethyl cellulose.[a,b]Means having different letters within a same row differ significantly ($P<0.05$).

Table 3: Effect of dietary essential oil components on plasma lipids in female broiler chickens fed diet containing CMC at 21 days[1]

Measure	Diet					SEM[2]	P value
	Base	CMC[3]	CMC + Carvacrol	CMC + Cinnam-aldehyde	CMC + Blend		
Total cholesterol, mmol/l	3.25	3.15	3.36	2.91	3.13	0.113	0.140
Phospholipids, mmol/l	2.75	2.53	2.68	2.39	2.40	0.083	0.070
Triglycerides, mmol/l	0.41	0.37	0.39	0.42	0.35	0.031	0.475
HDL cholesterol, mmol/l	2.35	2.25	2.49	2.06	2.23	0.097	0.102

[1]Results are expressed as means for three replicates per dietary group. [2]SEM = pooled standard error of mean.
[3]Carboxymethyl cellulose.

germ-free (Pell *et al.*, 1995; Smits, 1996) and conventionally raised animals (Van der Klis *et al.*, 1993; Smits *et al.*, 1997), indicating that CMC has a mechanical effect on the intestinal tract.

An unexpected result emerged from the present study. Birds given the blend of essential oil components gained 16% less weight than those fed on CMC-containing diet without supplement. In contrast, carvacrol or cinnamaldehyde alone did not significantly affect weight gain. It is not known why the blend of carvacrol and cinnamaldehyde had a negative influence on weight gain, whereas the individual compounds had no negative effect. In any event, the present data point at an interaction between the two essential oil components with regard to growth performance of broilers fed a CMC-containing diet. The essential oil components did not affect small intestinal weight and length. Feed additives containing antibiotic activity such as in-feed antibiotics (Jukes *et al.*, 1956; Hill *et al.*, 1957), and lactoferrin and lysozyme (Humphrey *et al.*, 2002) have been reported to affect small intestinal morphology, thereby leading to better nutrient digestion and absorption. In a previous experiment we have shown that cinnamaldehyde did not alter the CMC-induced increase in viscosity of intestinal contents (Lee *et al.*, 2004).

In keeping with an earlier study (Lee *et al.*, 2004), the feeding of CMC did not significantly modulate plasma cholesterol and triglyceride concentrations. In contrast, Smits *et al.* (1997) showed that inclusion of CMC in the diet reduced plasma cholesterol and triglycerides. When the essential oil components were added to CMC diet, no systematic effects on plasma lipids were observed. This result is at variance with that of Case *et al.* (1995) who reported that birds fed carvacrol had significantly lowered plasma cholesterol concentrations. We found earlier that both thymol, an isomer of carvacrol, and cinnamaldehyde did not lower plasma cholesterol concentrations in birds fed on a CMC-containing diet (Lee *et al.*, 2004). Case *et al.* (1995) used a slow-growing breed of chickens which might be more sensitive to agents affecting plasma cholesterol levels.

In conclusion, the present data show that in birds fed on a CMC-containing diet the ingestion of a blend of carvacrol and cinnamaldehyde tended to reduce weight gain, whereas the individual compounds had no such effect. It would appear that essential oil components have interactive effects.

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