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Cinnamaldehyde, but Not Thymol, Counteracts the Carboxymethyl Cellulose-induced Growth Depression in Female Broiler Chickens

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Abstract: The question addressed was whether dietary essential oils could antagonize the negative effect of Carboxymethyl cellulose (CMC) on growth performance in broilers. Diets without or with 1% CMC, and CMC containing diet with either 100 ppm thymol, cinnamaldehyde or a commercial essential oil blend were fed to female broiler chickens for 40 days. Chicks receiving the CMC diet showed significantly depressed weight gain for the period of 0 - 21 days. Addition of cinnamaldehyde or commercial essential oil to the CMC diet partially counteracted the negative effect on growth performance. Group mean feed intake was lower in CMC-fed chicks, but was raised when cinnamaldehyde or the commercial oil was added to the diet. Intestinal viscosity was increased by CMC inclusion, but was not lowered by the additives. Fat digestibility was significantly reduced by CMC, but cinnamaldehyde or commercial oil inclusion partially reversed this effect. This study indicates that cinnamaldehyde, but not thymol, may antagonize the negative effects of CMC on growth performance which may relate to improving fat digestibility.

Key words: Cinnamaldehyde, thymol, Carboxymethyl cellulose, growth, broilers

Introduction

The antimicrobial activity of essential oils derived from spices and herbs (Deans and Ritchie 1987; Hammer *et al*., 1999) is of interest as these oils could be used as feed additives alternative to antibiotics (Taylor, 2001). We have addressed the question whether selected principles derived from essential oils, i.e. thymol, carvacrol, beta-ionone and cinnamaldehyde, would stimulate growth performance in broiler chickens (Lee *et al*., 2003a,b,c). Under the conditions of our experiments, i.e. using female broiler chickens kept in a clean environment and fed a highly digestible diet, the compounds did not affect growth. It was reasoned that dietary essential oils could stimulate growth performance in broiler chickens fed a suboptimal diet. We decided to use a diet containing Carboxymethyl cellulose (CMC), which is a non-fermentable, viscous fiber that raises the viscosity of intestinal chyme and lowers growth performance in broiler chickens which is explained by depressed digestibility of macronutrients (Van Der Klis and Van Voorst, 1993; Smits *et al*., 1997). There is suggestive evidence that dietary essential oils may enhance macronutrient digestibility (Williams and Losa, 2001). Thymol [5-methyl-2-(1-methylethyl)phenol] is a major component of essential oils from thyme and cinnamaldehyde (3-phenyl-2-propenal) is a major component of that from cinnamon. In this study we investigated whether dietary thymol, cinnamaldehyde or a commercial blend of essential oil compounds would be able to counteract the CMC-induced growth depression in broiler chickens.

Materials and Methods

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

Experimental design: 45 one-day old female broiler chickens (Cobb) were obtained from a local hatchery. They were wing-banded, weighed and randomly allocated to one of 5 treatments. Each treatment had 3 pens with 3 chicks each so that there were 9 chicks per treatment. The 5 dietary treatments were as follows: base diet, base diet + 1% CMC (Akzo Nobel, Arnhem, The Netherlands), base diet + CMC + 100 ppm thymol, base diet + CMC + 100 ppm cinnamaldehyde, and base diet + CMC + 100 ppm commercial essential oil. The commercial preparation used was CRINA® Poultry (Crina S. A., Akzo Nobel, Switzerland); it contains 29% of active components, the composition being proprietary. The base diet contained 0.04% chromic oxide as a marker (Table 1) and 1% CMC was included at the expense of cellulose. The CMC preparation used has high viscosity and is not fermented (Smits *et al*., 1997). Corn oil was used as fat source and included at the level of 5% (Table 1). Thymol (99% purity, Acros Organics, Geel, Belgium), cinnamaldehyde (99% purity, Acros Organics), and the commercial essential oil blend were dissolved in corn oil and then gently mixed with the diets to arrive at 5% corn oil and 100 ppm of additives in the diet. The diets were prepared freshly each day. To compose the additive-free diets, the base and CMC-containing ingredients were mixed with corn oil only. Feed and water were provided for *ad libitum* consumption. The temperature of the room with the pens was 34°C on arrival of the chickens and was gradually decreased to 25°C after 3 weeks and then was kept constant. Lighting was on continuously. Individual

Table 1: Ingredient composition of the base diet and CMC-containing diets

Ingredient	g
Corn, yellow	300
Corn starch	211.6
Soybean meal, 48% CP	375
Corn oil	50
Sodium chloride	5
Calcium carbonate	15
Monocalcium phosphate	19
DL-Methionine	4
Premix[1]	10
Cellulose[2]	0/10
CMC[3]	10/0
Chromic oxide	0.4
Total	1000

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

body weights, 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.

From days 37-39, excreta were collected quantitatively, weighed and dried at 60°C until weight was constant. Dried excreta were pooled per pen and ground to pass a 1-mm sieve. On day 40, blood was collected by heart puncture. Plasma was obtained by centrifugation at 1700 × g at 15 min and stored at –70°C prior to analysis. Immediately after blood sampling, the birds were killed by cervical dislocation. Liver and pancreas were removed and weighed. Intestinal contents between Meckel's diverticulum and the ileocecal junction were collected in tubes on ice by gentle stripping of the segments. The samples were pooled per pen. Immediately after obtaining the digesta, the samples were mixed thoroughly, divided into several portions for various chemical analyses and stored at -20°C. For determination of the intestinal viscosity, one portion was kept on ice and processed on the day of sampling.

Measurements: For viscosity measurements, the ice-cold digesta samples were centrifuged at 12000 × g for 2 min. The viscosity of approximately 0.5 ml of supernatant was measured using a cone/plate geometry (cone angle, 1° and diameter, 40 mm) at 37°C in a Bohlin CS 50 Rheometer (Bohlin Reologi, Mühlacker, Germany). The shear stress was varied from 19 to 0.1 Pa. The measurement started at high shear stress, and was decreased stepwise to 0.1 Pa and then increased again to the initial value (down/up measurement). The viscosity at a shear stress of 1 Pa was taken as the viscosity value.

Isoprenoids such as carvacrol, thymol and β-ionone may influence plasma cholesterol concentrations (Case *et al.*, 1995), which prompted us to determine plasma lipids in this study. Plasma total cholesterol, phospholipids, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured as described (Beynen *et al.*, 2000).

Nitrogen in the ileal digesta and diet samples was analyzed with the Kjeldahl method. Crude protein content was calculated as 6.25 × N (g). Starch contents in diet and ileal chyme were determined enzymatically using amyloglucosidase (EC 3.2.1.4) from *Aspergillus niger* (Boehringer Mannheim Diagnostica, Mannheim, Germany) according to the method of Cone and Vlot (1990). For crude fat determination, diet and excreta were first treated with 3 N hydrochloric acid for 1 hr and then overnight extracted with diethylether. Extracted fat was measured after evaporating the solvent at 101°C. Chromium contents in diet and ileal chyme were determined according to Murthy *et al.* (1971) as outlined in the manual of the AAS 3300 (Perkin-Elmer Corp., Connecticut, USA).

The apparent ileal digestibilities of protein and starch were calculated as: $DC_{diet} = (1-[(Cr_{diet}/Cr_{ileum}) \times (P,S_{ileum}/P,S_{diet})]) \times 100$, where DC_{diet} = apparent digestibility of either crude protein (P) or starch (S) in the diet; Cr_{diet} = concentration of chromium in the diet; Cr_{ileum} = concentration of chromium in the ileal content; P,S_{ileum} = concentration of either crude protein or starch in the ileal content; P,S_{diet} = concentration of either crude protein or starch in the diet.

Statistical analysis: Pooled SEMs were calculated by one-way ANOVA. Treatment means were evaluated for statistically significant differences using Tukey test. A P-value < 0.05 was preset as criterion of statistical significance.

Results

For the period of 0-21 days, daily weight gain was significantly lowered in CMC-fed birds when compared to those fed the CMC-free base diet (Table 2). When the additives were introduced into the CMC diet, the CMC-induced depression of weight gain was either not affected or partially counteracted. Birds fed the CMC diets with cinnamaldehyde or the commercial essential oil performed better than those fed the diet with CMC only, but the group differences did not reach statistical significance. Thymol did not counteract the CMC effect. Feed intakes for the period 0-21 days showed a pattern similar to that of weight gain, but without statistical

Table 2: Effects of essential oils on growth performance in female broiler chickens fed on diets containing CMC

Measure	Diet					SEM	P value
	Base	CMC	CMC+ Thymol	CMC+ Cinnam -aldehyde	CMC+ Commercial preparation		
0 - 21 days							
Weight gain, g/d/bird	38.4[a1]	32.1[b]	33.3[b]	36.5[ab]	36.6[ab]	1.320	0.038
Feed intake, g/d/bird	54.2	48.4	48.0	52.5	54.0	1.841	0.092
Water intake, g/d/bird	113	106	114	105	132	9.398	0.332
Feed:gain, g/g	1.412	1.508	1.441	1.441	1.475	0.023	0.108
Water:feed, g/g	2.087	2.185	2.357	2.005	2.442	0.130	0.173
0 - 40 days							
Weight gain, g/d/bird	51.4	47.0	44.3	47.6	49.2	1.916	0.182
Feed intake, g/d/bird	88.5	83.1	77.5	86.4	88.0	3.123	0.156
Water intake, g/d/bird	181	178	174	166	206	12.331	0.278
Feed:gain, g/g	1.720	1.769	1.752	1.815	1.789	0.021	0.069
Water:feed, g/g	2.032	2.138	2.245	1.931	2.339	0.116	0.117

[1]Results are expressed as means of three replicates per dietary group. [a,b]Means having different superscripts within a same row differ significantly ($P<0.05$)

significance (Table 2). Feed:gain ratio was lowest for the chickens fed the base diet and highest for the CMC group. The feed:gain ratio for the period 0-21 days when expressed as percentage increase versus that for the CMC-free base diet was 6.8, 2.1, 2.1, and 4.5% for CMC alone and for CMC in combination with either thymol, cinnamaldehyde or the commercial essential oil, respectively. The statistically significant treatment effect on weight gain seen for the period 0-21 days was not found for the period 0-40 days. Water consumption was not significantly different among treatments. Birds fed the commercial essential oil blend exhibited the highest group mean water intake and those given cinnamaldehyde showed the lowest intake.

Relative organ weights and ileal viscosity are presented in Table 3. Liver and pancreas weight were not affected by dietary treatment. Group mean ileal viscosity was markedly increased by CMC inclusion, but due to the high inter-individual variation the increase was not statistically significant. The additives did not significantly influence the viscosity of ileal contents. Fecal fat digestibility was affected by dietary treatment: chickens fed the diet containing CMC only and those fed the CMC diet with thymol had significantly lower in fat digestibility than the control group (Table 3). The inhibitory effect of CMC on fat digestibility was partially counteracted by either cinnamaldehyde or the commercial essential oil, but the counteractive effect was not statistically significant. Ileal digestibilities of starch and crude protein were not significantly different between dietary treatments (Table 3). Both macronutrients were well digested at the level of the ileum, the apparent digestibility ranging from 92 to 95% for crude protein and being about 99% for starch. Plasma total cholesterol did not differ between dietary treatments (Table 4). Group mean triglycerides were somewhat lowered by the diets containing CMC, but the lowering was not statistically significant. HDL cholesterol and phospholipids were not influenced by the dietary variables.

Discussion

The present experiment was conducted to investigate the effect of thymol and cinnamaldehyde on growth performance in female broiler chickens fed a CMC-containing diet. CMC was added to the diet in order to depress growth performance as was reported earlier (Smits *et al.*, 1997, 1998). Indeed, the diet containing 1% CMC was found to lower weight gain by 16% during days 0-21 and by 9% during days 0-40. CMC is a non-fermentable, viscous fiber (Smits *et al.*, 1997) and in the present study ileal viscosity rose on average 5-fold when CMC was added to the diet. This observation corroborates earlier studies (Van Der Klis *et al.*, 1993; Smits *et al.*, 1997). As would be expected (Smits *et al.*, 1997), CMC significantly lowered fat digestibility, albeit that the lowering was relatively small (Smits *et al.*, 2000). Unlike earlier observations (Smits *et al.*, 1997) CMC feeding did not decrease the ileal digestibilities of starch and crude protein, which may be explained by the relatively small CMC-induced increase in viscosity of ileal digesta in this study as opposed to other studies (Van Der Klis *et al.*, 1993; Smits *et al.*, 1997, 2000). The decrease in macronutrient digestibility as mediated by CMC consumption is explained by the increase in viscosity of ileal digesta (Smits *et al.*, 1997). In any event, the observed effects of CMC generally agree with those reported earlier.

For the period of 0 - 21 days, the CMC-induced decrease in weight gain tended to be partially antagonized by either cinnamaldehyde or the commercial preparation in the diet. Weight gain in animals fed the CMC-containing diets with either cinnamaldehyde or the commercial preparation did not differ significantly from that in the birds fed the CMC-free base diet. Thymol was less

Table 3: Effects of essential oils on organ weights, ileal viscosity, and digestibilities of nutrients in female broiler chickens

Measure at 40 days	Diet					SEM	P value
	Base	CMC	CMC+ Thymol	CMC+ Cinnam-aldehyde	CMC+ Commercial preparation		
Liver, g / 100 g b.w.[2]	1.847[1]	1.707	1.742	1.841	1.834	0.090	0.719
Pancreas, g / 100 g b.w.[2]	0.157	0.159	0.155	0.172	0.153	0.009	0.621
Viscosity, mPa.s	1.56	8.20	8.83	9.17	10.30	2.076	0.078
Ileal digestibilities							
Crude protein, %	92.42	95.38	94.49	93.72	94.05	1.279	0.594
Starch, %	98.95	99.25	99.05	98.75	98.93	0.309	0.842
Fecal digestibility							
Crude fat, %	88.90[a]	86.77[b]	86.68[b]	87.23[ab]	87.45[ab]	0.432	0.028[1]

[1]Results are expressed as means of three replicates per dietary group. [2]b.w. = body weight

Table 4: Effects of dietary essential oils on plasma lipids in female broiler chickens fed diet containing CMC at 40 days

Measure at 40 days	Diet					SEM	P value
	Base	CMC	CMC+ Thymol	CMC+ Cinnam -aldehyde	CMC+ Commercial preparation		
Total cholesterol, mmol/l	3.183[1]	3.232	2.934	3.102	3.104	0.142	0.660
Phospholipids, mmol/l	3.013	2.949	2.644	2.857	2.837	0.213	0.780
Triglycerides, mmol/l	0.469	0.387	0.354	0.392	0.356	0.051	0.527
HDL cholesterol, mmol/l	2.852	2.742	2.586	2.771	2.787	0.156	0.805[1]

Results are expressed as means of three replicates per dietary group

effective in antagonizing the CMC effect than was either cinnamaldehyde or the commercial preparation. Feed intake showed the same pattern as weight gain, but the treatment effect failed to reach statistical significance. For the period 0 - 40 days, the CMC effect on weight gain was not statistically significant and the effect of cinnamaldehyde had disappeared. Group mean feed intakes for 0 - 40 days still showed the initial pattern in that CMC reduced group-mean feed intake and that cinnamaldehyde and the commercial preparation, but not thymol, tended to counteract the CMC effect. It is suggested that in the present experiment statistical power was not high enough to substantiate the observed treatment effects by statistical significance.

It is concluded tentatively that both cinnamaldehyde and the commercial preparation partially counteracted the CMC-induced reduction in weight gain and feed intake for the period of 0 - 21 days. The question then arises as to the possible underlying mechanisms. The inhibitory effect of CMC on growth performance is explained by raising the viscosity of ileal contents and subsequent decrease in digestibility of macronutrients (Smits *et al.*, 1997). However, cinnamaldehyde and the commercial preparation of essential oils did not lower the viscosity of ileal contents and thus had another mode of action. The CMC preparation used is not fermentable, but may raise microbial growth by increasing available substrates for microorganisms by inhibiting protein and starch digestibility (Smits *et al.* 1997). In this study, ileal digestibility of starch and protein was not lowered by CMC, which may relate to the highly digestible ingredients in the purified diet. Thus, under the conditions of this study, cinnamaldehyde and the commercial essential oil may not have counteracted the CMC effect by inhibiting CMC-induced proliferation of the gut microflora. CMC feeding significantly decreased apparent crude fat digestibility which may be explained by a reduced availability of bile salts for the process of fat digestion (Smits *et al.* 1998). Fortification of the CMC diets with either cinnamaldehyde or the commercial essential oil blend raised fat digestibility to values not significantly different from those in the birds fed the CMC-free diet. It is thus likely that the CMC-antagonizing effect of cinnamaldehyde and the commercial preparation on growth performance relates to maintaining fat digestibility at a higher efficiency. This reasoning is reinforced by the observation that thymol did not counteract the CMC effect and did not affect fat digestibility. The stimulatory effect of cinnamaldehyde on fat digestibility may relate to stimulation of bile secretion as shown in rat (Harada and Yano, 1975).

CMC has been reported to increase water intake and also the water:feed ratio in chickens (Van Der Klis and Van Voorst, 1993; Van Der Klis *et al.*, 1993; Smits *et al.*, 1997, 1998). It is not clear why CMC did not stimulate water intake in this study. Moisture contents of ileal

chyme were 82.8 and 85.0% for the control and CMC-fed chickens, indicating that water absorption in the birds fed CMC was impaired (Van Der Klis *et al.*, 1993) and that CMC exhibited its water-holding capacity (Smits *et al.*, 1998). The commercial blend of essential oils tended to raise water consumption, which agrees with earlier work (Lee *et al.*, 2003a). However, cinnamaldehyde did not lower water consumption as seen earlier (Lee *et al.*, 2003a), but it should be noted that in this study cinnamaldehyde was fed together with CMC.

CMC failed to lower plasma cholesterol levels, but plasma triglycerides were on average 18% lower in CMC-fed chickens. CMC may reduce plasma cholesterol and triglycerides as a secondary feature to a low bile salt availability (Smits *et al.*, 1997). The lack of effect of CMC feeding on plasma cholesterol in this study could relate to the use of corn oil as fat source. Dietary corn oil has hypocholesterolemic activity (Beynen and West, 1989) which may have masked any effect of CMC. Addition of either thymol, cinnamaldehyde or the commercial essential oil to the CMC diet did not affect plasma lipids. Isoprenoids such as thymol, carvacrol and β-ionone have been shown to lower plasma cholesterol concentrations in chickens (Case *et al.*, 1995). It is not clear why this study failed to show a hypocholesterolemic effect of thymol.

In conclusion, the present study shows that cinnamaldehyde, but not thymol, might counteract the negative effect of CMC on growth performance in broiler chickens. The counteractive effect of cinnamaldehyde may be explained by the relief of the CMC-induced inhibition of fat digestion, but the molecular basis remains unknown.

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