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Growth Performance, Intestinal Viscosity, Fat Digestibility and Plasma Cholesterol in Broiler Chickens Fed a Rye-containing Diet Without or with Essential Oil Components

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Abstract: The present study was conducted to investigate the effects of dietary essential oil components, i.e., thymol, cinnamaldehyde and a commercial blend of essential oil components (CRINA® Poultry) on growth performance, fat digestibility, intestinal viscosity and plasma cholesterol in female broiler chickens fed on a diet rich in rye. There were 5 dietary treatments: a diet containing corn, a diet with rye, the rye diet with 100 ppm thymol, the rye diet with 100 ppm cinnamaldehyde and the rye diet with 100 ppm CRINA® Poultry. Each treatment consisted of 3 cages with 5 birds per cage. Birds fed on the supplement-free diet containing rye instead of corn showed a significantly depressed daily weight gain between 1-14 days of age. The rye-induced suppression of weight gain between 1-14 days of age was partially overcome by the inclusion in the diet of cinnamaldehyde, but the effect failed to reach statistical significance. Birds fed on the diets with rye generally ate less than those fed on the corn diet. Cinnamaldehyde tended to increase voluntary feed intake. The water:feed ratio was increased by the feeding of rye, but the supplements had no effect. The viscosity of jejunal and ileal digesta were significantly elevated when the diet contained rye instead of corn, but there was no counteracting effect of the essential oil components. Fecal fat digestibility was significantly lowered in birds fed on the rye diet, but the supplements did not reverse this effect. Thus, it is concluded that cinnamaldehyde may counteract the antinutritional effect of rye, but without a simultaneous effect on intestinal viscosity or fat digestibility. Feeding rye instead of corn did not modulate plasma cholesterol. Cinnamaldehyde produced a significant increase in plasma cholesterol concentration.

Key words: Broilers, growth, fat digestion, intestinal viscosity, rye, essential oils

Introduction

It is well documented that soluble non-starch polysaccharides (NSPs) in barley, rye and wheat lower animal performance by raising intestinal viscosity, depressing fat digestibility and/or altering the composition of the intestinal microflora. Antimicrobial compounds have been shown to improve the nutritional value of rye and barley (Fernandez *et al*., 1973; Marusich *et al*., 1978; Misir and Marquardt, 1978) and to enhance fat digestion (Antoniou and Marquardt, 1982; Fengler *et al*., 1988). The fact that intestinal bacteria can de-conjugate bile salts (Feighner and Dashkevicz, 1988) and the observation that soluble NSPs reduced the intestinal bile acid pool (Smits *et al*., 1998) may explain the negative effect of soluble NSPs on fat digestion in broiler chickens.

We are studying the effects on broiler performance of essential oil components which are regarded as alternative growth promoters (Lee *et al*., 2003). In the present study with female broiler chickens, we used two pure essential oil components, i.e. thymol (5-methyl-2-(1-methylethyl)phenol) and cinnamaldehyde (3-phenyl-2-propenal) and addressed the question whether they would counteract the growth-suppressive effect of rye. An additional question to be addressed was whether any positive effect of the dietary essential oil components on growth would be associated with a reduction in viscosity of jejunal and ileal contents and/or an increase in fat digestibility. The evidence that dietary essential oils affect plasma cholesterol concentrations in chickens (Case et al., 1995) prompted us to also measure serum lipids in the broilers.

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 purchased from a local hatchery. They were weighed on arrival and randomly subjected to one of five dietary treatments. Each treatment consisted of 3 cages with 5 birds per cage. The temperature of the animal house was controlled and continuous lighting used throughout the entire experimental period.

A diet containing corn was used as a negative control because corn contains a negligible amount of NSPs.

Table 1: The composition of the diets containing either corn or rye

Ingredients	Corn	Rye
Corn (85 g/kg CP), g	400	0
Rye (93 g/kg CP), g	0	400
Corn starch, g	0	186.5
Wheat (115 g/kg CP), g	180	0
Soybean meal (485 g/kg CP), g	205	180
Soybean isolate (841 g/kg CP), g	74	108
Tallow, g	40	40
Corn oil, g	20	34
Iodized salt, g	4.2	4.2
Calcium carbonate, g	16	16
Monocalcium phosphate, g	17	17
DL-methionine, g	3.5	3.9
L-threonine, g	0.3	0.4
Premix [1], g	10	10
Cellulose [2], g	30	0
Total, g	1000	1000
Calculated contents		
Metabolizable energy, MJ/kg	13.2	13.2
Crude protein, %	21.6	21.6
Crude fat, %	8.2	8.2
Lysine, %	1.2	1.3
Methionine+cystine, %	0.9	0.9
Calcium, %	1.0	1.0
Available phosphorus, %	0.5	0.5

[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%); 2000.0 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 4842.94 mg corn meal as carrier. [2]Arbocel (Akzo Nobel, Arnhem, The Netherlands).

Table 2: Analyzed fatty acid composition of the diets containing either corn or rye

	Corn	Rye
C16:0, palmitic acid (%)[1]	20.8	19.1
C18:0, stearic acid (%)	5.8	5.4
C18:1, oleic acid (%)	32.8	32.7
C18:2, linoleic acid (%)	26.8	31.7
C18:3, α-linolenic acid (%)	1.2	1.8

[1]Percentage fatty acid methyl ester of total fatty acid methyl esters.

Corn and rye diets were formulated to be isoenergetic and isonitrogenous (Table 1). The corn and rye component were each added at the level of 40%. The diets were in a powdered form. Fatty acid composition of the corn and the rye diets was analyzed and found to be similar (Table 2) so that any difference in lipid digestibility between the two diets cannot be ascribed to fatty acid composition. According to Smits and Annison (1996), rye contains 4.6% soluble NSPs and 3.4% arabinoxylans in the dry matter fraction. It can be calculated that the rye diet used in this study contained 16 g NSPs /kg of diet and that the level of arabinoxylans was approximately 12 g/kg of diet.

To produce the experimental diets, 10 g of the corn oil component/kg of diet was added without or with supplement. There were five experimental diets; the corn diet without supplement; the rye diet without supplement; the rye diet with 100 ppm thymol (99% purity, Acros Organics, Geel, Belgium); the rye diet with 100 ppm cinnamaldehyde (99% purity, Acros Organics) and the rye diet with 100 ppm CRINA® Poultry. CRINA® Poultry (Akzo Nobel, Crina S.A., Gland, Switzerland) is a commercially available essential oil-based product. The feed supplements were first mixed with corn oil and then added to the rye diet to attain a dietary concentration of 100 ppm. The rye and corn diets without supplement were blended with corn oil only. Birds were subjected to their respective diets for 33 days. They were weighed individually on a weekly basis. Weekly feed and water intake were determined per cage. Feed:gain and water:feed ratios were determined for each cage. Feed and water were provided for *ad libitum* consumption.

Sampling: From 31-33 days, total excreta were collected, dried at 60 ºC for three days, weighed and pooled per pen. At the end of the experimental period, birds were individually weighed and blood was collected by heart puncture. Plasma was obtained by centrifugation at 1700g for 15 min and analyzed for plasma lipids. Immediately after blood sampling, birds were killed by cervical dislocation. Then, the intestinal tract was removed to obtain digesta by gentle finger stripping of the intestinal segments, and liver and pancreas were sampled and weighed. Jejunum was defined as the segment from the end of the duodenum to Meckel's diverticulum, and ileum as the part between Meckel's diverticulum and ileocecal junction. Intestinal digesta were pooled per pen, homogenized thoroughly and centrifuged at 12000g for 5 min to obtain supernatants. They were kept on ice prior to viscosity measurement.

Analysis: For viscosity measurements, the supernatants obtained were 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 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.

Plasma cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and phospholipids were

Table 3: Growth performance in female broiler chickens fed the experimental diets

Parameters	Treatments					Pooled SEM[1]	P value
	Corn	Rye	Rye+ Thymol	Rye+ Cinnam-aldehyde	Rye+ CRINA Poultry		
Weight gain, g/d/bird							
Days 1-14	25.0[a]	21.1[b]	20.2[b]	22.7[ab]	20.6[b]	0.837	0.013
Days 1-21	33.6	29.7	29.8	32.7	30.0	1.361	0.204
Days 1-33	46.5	43.3	44.4	47.0	43.7	1.796	0.510
Feed intake, g/d/bird							
Days 1-14	31.2[a]	27.2[abc]	25.1[c]	30.1[ab]	26.9[bc]	1.247	0.036
Days 1-21	44.6	39.5	38.2	44.2	40.0	1.642	0.067
Days 1-33	69.1	66.0	64.4	71.8	65.8	2.460	0.275
Water intake, g/d/bird							
Days 1-14	71.1	88.4	76.1	89.5	81.1	7.551	0.411
Days 1-21	97.4	117.5	103.2	121.5	110.6	8.187	0.282
Days 1-33	141.5	168.2	158.3	174.3	162.4	8.751	0.168
Feed:gain, g:g							
Days 1-14	1.25	1.29	1.24	1.32	1.31	0.021	0.071
Days 1-21	1.33	1.33	1.28	1.35	1.33	0.017	0.167
Days 1-33	1.49	1.52	1.45	1.53	1.51	0.027	0.335
Water:feed, g:g							
Days 1-14	2.28	3.22	3.02	2.98	3.01	0.209	0.074
Days 1-21	2.20	2.95	2.70	2.76	2.76	0.166	0.076
Days 1-33	2.05[b]	2.54[a]	2.46[a]	2.43[a]	2.47[a]	0.085	0.017

[1]SEM = Standard error of means. [ab]Means in the same row not sharing a common superscript are significantly different (P<0.05)

measured enzymatically on an autoanalyser (Cobas Bio, Roche, Switzerland) as described (Yeom *et al.*, 2002).

For crude fat determination in diet and excreta, the samples were first treated with 3 N hydrochloric acid for 1 h and further extracted with diethylether overnight. Total fat extracted was then measured after drying at 101°C overnight.

To determine fatty acid composition of corn and rye diets, a 10-g sample was extracted with a chloroform:methanol (2:1, v/v) mixture according to the method of Folch *et al.* (1957). Then, 20-25 mg of the extracted fat was saponified with 0.5 M methanolic sodium hydroxide and methylated with boronitrifluoride-methanol (Product no. 49370, Pierce, Illinois, USA) according to the method of Metcalfe *et al.* (1966). The methyl esters obtained were determined by gas liquid chromatography.

Statistical analysis: Pen was considered to be the experimental unit. The data obtained were evaluated by ANOVA using the SPSS/PC+ program. Treatment means were tested for statistically significant differences with the multiple range test of Duncan using the SPSS/PC+ program. The level of statistical significance was pre-set at P<0.05.

Results

Growth performance: Weight gain of birds fed the diet containing rye instead of corn was significantly lowered by on average 15.6% between 1-14 days of age (Table 3). The rye-induced suppression of weight gain between either 1-21 or 1-33 days of age did not reach statistical significance. Fortification of the rye diet with cinnamaldehyde tended to overcome the growth-suppressive effect of rye. In the birds fed rye diets with either thymol or CRINA® Poultry there were no signs of growth improvement. Birds fed on the rye diet without supplement tended to display a lower daily feed intake than their counterparts fed on the corn diet. When cinnamaldehyde was mixed into the rye diet there tended to be an increase in feed intake. Feed:gain ratio was not significantly different between dietary treatments. Daily water intake was higher in birds fed on the diet containing rye versus those given the corn diet and the increase was significant between 1-33 days of age. There were no effects of dietary essential oil components on water:feed ratio.

Organ weighs and intestinal viscosity: Wet liver weights of birds fed on the rye diet were numerically lighter than of those fed on the corn diet (Table 4). The rye-mediated reduction in liver weight was significantly reversed by dietary cinnamaldehyde, but was not affected by either thymol or CRINA® Poultry. Feeding rye to chickens significantly lowered pancreas weight. The reduction was not affected by the dietary essential oil components.

Table 4: Organ weights and intestinal viscosity in female broiler chickens fed the experimental diets

Parameters	Treatments					Pooled SEM[2]	P value
	Corn	Rye	Rye+ Thymol	Rye+ Cinnam-aldehyde	Rye+ CRINA Poultry		
Wet organ weight							
Liver, g	34.4^{ab}	30.9^{b}	32.8^{b}	37.3^{a}	32.6^{b}	1.171	0.027
Pancreas, g	3.87^{a}	3.38^{b}	3.44^{b}	3.55^{ab}	3.36^{b}	0.105	0.033
Relative organ weight[1]							
Liver	2.20	2.11	2.18	2.35	2.21	0.065	0.183
Pancreas	0.25	0.23	0.23	0.23	0.23	0.013	0.732
Viscosity, mPa.s							
Jejunum	1.26^{b}	7.82^{a}	7.40^{a}	8.65^{a}	8.03^{a}	0.650	0.000
Ileum	1.62^{b}	30.7^{a}	22.5^{a}	24.2^{a}	33.2^{a}	5.449	0.016

[1]g/100 g of body weight. [2]SEM = Standard error of means. ^{ab}Means in the same row not sharing common letters are significantly different (P<0.05)

Table 5: Apparent fat digestibility and plasma lipid profiles in female broiler chickens fed the experimental diets

Parameters	Treatments					Pooled S.E.M[1]	P value
	Corn	Rye	Rye + Thymol	Rye + Cinnam-aldehyde	Rye + CRINA Poultry		
Fecal digestibility , % of intake							
Crude fat	81.8^{a}	74.6^{b}	76.9^{b}	74.1^{b}	75.3^{b}	1.077	0.003
Plasma lipids							
Total cholesterol, mmol/L	2.94^{c}	3.14^{bc}	3.17^{b}	3.40^{a}	3.16^{bc}	0.065	0.009
Triglycerides, mmol/L	0.38	0.40	0.40	0.47	0.35	0.041	0.382
Phospholipids, mmol/L	2.72	2.91	2.90	3.14	2.94	0.087	0.074
HDL cholesterol, mmol/L	2.04^{c}	2.20^{abc}	2.17^{bc}	2.36^{a}	2.23^{ab}	0.052	0.017
HDL cholesterol, % of total	69.3	70.1	68.6	69.5	70.6	1.174	0.791

[1]SEM = Standard error of means, ^{abc}Means in the same row not sharing common letters are significantly different (P<0.05)

Relative liver weight (g/100 g of body weight) followed the same trend as did absolute wet liver weight, but the relative pancreas weights did not differ between dietary treatments. The feeding of rye instead of corn produced a significant, 6-fold increase in jejunal viscosity (Table 4). Ileal viscosity was even more pronouncedly increased by rye inclusion into the diet. There was no indication of an influence of essential oil components on intestinal viscosity.

Fat digestibility and plasma lipids: The feeding of rye instead of corn significantly impaired fat digestibility by 7.2% units. The rye-mediated inhibition of fat digestion was not relieved by any of the dietary essential oil components.

No effect of rye versus corn on plasma cholesterol was observed (Table 5). Supplementation of the rye diet with cinnamaldehyde significantly increased plasma total cholesterol. by 8.3%. Thymol and CRINA® Poultry did not affect plasma cholesterol. Plasma phopholipids and HDL cholesterol were not significantly elevated by dietary cinnamaldehyde. Plasma triglycerides were not affected by dietary treatments. HDL cholesterol accounted for approximately 70% of total plasma cholesterol.

Discussion

The present study was conducted to investigate whether thymol and/or cinnamaldehyde could overcome the anti-nutritional effect of rye. As would be expected, chickens fed a diet containing rye instead of corn showed a significantly lowered daily weight gain between 1-14 days of age. The rye-mediated growth suppression was continued during the intervals of 1-21 and 1-33 days of age and on average was as large as 11.6 and 6.9%, respectively. However, the reductions were not statistically significant which probably relates to insufficient statistical power caused by a relatively large inter-individual variation. Daily feed intakes of chickens fed on rye versus corn were non-significantly lowered by 12.8 and 11.4% between 1-14 and 1-21 days of age. Feeding rye to chickens significantly increased the water:feed ratio. Jejunal and ileal viscosities were significantly elevated by the rye inclusion. Fecal fat digestibilities of chickens fed the diet rich in rye were

significantly lower when compared to those of the chickens fed the corn diet. It can be concluded that the present results confirm the detrimental effect of rye on growth performance, intestinal viscosity and fat digestibility as reported earlier (MacAuliffe and McGinnis, 1971; Misir and Marquardt, 1978; Patel *et al.*, 1980; Antoniou and Marquardt, 1982; Honeyfield *et al.*, 1983; Bedford *et al.*, 1991).

Supplementation of the diet with either thymol or CRINA® Poultry did not counteract the growth-suppressive effect of rye. Cinnamaldehyde at the level of 100 ppm numerically improved weight gain and feed intake between 1-14 and 1-21 days of age, but the effects did not reach statistical significance. In contrast, water intake, water:feed ratio, intestinal viscosity and apparent fat digestibility were not affected by dietary cinnamaldehyde. It is generally accepted that the antinutritional effect of rye is mediated by its NSP constituents that raise the viscosity of gut contents and alter the microflora (Smits *et al.*, 1998; Langhout *et al.*, 1999). An increase in intestinal viscosity associated with enhanced bacterial fermentation may depress fat digestion. There is suggestive evidence that the low lipid digestibility seen in broiler chickens fed rye may be due to bacterial overgrowth in the small intestine and subsequent excessive deconjugation of bile acids (Smits and Annison, 1996). Dietary cinnamaldehyde had no effect on fat digestibility. It is thus likely that under the conditions of this study cinnamaldehyde not only left intestinal viscosity unchanged, but also the intestinal microflora.

Contrary to the reported hypocholesterolemic effect of dietary soluble fiber (Fadel *et al.*, 1987; Newman *et al.*, 1991; Petterson and Åman, 1991; Martinez *et al.*, 1992; Wang *et al.*, 1992) the feeding of rye instead of corn did not affect plasma cholesterol concentrations. It has been suggested that the increase in intestinal viscosity as caused by soluble NSPs is responsible for their hypocholesterolemic effect (Wang *et al.*, 1992). Rye consumption drastically raised intestinal viscosity. The lack of effect of rye consumption on plasma cholesterol may relate to the use of cholesterol-free diets. Those studies showing hypocholesterolemic effects all used diets fortified with cholesterol (Fadel *et al.*, 1987; Newman *et al.*, 1991; Pettersson and Åman, 1991; Martinez *et al.*, 1992; Wang *et al.*, 1992).

Supplementation of the rye diet with cinnamaldehyde significantly increased plasma cholesterol. In contrast, it has been shown that d-limonene, a component of essential oil derived from *Citrus sinensis*, depressed the rate-determining enzyme in *de novo* cholesterol synthesis, HMG-CoA reductase, which caused a significant reduction in plasma cholesterol concentration (Qureshi *et al.*, 1988). Sambaiah and Srinivasan (1991) reported that powdered-cinnamon at the level of 500 ppm increased serum cholesterol in rats, which would corroborate the present results. However, the addition of cinnamaldehyde to a corn-soybean basal diet did not affect plasma cholesterol (Lee *et al.*, 2003). Unlike in the study of Case *et al.* (1995), dietary thymol did not lower plasma cholesterol in this study.

In summary, the present study confirmed the antinutritional effects of rye on growth performance, intestinal viscosity and fat digestibility. Addition of cinnamaldehyde to the rye diet tended to improve growth performance, but this was not associated with either a reduction in gut viscosity or a relief of the impairment of fat digestibility, but rather to an increase in voluntary feed intake. Thymol added to the diet at the same level as cinnamaldehyde had no effect on growth performance.

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