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Full Length Research Paper

Influence of dietary thymol and carvacrol preparation and/or an organic acid blend on growth performance, digestive organs and intestinal microbiota of broiler chickens

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This experiment was designed to investigate the effects of supplementation with plant extract, either alone or in combination with an organic acid on growth performance, intestinal organ measurements and intestinal microbiology. One-day-old male Ross 308 strain broiler chicken (n=96) were allocated to 4 dietary treatments in a randomized complete block design. Dietary treatments were: (i) basal diet (as a control) (C), (ii) basal diet + organic acid mixture feed (OA), (iii) basal diet + plant extract (PE) and (iv) basal diet + organic acid mixture + plant extract feed (OA+PE). Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were not improved by supplementation of OA or PE to the diets. Proventriculus, gizzard, hearth, liver, pancreas, abdominal fat and bursa of Fabricious weights were not significantly affected by dietary treatments. OA and PE diets resulted in increased weight and length of duodenum, jejenum, ileum and caceum. However, differences between treatments were not statistically significant (P>0.05). Supplementation of organic acid has positive effect on ileal microbiology. In ileal digesta, LAB and yeast counts were significantly (P<0.001) increased for birds fed OA and PE, whereas *Escherichia coli* counts were significantly (P<0.001) decreased in OA group. In conclusion, as OA and PE supplementation had any positive effects on the performance. Ileum microflora of OA supplemented group changed for the benefit of non-pathogenic bacteria, probably due to the decrease in pH levels of ileum. *E. coli* count was found lower for OA treatment than the other groups. These results indicate that the OA can improve gut health. The products that researched show promising effects, however, further researchers may be useful to understand their effects better.

Key words: Broiler, plant extract, thymol, carvacrol, organic acid.

INTRODUCTION

With the removal of antibiotic growth promoters (AGP) from poultry diets in various regions of the world, it is of interest to investigate potential alternatives to maintain good growth performance and improvement of feed efficiency, good intestinal microbial populations, particularly to prevention or control of infectious disease. Organic acids and herbal extracts are two important alternatives. These alternatives are of great interest to the poultry industry.

Organic acids have been used for decades in feed preservation, protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feed, e.g. silages. In particular formic acid and propionic acid have been used extensively for this purpose. Some researchers have suggested that organic acids can be used to control intestinal microbial growth (Dibner and Buttin, 2002; Ricke, 2003). Most proposed hypotheses for mode of action for controlling microbial growth with organic acids involve depolarization of the bacterial membrane, a change in internal pH, and alterations in the nutrient transport and synthesis within the bacterium (Biggs and Parsons, 2008). Moreover, organic acids feeding are believed to have several

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beneficial effects such as improving feed conversion ratio, growth performance, enhancing mineral absorption and speeding recovery from fatigue (Boling et al., 2000). The beneficial effects of organic acids on the productive traits of pigs have been demonstrated in many studies, but consistent enough data have not been obtained for poultry (Samanta, 2008).

On the other hand, various plant extracts, especially essential oils, have been investigated on the basis of their demonstrated *in vitro* antimicrobial activity (Griggs, and Jacob, 2005). In poultry production, the main effects of essential oils are focused on the intestinal tract (Wenk, 2002). Essential oils have been studied as a tool to reduce unwanted bacteria on the basis of their demonstrated *in vitro* antimicrobial activity (Griggs and Jacob, 2005; Cowan, 1999).

The use of essential oils in broiler diets is a new issue and there is an enormous variety of these products. The results of inclusions of plant extracts on the growth performance of broilers (Botsoglou et al., 2002) or pigs (Turner et al., 2001) are controversial.

In recent years, there is an increasing trend in using organic acid mixtures, or their salts in combination with essential oils and herbal extracts as alternatives to antibiotic growth promoters due to their inhibiting activity on the growth and development of pathogens in animal feed and gastrointestinal tract (Isabel and Santos, 2009; Zhang et al., 2005; Cardinali et al., 2008). This study was conducted to assess the potential of organic acid mixture, plant extracts (essential oils), and the combination of organic acid mixture with essential oils as possible alternatives to improve broiler chicken growth performance, feed conversion ratio (FCR) and intestinal microbiology.

MATERIALS AND METHODS

Animals and housing

Male Ross 308 broilers were obtained from a local parent stock supplier and randomly transferred to compact-type three-tier cage, four chicks per cage. Battery cages were equipped with wire mesh, nipple drinkers and through feeders. The experiment consisted of four dietary treatments and was set up in completely randomized design in which 24 chicks were randomly assigned to each of the four treatments, six replicates each. The experiment lasted for 21 days and the chicks were fed the experimental diets throughout the experimental period. Feed and water provided *ad libitum*. The lighting regime was 23 h/day. The birds were weighed individually 1, 7, 14 and 21 days of age.

Diets

Four experimental treatments included diet C, a control diet without additive supplementation; diet OA with 20 000 mg/kg of propionic acid, 85 000 mg/kg of ammonium propionate, 160 000 mg/kg of ammonium propionate and 35 000 mg/kg of formic acid (Salgard® was provided by optivite LTD., Notts, UK); diet PE with 250 g/kg thymol and 250 g/kg carvacrol (Next Enhance 200® was provided by Ekol Gida, Levent 34330 Istanbul/Turkey) and diet OA + PE containing a blend of 20 000 mg/kg of propionic acid, 85 000 mg/kg of ammonium propionate, 160 000 mg/kg of ammonium propionate and 35 000 mg/kg of formic acid and 250 g/kg thymol and 250 g/kg carvacrol. The diets were prepared consecutively using the same batch of ingredients, and the different additives used were added together with the micro ingredients.

Experimental diets were formulated using ration-formulation software (UFFDA, 1992) to be isocaloric and isonitrogenous following National Research Council recommendations (NRC, 1994). Diets were formulated to contain 23% crude protein and 3152 kcal/kg, and other essential nutrients (Table 1). Birds were fed with experimental diets *ad libitum* in mash form. Feed intake was recorded weekly. The FCR was calculated as grams of feed consumed per chick divided by grams of weight gain per chick.

Digestive organ measurements

At 21 days of age, two birds taken randomly from each cage (14 birds per treatment) were starved overnight and killed by cervical dislocation. The birds were then weight and their organs harvested. Organ analyses included proventriculus, gizzard, abdominal fat, hearth, pancreas, liver, duodenum, jejenum, ileum, ceca, thymus, bursa of Fabricious and spleen weights and duodenum, jejenum, ileum and ceca lengths. The gastrointestinal tract was portioned into proventriculus, gizzard, pancreas, duodenum, jejenum, ileum and ceca. The ileum, defined as the region from Meckel's diverticulum to a point 40 mm proximal to the ileocecal junction. The jejenum was defined as the portion of intestine extending from the bile duct enterance to Meckel's diverticulum. All organs weights and lengths were expressed as a percent of body weight.

Intestinal microbiota

Ileal contents of two chicks from each replicate were immediately collected. The entire contents were transferred under aseptic conditions into sterile glass tubes in which they kept on ice until subsequent inoculation into agar. Accordingly, as the incubation medium; MRS agar (MERCK, 1.10660) was used for lactic acid bacteria (LAB) and malt extract agar (MERCK, 1.05398) was used for yeast. LAB and yeast counts of the ileum contents were obtained at 30°C following a 3 day incubation period. *E. coli* were grown aerobically on VRB agar (MERCK, 1.01406) at 37°C for 24 to 48 h. The LAB, yeast and *E. coli* were counted, and the average number of live bacteria determined per gram of original ileal contents. All quantitative data were converted into logaritmic colony forming.

Statistical analysis

Collected data were recorded on a weekly basis and statistically subjected to ANOVA using the PASW Statistics18 packed program for windows (PASW Statistics18, 2010). The differences between group means were evaluated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Performance

The result of growth performance is shown in Table 2. The effect of dietary treatments was significant (P<0.001) on body weight gain (BWG) from d 0 to 21. From 0 to 21

Table 1. The ingredients and chemical composition of experimental basal diet (as-fed basis).

Ingredients (%)	Basal diet
Corn	49.356
Soybean meal (44% CP)	29.210
Full-fat soybean (37.5% CP)	13.000
Soybean oil	4.218
Di-calcium phosphate	1.918
Limestone	1.499
Sodium chloride	0.321
DL-Methionine	0.201
L-Lysine	0.027
Vitamin-Mineral Premix[1]	0.250
Nutrient content[2]	
ME (kcal/kg)	3100
Crude protein (%)	23.00
Crude fiber (%)	3.39
Ether extract (%)	7.46
Crude ash (%)	5.42
Methionine+Cystine (%)	0.87
Lysine (%)	1.20
Methionine (%)	0.52
Trytophan (%)	0.30
Calcium (%)	1.05
Total phosphorus (%)	0.67
Nonphytate phosphorus (%)	0.50
Linoleic acid (%)	4.51

[1]Provided per kilogram of diet: vitamin A, 8,000 IU (as retinyl acetate); vitamin D_3, 2,500 IU (as cholecalciferol); vitamin E, 30 mg (as α-tocopheryl acetate); vitamin K_3, 2.5 mg (as menadione sodium bisulfite); vitamin B_1 2 mg (thiamine); vitamin B_2, 5 mg (as riboflavin); vitamin B_6, 2 mg (as pridoxamine); vitamin B_{12}, 0.01 mg (as cyanocobalamin); niacin, 30 mg; calcium-D-pantothenate, 8 mg; folic acid, 0.5 mg; D biotin, 0.045 mg; choline chloride, 300 mg; vitamin C, 50 mg; MnO_2, 70 mg; $FeSO_4\_7H_2O$, 35 mg; ZnO, 70 mg; $CuSO_4\_5H_2O$, 8 mg; $Ca(IO_3)_2$. [2]Based on NRC (1994) values for feed ingredients.

Table 2. Effects of experimental diets on broiler performance (0 to 21 days).

Treatment	Weight gain (g/bird)	Feed intake (g/bird)	FCR
C	766.92^{b}	1070.43^{b}	1.396
PE	615.23^{a}	946.98^{a}	1.539
OA	686.36^{a}	969.58^{a}	1.413
OA+PE	641.49^{a}	967.51^{a}	1.508
SEM	16.186	14.394	0.026
P level	0.001	0.004	0.117

a-b: Means with different superscripts in a column are significantly different.

days, all treatments depressed growth and feed intake (FI) of chicks when compared with the control diet. Dietary treatments did not have any significant effect on FCR (P>0.05). BWG, FI and FCR of birds fed only organic acid diet among experimental diets was numerically better than those of birds fed on PE and OA + PE diets. The present finding are in conformity with the report of Fancher and Jensen (1988) that reported when propionic acid is supplemented up to 3% in the diet, no significant effects on feed intake. Liem et al. (2008)

Table 3. Digestive organ measurements of broilers.

Organ	C	PE	OA	OA+PE	SEM	P level
Organ weight, g/100 g of BW						
Gizzard	3.003	3.019	3.142	3.222	0.092	0.834
Proventriculus	0.639[b]	0.605[b]	0.501[a]	0.582[b]	0.160	0.008
Heart	0.725	0.828	0.763	0.770	0.017	0.192
Liver	2.828	2.806	2.812	3.011	0.053	0.478
Pancreas	0.412[b]	0.386[b]	0.328[a]	0.362[ab]	0.010	0.015
Abdominal fat	0.783	0.845	0.813	0.909	0.046	0.817
bursa of Fabricious	0.235	0.234	0.257	0.227	0.012	0.855
Duodenum	1.443	1.557	1.524	1.582	0.027	0.313
Jejunum	3.917	3.719	4.080	3.943	0.135	0.844
Ileum	2.204	2.342	2.098	2.398	0.083	0.599
Ceca	0.907[a]	1.368[ab]	1.290[ab]	1.546[b]	0.104	0.166
Organ length, cm/100 g of BW						
Duodenum	3.696	4.235	3.999	4.356	0.112	0.163
Jejunum	9.059	9.882	9.915	9.917	0.207	0.391
Ileum	6.599	7.862	7.404	7.437	0.222	0.245
Ceca	1.313[a]	2.621[b]	2.417[b]	2.498[b]	0.161	0.005

a-b: Means with different superscripts in a row are significantly different.

showed that addition of citric acid, malic acid and fumaric acid did not increase the 16 days body weight and gain-feed. The dietary inclusion of benzoic acid at 0.2% depressed the growth of broiler chickens (P<0.05) (Jozefiak et al., 2008). Similar to our results, Hernandez et al. (2006) failed to observe any effect on the performance of chickens when formic acid (5, 000 or 10, 000 ppm) was added to the feeds. On the other hand, Lee et al. (2003) found that dietary carvacrol and thymol at a concentration of 200 ppm lowered BWG and FI but improved FCR when female broilers were fed the respective diet for 4 weeks. In contrast, Botsoglou et al. (2002) reported that when dietary oregano essential oils, at concentrations of 50 and 100 ppm, were fed to broiler chickens for a period of 38 days, hardly any effects on BW and FCR could be demonstrated. Moreover, Case et al. (1995) reported that dietary carvacrol and thymol, at 150 ppm, did not influence BW gain of cockerels with initial weights of 126 g that were followed during a 21 days feeding trial.

The findings from this experiment indicated that the supply of essential oil and organic acid to broiler chicks feeds for 21 days have limited effect on chick performance. The study is realized with minimum stress and maximum hygienic conditions, and it would get more positive results under similar circumstances in practical conditions.

Digestive organ measurements

The effect of dietary treatments on the relative weight and length of different sections of digestive organs from 21 days old birds are presented in Table 3. No treatments effects were observed with any feed supplements within the diets on organ weights except for proventriculus, pancreas and ceca weight and ceca lengths. The supplementation of organic acid decreased proventriculus, pancreas and ceca weight compared with PE and OA + PE supplementation. In addition, ceca length tended to decrease in birds on the OA diet compared with those given PE and OA + PE diets but differences are not significant (P>0.05).

No differences were noticed for liver, gizzard, abdominal fat, and other edible parts in experiments. However, in experiment, birds fed OA + PE had numerically higher liver weight percent compared to the other treatments. It has been reported that water-soluble extract from rosemary enhanced hepatic metabolism and increased liver weight in rats (Debersac et al., 2001). However, other studies in broilers have reported no effects on plant extracts on organ weights (Hernandez et al., 2004). The organic acid dietary regimens had no effect (P>0.05) on the abdominal fat percentage and liver weight at the end of experiment compared with control and other groups. Skinner et al. (1991) compared the effects of dietary fumaric acid supplementation at 0.125, 0.25 and 0.50% on broiler performance from 0 to 49 days. They found similar results in our present study.

Intestinal microbiota

The effects of dietary treatments on ileum microbiota (log

Table 4. Effects of experimental diets on ileum microbiota (log cfu/g ileal content).

Treatment	LAB	Yeast	E. *coli*
C	3.623[a]	3.722[a]	5.386[b]
PE	4.975[b]	5.035[c]	5.653[b]
OA	5.250[bc]	4.402[b]	4.086[a]
OA+PE	5.462[c]	3.978[a]	5.688[b]
SEM	0.159	0.115	0.157
P level	0.001	0.001	0.001

a-c: Means with different superscripts in a column are significantly different.

cfu/g contents) is shown in Table 4. In ileal digesta, LAB counts were significantly increased for birds fed OA + PE and OA (P<0.001), whereas E*. coli* were significantly decreased compared to other groups for birds fed OA (P<0.001). A decrease in the population of yeast in ileal digesta was observed in control and OA + PE groups (P<0.001). The results of present study demonstrate that the addition of organic acid had a limited effect on intestinal microbiology. Organic acid activity would reduce the total microbial population but would be particularly effective against *E. coli* and other acid intolerant microorganism (Dibner and Buttin, 2002).

Plant extracts and organic acids could control and limit the growth and colonization of numerous pathogenic and non-pathogenic species of bacteria in the gut. It has been reported that Gram positive bacteria are more sensitive to essential oil compounds (Smith-Palmer et al., 1998). Organic acids are one of the most efficient feed additives for mould prevention. For example, propionic acid inhibits the growth of fungi and prevents production of mycotoxins (Paster et al, 1988; Marin et al., 1999). Usage of some acids, such as formic, propionic, and HMB have broader antimicrobial activities and can be effective against bacteria and fungi, including yeast (Partanen and Mroz, 1999; Doerr et al.,1995;

Conclusion

In conclusion, the results of the present study confirm previous studies showing little benefit from organic acid and/or plant extract supplementation to broiler diets. The study is realized with minimum stress and maximum hygienic conditions, and it would get more positive results under similar circumstances in practical conditions. However, addition of organic acid mixture to broiler diets may result in a reduced *E. coli* in ileal digesta.

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