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EFFECTS OF DIETARY ORGANIC ACIDS ON PERFORMANCE, CARCASS CHARACTERISTICS AND GUT FLORA OF BROILER CHICKS

E. Öztürk[1], A. Yildirim[2] and C. Eroğlu[3]

[1] *Department of Animal Science, Faculty of Agriculture, 19 Mayıs University, 55139 Samsun, Turkey.*
[2] *Department of Animal Breeding and Health, Şarkışla Aşık Veysel Vocational College, Cumhuriyet University, 58400 Sivas, Turkey.*
[3] *Department of Clinical Microbiology and Infectious Diseases, Medicine Faculty, 19 Mayıs University.*

ABSTRACT

In this study, 360 day-old mixed sex Ross 308 broilers were allocated to 4 experimental groups. Organic acid (Genex) at 0, 0.1, 0.2 and 0.3 % levels were added to the experimental diets. No difference was observed for live weight gains among treatments at the end of the 6th week (P>0.05) while maximum feed consumption were observed at 0.1% organic acid levels and the poorest feed efficiency were observed at 0.2 and 0.3 % organic acid levels (P<0.05). Organic acids did not affect either carcass parameters carcass weights, dressing out percentage and edible organs (P>0.05) or intestinal pH and bacterial population (P>0.05) while there were evident decreases in gram (-) bacteria counts on 21st day with dietary organic acid supplementation. In conclusion, the dietary supplementation of organic acid had no beneficial effect on either the performance or intestinal microflora.

Key words: Organic acid, broiler chickens, gut microflora, diet.

INTRODUCTION

The hygienic conditions provided in the poultry farms decreases the size of population of intestinal gut flora. So animals are more sensitive against pathogen bacteria from outside in comparison to wild life or free range birds. Consequently, the growth performance of broilers decreases additionally with stress factors (Mulder, 1996; Bilal *et al*., 1999). This situation leads animal nutritionist to investigate solutions alternative to therapeutic and/or prophylactic (Campenhout *et al*., 2001). In the young animals, hydrochloric acid production in the stomach is not sufficient and acidifiers (organic or inorganic acids) are used to modify gut flora in animal feeds (Ivanov, 2003). The-pH – effect of organic acids in feed potentially encourages the establishment of a beneficial microflora in the upper intestine while at the same time reducing the growth rate of pathogenic bacteria (Ratcliff, 2000). Organic acids (OA) are added to feed in order (1) to improve intestinal gut flora and (2) to vanish digestive disorders from stress conditions in animals. However, in order to clear the effects of dietary organic acids on gut flora, it has to be determined the effects of organic acids on the size of bacteria strain and numbers. This study was carried out to determine whether dietary organic acid have impact on growth performance, carcass and intestinal parameters on total and gram-negative bacteria numbers.

MATERIALS AND METHODS

The study was conducted in the research farm of 19 Mayis University. One-day-old mixed sexes 360 broiler chicks (Ross 308) were used in this study. The chicks were fed with the starter diet (12.87 mj ME kg^{-1}, 240 g CP kg^{-1} as fresh matter basis) for the first three weeks of the experiment. Consequently, they were fed on grower diet (13.40 mj ME kg^{-1}, 200 g CP kg^{-1} as fresh matter basis) during the period of 4-6 weeks of the experiment (Table1).
The organic acid (Genex) used in the study is a mixture of propionic acid, ammonium propionate, formic acid, ammonium format, sodium aluminium silicate, plant extract and essential fatty acids. The chicks were randomly subjected to four experimental groups with three replications. The experimental feeds (feed 1 and feed 2) were given to animals ad libitum in mash form.

Control group was fed with basal diet without containing organic acid while the 2nd, 3rd and 4th groups were fed with the feed supplemented @ 0.1% (0.1 OA), 0.2 (0.2 OA) and 0.3 (0.3 OA). The experimental feeds were chemically analysed according to the Weende Method. A total 48 representative birds (4 birds from each sub-group 2 males and 2 females) were slaughtered to determine carcass parameters. For determination of intestinal parameters, 24 male birds (2 males from each sub-group) were slaughtered. For intestinal parameters, 2 male

[1]Corresponding Autor: Ondokuz Mayis University Ziraat Fakultesi Kurupelit, 55139 Samsun, Turkey. eozturk@omu.eu.tr

broilers representing each group (total 6), and 24 chicken broilers for total groups were also slaughtered on the 43rd day. Full digestive system, pharynx, oesophagus, proventriculus, gizzard, caecum, colon, and small intestines were eviscerated to be weighted.

The relative small intestine weight was calculated by the criterion of % of the body weight. pH values for the intestinal samples were measured in duodenum, ileum and caecum by using electronic pH device after diluted with deionised water in blood tubes at the rate of 1:10 (Alp *et al*., 1999). For microbiological analysis on the 21st day of the study total 12 chicks (one male from each group), and on the 42nd day total 24 animals (two males from each subgroup) were selected slaughtered after being weighted. For gram-negative and total bacteria count in the laboratory, the full gut was kept in different plastic bags.

Table 1. The composition and nutrient contents of experimental diets.

Feeds / experimental periods	Starter diet (0 to 3 weeks)	Grower diet (4 to-6 weeks)
Maize	42.5	46.3
Barley	8	5
Soybean meal	22.5	13
Hazelnut kernel oil meal	5	4
Full fat soybean	8	12
Whest middlings	5	4
Corn bran	-	5
Corn gluten	-	3
Vegetable oil	2.4	1.6
Meat-bone meal	3	4
Fish meal	2	1
Vitamin premix[1]	0.2	0.2
Mineral premix[2]	0.1	0.1
Salt	0.22	0.21
DCP	0.7	0.2
Sodium bicarbonate	0.1	0.1
Anticocsidiostat[3]	0.1	-
DL-Methionine	0.13	0.15
Choline chloride	0.05	0.05
L-Lysine	-	0.09
Total	100	100
Chemical contents of experimental diets		
Calculated		
ME (MJ/kg)	12.87	13.40
Crude protein, %	24	20
Calcium, %	1.00	0.90
Available phosphorus,%	0.45	0.45
Lysine, %	1.38	1.20
Methionine, %	0.48	0.44
Calorie/ Crude protein	125.4	160
N free extracts, %*	48.02	49.45
Analysed		
Dry matter, %	87.90	87.33
Crude protein, %	24.3	21.9
Crude fat, %	7.41	7.85
Crude fibre, %	2.31	2.34
Cost ($/kg) [4]	0.279	0.219

[1]: Vitamin (Vit) premix contains per kg diet: 6 500 000 IU Vit. A, 1 500 000 IU Vit. D_3, 25 000 mg Vit. E, 2500 mg Vit. K_3, 1500 mg Vit. B_1, 3000 mg Vit. B_2, 2500 mg Vit. B_6, 15 mg Vit. B_{12}, 25 000 mg Vit. C, 5000 mg Ca D-Pantotenate, 15 000 mg Niasin, 500 mg Folic acid, 38 mg Biotin, 250 mg Apo carotenoic acid ester, 62 500 mg Endox

[2] :Mineral premix contains for per kg diet: 80 000 mg Mn, 60 000 mg Fe, 60 000 mg Zn, 5000 mg Cu, 200 mg Co, 1000 mg I, 150 mg Se, 300 000 mg choline chloride.

[3]: 1000 mg diclazuril contains Clninacox coccidiostat wasn't added to diets for 4-6 week period.

[4]: This calculation was made based on the price (the Turkish currency) of ingredients on the 1 December 2001.

On the 21st day of the study, the samples were taken from ileum and caecum while the same procedure was done only in caecum on the 42nd day. These samples were weighted on the electronic scale after the intestines was cut through with a help of a scalpel and placed into 1,5 ml sterile eppendorf tubes (10 mg).

For gram-negative and total bacteria count in the laboratory, 1 ml sterile saline was added to taken samples. After saline addition, they were vigorously vortexed for homogenisation (60 second). Homogenized solutions (5 µl) were inoculated to eosin methylene blue (EMB) and blood agar plates. After that, these plates were exposed to 48 h incubation at 37 ^{o}C (Watkins and Kratzer, 1983). Then each bacterial colony was counted on every plate. The number of total and gram (-) bacteria count were recorded with calculation of multiplying these counts with diluting rates at the beginning.

The data were analysed by using Generalized Linear Model (GLM) procedures of the SPSS, release 10.0. Because experimental animals were in both sexes, sex factor was regarded as co-variant. The treatment means were compared using Duncan's multiple range test (SPSS, release 10.0).

RESULTS AND DISCUSSION

During experimental period, body weight gain did not change by organic acid supplementation to diet of broilers, but 0.3% OA decreased body weight gain on the days of 14 and 35th in comparison to other doses (Table 2). The effect of organic acid supplementation on body weight gain may not be seen for the first weeks (Namur *et al.*, 1988), because of the age of experimental birds in which microbial population may not be develop sufficiently. It was hypothesised that OA can optimise the intestinal pH for beneficial bacterial flora (gram +).

Table 2. Body weights of broiler chickens fed diets containing organic acids, g per chicken.

Old, days	Control	0.1OA	0.2OA	0.3OA	SEM
1	41	40	40	41	0.4
7	89	90.	88	87	1.7
14	325 a	326 a	323 ab	310 b	4.7
21	721	721	715	706	9.5
28	1207	1217	1213	1198	15.8
35	1773 a	1789 a	1758 a	1735 b	24.1
42	2342	2347	2274	2321	30.9

SEM: standard error of the mean.
a, b. Values within a row with unlike superscripts differ significantly (P < 0.05).

These results are in line with those of Izat *et al.*, (1989), Izat *et al.*, (1990 a,b), Waldroup *et al.*, (1994), Huff *et al.*, (1994), Alp *et al.*, (1999) and Hadorn *et al.*, (2000). It can be assumed that the shorter and small caecum and colon and the high rate of feed transit may not allow the appearance of the impact of organic acids on performance of broiler chicks.

The different results from literature might be related to different hygienic conditions of animal houses, the health of animals, the structure of the diet, the combination of feedstuffs and feed supplementation factors (Alp *et al.*, 1993; Eren *et al.*, 1999). Feed intake increased with increased supplementation of organic acids while there was no difference at the age of 7th, 21st, 28th and 35th days (Table 2). A 3% OA supplemented group consumed the higher amount of feed than control group on 14th day ($P<0.05$). The similar difference was observed on 42nd day. In this period, 0.3% OA supplemented group consumed higher amount of feed than control group. Some scientists thought the feed intake were related to organoleptic properties of diet (Şahin, 2003) and its supplementation (Skinner *et al.*, 1991). During experimental period, feed conversion ratio increased with organic acid supplementation (Table 2). This increase was clear in 0.3 % OA group in comparison to that of control group on 14th day ($P<0.05$) while there was no significant difference afterwards until 35th($P>0.05$). On the other hand both 0.2 and 0.3 % OA groups (4.24 vs 4.74 % lower than that of control) showed the lower feed efficiency in comparison to controls at the age of 42nd day. This reduction in feed efficiency was a result of increase in feed intake by organic acid. There have been contradictory findings on literature: Some researchers informed that organic acids added to feed did not affect feed efficiency in broilers (Izat *et al.*, 1990b; Skinner *et al.*, 1991; Kahraman *et al.*, 1997; Kırkpınar *et al.*, 1999; Hadorn *et al.*, 2000) while some reported organic acids enhanced feed efficiency in broilers (Patten and Waldroup, 1988; Izat *et al.*, 1990a; Alp *et al.*, 1999; Versteegh and Jongbloed, 1999).

The mortality of broiler chicks was not affected by organic acid supplementation, agreeing with findings of Izat *et al.*, (1990b), Skinner *et al.*, (1991), Hadorn *et al.*, (2000) in the literature.

The price of diet was estimated and with organic acid supplementation as 0.279, 0.282, 0.286 for starter and 0.289; 0.219, 0.223, 0.226 and 0.229 $ per kg grower diets, respectively.

The data regarding final live weight, carcass weight and edible inner organs showed that there was no effect of organic acid on these parameters (Table 3). While the relative abdominal fat pad (% of body weight) was increased in a dose related manner (P<0.05).
The increase in abdominal fat pad with organic acid supplementation could be sign of increasing of feed intake and poorer feed efficiency. This increase in feed intake did not contribute positively to weight gain increase because it increases abdominal fat pad with negatively affecting feed efficiency, assuming that the metabolic cost of fat synthesis.

Table 3. Feed intake and feed conversion ratio (g feed / g gain) of broiler chickens fed diets containing organic acids.

Old, days	Control	0.1OA	0.2OA	0.3OA	SEM
Feed intake, g/bird					
7	129	129	131	127	2.7
14	483 b	493 ab	488 ab	504 a	5.1
21	1045	1060	1062	1081	12.3
28	1870	1902	1909	1935	17.2
35	2924	2963	2983	3010	26.9
42	4196 b	4201 b	4249 ab	4353 a	33.6
Feed conversion ratio, feed/gain					
7	1.45	1.43	1.49	1.47	0.03
14	1.49 b	1.52 b	1.51 b	1.62 a	0.02
21	1.45	1.47	1.49	1.53	0.04
28	1.55	1.57	1.58	1.62	0.05
35	1.65	1.66	1.70	1.74	0.04
42	1.79 b	1.79 b	1.87 a	1.88 a	0.03

SEM: standard error of the mean.
a, b. Values within a row with unlike superscripts differ significantly (P < 0.05).

Intestine length, weight and its relative weight for 100 g body weight, pH values in duodenum, ileum, and caecum were not affected by organic acid supplementation while there were insignificant declines in pH values in treatment groups in comparison to control group (Table 5). The data regarding pH values were revealed numbers reductions according to control groups. Pathogen micro-organism population such as *Enterobacteriacea* and *Salmonella* are known to be reduced by increasing volatile fatty acids at the level of intestinal pH 6.0 (Edens *et al*., 1997). Reducing effect of organic acids on pH values makes micro-organism population harmless and consequently organic acids will lead to increasing of enzyme activity along digestive tract (Tellez *et al*., 1993).

Table 4. Slaughter body weight (SBW), carcass weight (CW), dressing out percentage (DP), heart weight, liver weight, gizzard weight, abdominal fat pad (AFP), AFP per 100 g body weight (BW) of broiler chickens fed diets containing organic acid.

Variable	Control	0.1OA	0.2OA	0.3OA	SEM
SBW, g	2387	2513	2351	2403	65.3
CY, g	1745	1847	1744	1778	46.0
DP, %	73.1	73.5	74.2	74.3	0.52
Heart, g	11.2	11.8	11.0	11.8	0.53
Liver, g	44.6	44.9	42.8	42.6	1.79
Gizzard, g	31.8	31.8	31.1	33.0	1.30
AFP, g	35.5 b	40.6 a	38.9 a	45.8 a	2.54
AFP, g/100 g BW	1.49 b	1.64 ab	1.67 ab	1.95 a	0.030

SEM: standard error of the mean.
a, b. Values within a row with unlike superscripts differ significantly (P < 0.05).

Table 5. Small intestine length (SIL), small intestine weight (SIW), SIW per 100 g body weight (BW) and pH values of duodenum, ileum and caecum of broiler chickens fed diets containing organic acids.

Variable	Control	0.1OA	0.2OA	0.3OA	SEM
SIL, cm	195.7	194.3	189.2	194.2	5.76
SIW, g	46.1	47.8	42.2	47.2	1.72
SIW, g/100 g BW	1.82	1.87	1.68	1.80	0.054
pH of duodenum	6.6	6.5	6.5	6.6	0.06
pH of ileum	6.6	6.4	6.4	6.6	0.11
pH of caecum	7.1	6.7	7.1	6.7	0.06

SEM: standard error of the mean.

There was no difference between groups with respect to the number of total and gram (-) bacteria on the 21st day in ileum and caecum; on the 42nd day in caecum of broiler chicks (Table 6). This means that dietary organic acid supplementation did not affect the intestine bacteria population. However Izat *et al*., (1990b) found a reduction of ileum pH reduced the population of gram-negative *Enterobacteriaceae.*

These results showed that organic acids supplementation did not affect the performance of broiler chicks especially 0.20 % and 0.30% dietary organic acid supplementation decreased feed efficiency without affecting intestinal gram-negative bacteria populations. In conclusion, the dietary supplementation of organic acid had no beneficial effect on either the performance or intestinal flora.

Table 6. Number of total and gram (-) bacteria of ileum and caecum in broiler chickens, fed diets containing organic acid, log coloni forming unit (cfu)/g.

Old, days	Control	0.1OA	0.2OA	0.3OA	SEM
21st day bacteria counts					
Total in Ileum	9.54	8.82	9.78	9.80	0.266
Total in Caecum	10.01	9.72	9.89	9.96	0.127
Gram (-) in Ileum	8.98	8.11	7.81	8.28	0.566
Gram (-) in Caecum	9.96	9.52	9.67	9.85	0.165
42nd day bacteria counts					
Total in. Caecum	9.17	9.22	9.18	9.35	0.075
Gram (-).in Caecum	8.59	8.63	8.45	8.77	0.174

SEM: standard error of the mean.

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