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Effects of Dietary Supplementation with Organic Acids and Zinc Bacitracin on Ileal Microflora, pH and Performance in Broilers

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Abstract: An experiment was conducted to determine the effects of an organic acid combination (Acid Lac Dry: lactic, fumaric, propionic, citric and formic acid) and/or zinc bacitracin on the body weight gain, feed efficiency, microflora and pH in the ileum content of broiler-type chicks. Ross PM3 broiler chicks were placed in an experimental design that consisted of four dietary treatments with 48 broilers in each group. One-day-old chicks were fed either a control diet or a similar diet supplemented with 3 g/kg Acid Lac Dry, 0.1 g/kg zinc bacitracin or both additives in combination during a 42-d feeding trial. Acid Lac Dry+zinc bacitracin supplemented diet, significantly ($P<0.05$) increased body weight at 3 weeks of age but not at 6 weeks of age when compared with control and Acid Lac Dry supplemented diets. The best feed to gain ratio was obtained in group fed Acid Lac Dry+zinc bacitracin supplemented diet during this experiment. Carcass weight and dressing percentage were not affected by any treatment. Mean ileal pH was significantly ($P<0.05$) higher in group fed the additives in combination than the animals treated with either Acid Lac Dry or zinc bacitracin. However, the combination had the lowest number of *Enterobacteriaceae* in the intestinal material.

Key Words: Organic acid, zinc bacitracin, broiler, performance, ileal pH, ileal *Enterobacteriaceae* level

Broylerlerde Yeme Organik Asit ve Zinc Bacitracin İlavesinin İleum Mikroflorası, pH'sı ve Performansa Etkisi

Özet: Bir organik asit karması (Acid Lac Dry: laktik, fumarik, propiyonik, sitrik ve formik asit) ve/veya zinc bacitracinin broylerlerde canlı ağırlık artışı, yemden yararlanma ile ileum mikroflorası ve pH'sı üzerine etkilerini incelemek üzere bir araştırma yürütülmüştür. Ross PM3 broyler civcivler, her birinde 48 civciv bulunan dört deneme grubuna ayrılmıştır. Günlük civcivler, kontrol rasyonu veya buna 3 g/kg Acid Lac Dry, 0.1 g/kg zinc bacitracin ya da her ikisinin birden eklendiği deneme rasyonları ile 42 gün süresince beslenmişlerdir. Acid Lac Dry+zinc bacitracin katkılı yem, ilk 3 haftanın sonunda, kontrol ve sadece Acid Lac Dry katkılı gruplara göre canlı ağırlığı önemli düzeyde artırırken ($P<0.05$), 6. haftanın sonunda önemli bir farklılık saptanmamıştır. Deneme süresince en iyi yemden yararlanma oranı Acid Lac Dry+zinc bacitracin grubunda bulunmuştur. Karkas ağırlığı ve karkas randımanı, gruplar arasında farklılık göstermemiştir. Ortalama ileum pH'sı iki katkı maddesinin birlikte verildiği grupta, sadece Acid Lac Dry ya da zinc bacitracin verilen gruplara göre önemli düzeyde yüksek bulunmuştur ($P<0.05$). Buna karşın, iki katkı maddesinin birlikte kullanıldığı grupta bağırsak içeriğindeki toplam bakteri sayısı, tüm gruplar içerisinde en düşük değerde saptanmıştır.

Anahtar Sözcükler: Organik asit, zinc bacitracin, broyler, performans, ileum pH'sı, ileum *Enterobactericeae* düzeyi

Introduction

Acidification has the potential of controlling all enteric bacteria, both pathogenic and non-pathogenic (1). Various organic acids including formic (2), fumaric (2, 3, 4), propionic (5) and sorbic (6) have been added to broiler feed resulting in positive response. These acids may enhance growth and feed efficiency by eliminating organisms that compete with the bird for nutrients, a benefit attributed to antibiotics. However, the use of antibiotics causing some concern because of drug resistance.

Several studies have suggested that the addition of organic acids such as formic or propionic acids to diets of chickens reduces the incidence of *Salmonellae* on the carcass (7, 8, 9). The primary source of carcass contamination is thought to derive from the intestinal tract of the broiler (10). Therefore, reducing the number of enteric pathogens is very important for public health concern.

The objective of this study was to demonstrate the effects of continuous feeding of organic acids and antibiotic supplemented diet on the *Enterobacteriaceae* level and pH of intestinal contents, growth and feed efficiency of the broilers.

Materials and Methods

Hundredninety two, day-old sexed chicks of a commercial broiler strain (Ross PM3) were obtained from a local hatchery. The chicks (24 males and 24 females) were randomly assigned to each of four floor pens (6 m^2) in a broiler test house. The pens contained litter

composed of new wood shavings. Each pen was equipped with one hanging feeder and one automatic trough waterer. For the first 2 wk, supplemental heat was provided, and the birds were confined to the heated area. Supplemental feeder flats and waterers were used during the first 7 days. Both feed and water were given *ad libitum* and, florescent lighting was provided continuously.

The untreated control diet was formulated by linear programming to meet the nutrient needs suggested by the National Research Council (11). The composition of the basal diet is shown in Table 1. For each period (0 to 21, 22 to 42 days), a large batch of the basal diet was mixed, and aliquots were used to mix the test diets. Four diets were formulated in total: diets with Acid Lac Dry (3 g/kg), which contains lactic acid, fumaric acid, propionic acid, citric acid and formic acid or with a zinc bacitracin (0.1 g/kg) addition or both additives in combination and the control diet. Basal diet was chemically analyzed for crude protein, Ca and total P according to the methods of the AOAC (12) methods.

Chicks were fed *ad libitum* with starter and grower diets for 3 weeks during each period. Body weight and feed consumption were monitored weekly and feed conversion rate was calculated as feed consumed per unit of gain. Birds were removed from feed, but not water, for 12 h before weighing. Mortality was recorded, and birds that slaughtered were weighted for correction of feed conversion. At the end of the experiment, 10 males from each pen were randomly selected for processing. Feed and water were not withdrawn from the birds prior to processing. Dressing percentage was calculated as prechill carcass weight divided by live weight.

The birds were manually eviscerated, taking care not to rupture the intestinal tracts. Following evisceration, the intestinal tracts from ten males taken from each pen were divided into segments. A sample from the contents from ileum was gently expelled into containers. Each

Table 1. Composition and Nutrient Content of Experimental Diets.

Ingredients and composition	Starter diet 0 to 21 days	Grower diet 22 to 42 days
	%	
Yellow corn	15.00	14.00
Barley	10.00	13.00
Wheat	36.00	36.00
Soybean meal (44% CP)	18.00	17.00
Sunflower meal (32% CP)	6.00	6.00
Fish meal (68% CP)	5.00	–
Poultry byproduct meal (56% CP)	3.50	6.50
Vegetable oil	3.00	4.20
Limestone	1.30	1.04
Dicalcium phosphate	1.20	1.30
Salt	0.25	0.20
Premix[1]	0.40	0.40
Lysine	0.08	0.10
DL-methionine	0.17	0.16
Coccidiosit	0.10	0.10
Total	100.00	100.00
Calculated values		
AME, kcal/kg	2970	3270
Lysine, %	1.35	1.16
Methionine+Cystine, %	0.90	0.80
Analyzed values		
Crude protein, %	22.20	20.10
Calcium, %	1.96	1.37
Total phosphorus, %	0.75	0.83

[1] Premix provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 1,500 IU; vitamin E, 10 IU; vitamin K, 0.5 mg; vitamin B_{12}, 0.007 mg; thiamin, 0.4 mg; riboflavin, 6 mg; folic acid, 1 mg; biotin, 0.15 mg; pantothenic acid, 12 mg; niacin, 35 mg; pyridoxine, 4 mg; choline, 1000 mg; iodized salt, 2 g; manganese, 60 mg; copper, 5 mg; zinc, 50 mg and selenium, 0.1 mg.

sample was mixed with 10 ml of deionized water and the pH was determined. To determine *Enterobacteriaceae* level, one sample of ileum contents was also collected from the same birds. Then, the contents of ileum were evaluated for the *Enterobacteriaceae* level using standard enumeration and identification methods (13). All quantitative data were transformed to log 10 prior to statistical analyses.

Data for the feed conversion ratio could not be statistically analyzed because of the group feeding. Differences among the treatments for body weight gain, total number of *Enterobacteriaceae* and pH of ileal content were determined by Student's t-test whenever the respective analysis of variance were significant (14). Statistical difference was considered to be significant when the *P* value was ≤ 0.05.

Results

The average weekly weights of broilers from 1 to 42 days of age fed diets with Acid Lac Dry and/or zinc bacitracin are given in Table 2. Mean weights of broilers which were fed Acid Lac Dry supplemented diet were the lowest at all weeks except wk 4 and 5 among treatments. At the end of the experiment, the addition of either zinc bacitracin or Acid Lac Dry+zinc bacitracin to the diet resulted in chicks with numerically increased body weights over chicks fed the control diet, but not Acid Lac Dry alone. When Acid Lac Dry and zinc bacitracin were added, in combination to the diet, the body weights of the broilers were numerically higher than those of the broilers fed either supplement alone and also than the control group.

Feed efficiency ratio was numerically increased with the addition of either supplement to the diet (Table 3).

Table 2. Effects of Acid Lac Dry and/or Zinc Bacitracin on Body Weights (g) of Broilers.

	Age, wk													
	0		1		2		3		4		5		6	
Treatment[1]	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$
1	34.88	0.35	86.33^{a}	1.52	185.34	4.45	379.77^{bc}	8.25	780.23^{b}	12.81	1280.17	17.88	1795.03	27.73
2	33.72	0.35	82.31^{b}	1.66	177.67	4.56	372.59^{c}	7.56	799.27^{ab}	13.01	1309.57	19.51	1785.48	29.94
3	34.26	0.43	86.26^{a}	1.48	188.75	3.85	392.71^{ab}	7.72	808.13^{a}	13.74	1309.17	20.68	1801.67	29.23
4	33.92	0.37	87.11^{a}	1.80	192.13	3.85	408.15^{a}	8.28	834.32^{a}	15.72	1323.52	21.83	1810.23	26.21

$^{a-c}$ Means within columns with no common superscripts differ significantly (P≤0.05).

[1] 1) Control; 2) 3 g/kg Acid Lac Dry (ALD); 3) 0.1 g/kg Zinc bacitracin; 4) ALD+zinc bacitracin.

Table 3. Effects of Acid Lac Dry and/or Zinc Bacitracin on Feed Convertion Ratio of Broilers.

	Week					
Treatment	0-1	0-2	0-3	0-4	0-5	0-6
Control	1.63	1.51	1.75	1.71	1.79	1.95
3 g/kg Acid Lac Dry (ALD)	1.80	2.04	1.94	1.81	1.89	1.94
0.1 g/kg Zinc bacitracin	1.72	1.96	1.87	1.78	1.83	1.94
ALD+zinc bacitracin	1.55	1.48	1.57	1.66	1.74	1.93

Table 4. Effects of Acid Lac Dry and/or Zinc Bacitracin on Dressing Percentage of Broilers[1].

		Body weight,g		Carcass weight,g		Dressing percentage,%	
Treatment	n	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$
Control	10	2096.6	49.14	1501.6	36.28	72.62	0.26
3 g/kg Acid Lac Dry (ALD)	10	1965.2	53.58	1411.8	37.62	71.84	0.28
0.1 g/kg Zinc bacitracin	10	1989.5	41.99	1434.0	31.46	72.08	0.45
ALD+zinc bacitracin	10	2042.5	37.89	1480.0	30.41	72.45	0.41

[1] Prechill carcass including abdominal fat (excluding neck and giblets) as percentage of live weight.

Table 5. Effects of Acid Lac Dry and/or Zinc Bacitracin on log10 *Enterobacteriaceae* and pH in Ileum Contents of Broilers (n=10).

	pH		Log_{10}	
Treatment	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$
Control	6.37 [a]	0.06	7.68[a]	0.56
3 g/kg Acid Lac Dry (ALD)	6.23 [b]	0.04	6.41[bc]	0.22
0.1 g/kg Zinc bacitracin	6.16 [b]	0.08	6.98[b]	0.13
ALD+zinc bacitracin	6.52 [a]	0.08	5.89[c]	0.61

[a-c] Means within columns with no common superscripts differ significantly ($p<0.05$).

However, the addition of Acid Lac Dry and/or zinc bacitracin to diets did not result in any differences on carcass dressing percentage among treatments (Table 4).

The addition of either zinc bacitracin or Acid Lac Dry to the diet reduced *Enterobacteriaceae* count in the ileum contents of broilers compared to the control group (Table 5) ($P<0.05$). However, the addition of Acid Lac Dry alone reduced *Enterobacteriaceae* level to a greater extent than the addition of zinc bacitracin alone and to a lesser extent than that obtained when both supplements in combination was added. Mean ileal pH was significantly ($P<0.01$) higher in group fed the additives in combination and the control than the animals treated with Acid-Lac Dry or zinc bacitracin alone.

Discussion

In this trial, the addition of Acid Lac Dry+zinc bacitracin to the diet resulted in broilers greater weights during experimental period when compared with those of other groups (Table 2). However, there were no significant treatment effects on any of the performance variables due to feeding Acid Lac Dry and/or zinc bacitracin on 42-day body weights of broilers. Results from the present study with an agreement results obtained by others, which suggest that feeding organic acid (8, 15, 16) or zinc bacitracin (17, 18, 19) to broilers results no significant differences on weight gain and feed conversion. It has been suggested that when chicks were housed in a clean environment organic acid or antibiotic supplementation were unaffective on performance (1).

In contrast to our findings, Cave (20) reported that increasing level of propionic acid were depressed feed consumption and weight gain. At the higher levels of added propionic acid, mortality increased. However, several investigators reported that organic acids have positive effect in broiler body weight gain and feed conversion rate (2, 3, 4, 5, 6).

Carcass dressing percentage was unaffected by organic acids and zinc bacitracin supplementation regardless of whether they were fed alone or in combination (Table 4). In this regard, the results of the present experiment agree with previous experiments we have conducted which showed no improvement in dressing percentage from organic acid (15) and zinc bacitracin (17, 18) supplementation of diets fed to broilers.

There appeared to be synergism between organic acid and zinc bacitracin supplementation since *Enterobacteriaceae* level in the ileum was siginificantly less by feeding the combination of additives when compared with the results obtained with either additive separately (Table 5). Previous studies have also shown that different organic acid supplementation to broiler diets significantly reduced the total number of bacteria within the intestinal tract (2, 5, 21, 22). However, reducing number of *Enterobacteriaceae* by the treatments in the present study may not be related to reduction in the pH of the digestive tract. Because, the greatest reductions in *Enterobacteriaceae* number were in group fed Acid Lac Dry+zinc bacitracin supplemented diet and also this group resulted with the highest pH level in the ileum content among treatments. No explanation exists as to why pH levels in the ileum were increased when birds were fed the combination of additives. Izat et al. (8) also reported there were no differences in pH intestinal material between the feeding of various levels of propionic acid treatments indicating that microbial reductions observed were not due to a decrease in pH. In contrast, either Acid Lac Dry or zinc bacitracin supplementation to the diet resulted significantly decreased pH level in ileum content when compared control and both additives in combination, as expected.

In conclusion, the results of the present study confirm previous studies showing little benefit from organic acid and/or zinc bacitracin supplementation to broiler diets. There also appeared to be a synergism between Acid Lac

Dry and zinc bacitracin supplementation. Acid Lac Dry and zinc bacitracin in combination resulted greater reduction in the number of *Enterobacteriaceae* of ileum content and greater weights during experimental period when compared with those of other groups.

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