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Effect of different levels of organic acids in the diets of hens on laying performance, egg quality criteria, blood parameters, and intestinal histomorphology

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ABSTRACT

The objective of the current study was to evaluate the effects of different levels (1.5, 3.0, 4.5 g/kg) of organic acid mixture (OAM) (60% formic acid, 20% propionic acid, and 20% soft acid) supplementation in the diets of laying hens on egg production, egg quality parameters, and intestinal histomorphology. Seventy-two Lohman strains of layers (26-week-old) were divided into four treatment groups. The hens were fed either a control diet or the control diet supplemented with 0, 1.5, 3 or 4.5 kg/t level of OAM from 26 to 38 weeks of age. The OAM supplementation did not affect feed consumption, egg production, egg weight, feed conversion ratio and body weight. Except for yolk index none of egg quality parameters and metabolic profile were affected by the dietary treatments. Inclusion of OAM into the basal diet increased villus height (quadraticaly), villus weight (quadraticaly), and tunica mucosal width (linearly) eexcept for crypt depth. Based on the results, dietary supplementation with OAM containing 60% formic acid, 20% propionic acid, and 20% soft acid appears to exert neither positive nor negative effects on laying performance, egg quality parameters, or serum blood parameters. But the OAM supplementation into basal diet had a positive effect on the intestinal histomorphology except for crypt depth.

Key words: Egg production, Laying hens, Laying performance, Organic acid mixture, Villus height

INTRODUCTION

Following the ban on the use of antibiotics in animal nutrition by the European Union in 2006, researchers and nutritionists have attempted to find alternatives to enhance the performance of laying hens with respect to egg quality and egg production (Dehghani and Jahanian, 2012; Adil *et al*., 2010). Some animal nutritionists have focused on alternative feed additives, particularly probiotics, prebiotics, essential oils (plant extracts), and organic acids (OAs) (Soltan 2008; Yesilbag and Çolpan 2006). Antibacterial effect of OAs has also attracted interest in their application to poultry diets, for which effective salts and dosages are rapidly being established (Lückstädt and Mellor 2011). OAs has been used recently as natural feed additives to increase eggshell quality and enhance the quality of feces. Egg quality parameters are one of the most crucial issues in the laying hen industry because it influences both the economic profitability of egg production and hatchability (Swiatkiewicz and Arczewska-Wlosek 2012). Studies have shown that OAs, such as lactic acid, sorbic acid, fumaric acid, and propionic acid, and their salts exerts variable effects on egg production and egg quality parameters (Gama *et al*., 2000, Yalcin *et al*., 2009). The variations may be related to the source and the amount of OAs used (Yalcin *et al*., 2009). Rations and some animal raw materials are possibly the most important sources of contamination by pathogen microorganism in poultry. OAs have also been used as food preservatives (Dibner and Buttin 2002; Salgado *et al*., 2011). Furthermore, OAs added to feed can lower the gastric pH. The antibacterial activity of OAs is related to the reduction of pH (Wong and Zahari, 2009). By modifying the intestinal pH; OAs improves the solubility of the feed ingredients, in addition to digestion and nutrient absorption (Sacakli *et al*., 2006). Studies have reported that OAs reduce the production of toxic components produced by bacteria and the colonization of pathogens on the intestinal wall, thus preventing damage to epithelial cells (Dehghani and Jahanian, 2012; Samanta *et al*., 2010). The health of the gut is the most important factor governing the performance of poultry (Soltan, 2008). Martinez *et al*. (2004) was evaluated the performance of male broiler chickens receiving different levels (0, 0.25, 0.50, 1.0 and 2.0%) of a mixture of organic acids (70% formic acid and 30% propionic acid) in the diet. They mentioned that only feed intake was affected at the age one to 42 days, showing a quadratic effect,

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increasing at the levels of 0.25 and 0.5% and decreasing at 2% of OAs and the inclusion of 1% OAs in the diet resulted in a performance similar to that of untreated birds. It reported that addition of OAs (80% formic acid and 20% propionic) at 1% levels (Kaniawati *et al.*, 1992) and addition of 1% formic acid or 1.45% calcium formate (Izat *et al.*, 1990) to broiler chicken ration did not affect live weight and feed conversation ratio at 42 days.

Organic acids have been used for decades in commercial compound feeds, mostly for feed preservation, for which formic and propionic acids are particularly effective. There are a number of difficulties with adding OAs to poultry rations due to properties such as corrosiveness and odor. For OAs to be used more frequently in livestock feedstuffs there is a need for an improved form that is less corrosive, safe, effective, and easy to use. Users of OAs should not have to compromise between the safety and efficacy of their chosen product. With this in mind, Borregaard LignoTech has developed a unique and patented technology called soft acid. Soft acids are a product group consisting of OAM and modified lignosulphonic acid. The presence of the latter gives the product its name "soft" due to its ability to moderate the corrosive properties of the OAs (Kaya *et al.*, 2014). The objective of this study was to determine the effects of different levels of OAM on egg production, egg quality parameters, blood biochemical parameters, and small intestinal histomorphology.

MATERIALS AND METHODS

Animals, diet, and management: This study was conducted with seventy-two 26-week-old Lohman strains of layers. The layers were blocked according to the location of the cages (50 × 46 × 46cm) and then assigned randomly to receive one of four diets, a control diet or one of three dietary treatments, for 70 days. The diets were prepared from a basal diet by adding different levels of OAM supplementation (Sinerji Tarim AS, Izmir) (60% formic acid, 20% propionic acid, 20% soft acid; 1.5, 3.0, or 4.5 g/kg).The chemical analyses of the diets were determined as per the recommendations of the AOAC (AOAC, 2000). The ingredients and the chemical composition of the basal diets are given in Table 1. During the experiment, the hens were provided with food and water ad libitum.

Sample collection and analytical procedure: The feed intake (FI) and the egg production (EP) of the birds were recorded daily, their egg weight (EW) was measured bi-weekly, and their body weight was measured at the end of the experimental period. The feed conversion ratio (FCR) was expressed as kilogram of feed consumed per kilogram of eggs produced. To evaluate the egg quality parameters, 12 eggs were randomly selected from each experimental group every month. The samples were stored for 24 h at room temperature before determining the following egg quality parameters: shape index, shell strength, shell thickness, albumen index, yolk index, yolk color, and Haugh unit. The yolk color was assessed using the CIE standard colorimetric system (Yolk Colour Fan, F. Hoffman-La Roche Ltd., Basel, Switzerland).

Blood samples were taken from the bird's wing vein into blood tubes containing cloth activator at the end of the experimental period to determine serum blood parameters. The blood analysis was performed according to the methods of (Hara and Radin, 1978). Tissue samples (small intestine) were immediately collected and fixed in 10% neutral buffered formalin. The histomorphological evaluation was made with an Olympus BX51 microscope with an image analysis system (DP72-BSW). In each case, five to eight randomly selected villi were examined under 10 × magnifications.

TABLE 1. Chemical composition of experimental chicken diets and calculated major components

Ingredients	(%)	Chemical composition (analyzed on dry matter basis)	
Corn	52.8	Dry matter, (%)	89.4
Soybean meal, (48% CP)	18.1	Crude protein, (%)	17.1
Wheat	6.00	Crude fibre, (%)	3.43
Fish meal	1.65	Ether extract, (%)	3.18
Vegetable fat	7.50	Crude ash, (%)	13.3
Dicalcium phosphate[1]	2.04	Non-Nitrogenous Constituents, (%)	52.4
Marble	1.60	Metabolizable energy (kcal/kg)	2720
Vitamin-mineral premix[2]	6.82		
Salt	0.30		
Methionine[3]	2.65		
Lysine[4]	0.150		

[1]Each kilogram contained: Ca, 24% and P, 17.5%[2]The premix provided per 1 kg of diet: Vitamin A, 15.000 IU; cholecalciferol, 1500 ICU; DL-α-tocopheryl acetate, 30 IU; menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; panthotenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B_{12}, 15 µg; Mn, 80.0 mg; Zn, 60.0 mg; Fe, 30.0 mg; Cu, 5.0 mg; I, 2.0 mg; and Se, 0.15 mg.[3]DL-methionine.4L-lysine hydrochloride.

Statistical analysis: The experimental diets were allocated in a complete randomized block design. The data were analyzed using the General Linear Models procedure of SPSS software ($P < 0.05$) (SPSS 10.0, 1999). Linear, quadratic, and cubic polynomial contrasts (regression) were used to evaluate treatment effects.

RESULTS AND DISCUSSION

Laying performance: Table 2 summarizes the effect of OAM supplementation on laying performance parameters. The dose levels of supplemental OAM had no effect on FI, EP, EW and FCR ($p>0.05$). Body weights prior to and after the experiment did not differ across the experimental diets ($p>0.05$).

The results of the current study with respect to these parameters are in agreement with findings obtained previously (Salgado *et al*., 2011; Wong and Zahari 2009). Previous research found that different OAs, such as lactic acid, butyric acid, formic acid, fumaric acid, and citric acid, and different levels of these acids have different effects on laying performance parameters in poultry (Martinez et al., 2004; Nezhad *et al*., 2008). To evaluate the efficacy of organic acids as a growth promoter for broiler chickens relative to antibiotic growth promoters, Samanta *et al.*, (2010), supplemented broiler diets with graded doses of an organic acid mixture (1 g and 2 g/kg diet) contained orthophosphoric acid (400 g/kg), formic acid (150 g/kg), propionic acid and calcium propionate (30 g/kg resp.). They stated that live weight, live weight gain and feed consumption ratio increased linearly. Kaya *et al*., (2014) reported that there was a cubic effect on feed consumption, egg weight and feed conversion ratio with increasing levels of OAM (70% propionic acid, 5% citric acid, 25% soft acid) supplementation but the egg production, final body weight and body weight change did not differ. Sacakli *et al*. (2006) also reported that the addition of 2.5 g/kg OAs to the diets of quail did not affect laying performance parameters. Other researchers reported that butyric acid did not depress the FI, whereas the FI was depressed by propionate acid (Leeson *et al*., 2005). High levels of OAs added to the diets of laying hens also reduced the FI (Dibner and Buttin 2002; Esmaeilipour *et al*., 2011). They may exert this effect on laying hens by changing the palatability of the food. The differences in the findings may be due to variations in the OA level, the content of the OAs, chick strains, breeding conditions, and housing conditions.

Egg quality: The effects of supplemental OAM on the egg quality parameters are shown in Table 3. As can be seen from Table 4, except for yolk index, none of the egg quality parameters such as shape index, shell strength, shell thickness, albumen index, yolk color, and haugh unit was affected by the dietary treatments. Yolk index for hens fed the control diet was lower than hens fed OAM2 and OAM3 supplemented diets and yolk index increased linearly with increasing level of supplemental OAM ($P < 0.05$). The results related to egg quality parameters in the current study are in agreement with findings obtained earlier (Yesilbag and Çolpan 2006; Gama *et al*., 2000). Nezhad *et al*. (2008) found that the addition of citric acid and phytase to laying hen rations had positive effects on the eggshell strength and on eggshell thickness. Yalcin *et al*. (2000) reported that supplementing the diets of laying hens with 1.0% lactic acid produced significant differences in the albumen index, yolk index, and Haugh unit. Kaya *et al*. (2014) stated that shell strength and yolk color linearly increased as level (0, 1.5, 3, 4.5) of OAM supplementation increased.

TABLE 2. The effects of experimental diets on laying performance parameters of hens.

Treatment	Response variables						
	Feed consumption	Egg production	Egg weight	FCR[1]	Body weigth		
					Initial	Final	Change
	(g/d)	(%)	(g)	(kg feed/kg egg)	(g)		
Control	132.9	91.7	61.2	2.38	1446	1652	206
OAM1	123.9	91.1	62.1	2.21	1479	1697	219
OAM2	125.7	94.4	60.4	2.23	1470	1657	187
OAM3	130.1	93.1	60.6	2.34	1465	1680	215
SEM	6.23	3.97	0.970	0.120	15.7	20.7	24.4
Polynomial Analyze							
Linear	0.870	0.762	0.388	0.837	0.496	0.649	0.959
Quadratic	0.317	0.996	0.806	0.350	0.245	0.601	0.755
Cubic	0.765	0.601	0.333	0.737	0.517	0.120	0.350

OAM 1= 1.5g/kg Organic Acid Mixture, OAM 2=3.0g/kg Organic Acid Mixture, OAM 3=4.5 g/kg Organic Acid Mixture
[1]FCR= feed conversion ratio (kg feed consumed per kg egg produced).
SEM= Standart error of mean

Table 3. The effects of experimental diets egg quality parameters of hens.

Treatment	Response variables							
	Shape index (%)	Shell strenght (kg/cm^2)	Shell thickness ($mmx10^{-2}$)	Shell weight (g)	Yolk color	Yolk index (%)	Albumen index (%)	Haught unit
Control	76.4	2.69	0.370	7.72	12.4	42.1	8.15	81.1
OAM1	76.2	3.08	0.370	7.70	11.9	42.0	7.87	80.7
OAM2	77.1	2.97	0.370	7.76	12.0	42.67	8.85	83.6
OAM3	76.3	3.12	0.360	7.60	12.4	43.3	8.31	82.4
SEM	0.370	0.140	0.010	0.151	0.208	0.440	0.340	1.19
Polynomial Analyze								
Linear	0.705	0.074	0.592	0.897	0.558	0.046	0.349	0.215
Quadratic	0.486	0.411	0.196	0.073	0.955	0.440	0.694	0.717
Cubic	0.114	0.242	0.788	0.698	0.902	0.628	0.081	0.179

OAM 1= 1.5 g/kg Organic Acid Mixture, OAM 2= 3.0 g/kg Organic Acid Mixture, OAM 3= 4.5 g/kg Organic Acid Mixture
SEM= Standart error of mean

Blood parameters: Table 4 summarises the concentrations of albumin, cholesterol, high-density lipoprotein, total protein, glucose, alkaline phosphatase, aspartase aminotransferase, alanine aminotransferase, calcium, and phosphorous in serum. There were no significant differences among the treatment groups ($p>0.05$).Grashorn (2010) stated that OAs stimulates the immune system besides antimicrobial effects. But feeding increased levels of OAM did not affected blood parameters in this experiment. Kaya *et al*. (2014) reported that serum parameters containing albumin, cholesterol, high density lipoprotein, total protein, AST and ALT did not change as OAM level increased but ALP (linear), glucose (linear), blood Ca (cubic) and P (quadratic) concentration increased due to increased OAM level in laying hen diet. Yesilbað and Çolpan (2006) also found that OAs did not affect some blood parameters (alanine aminotransferase, cholesterol, high-density lipoprotein, triglyceride, very low-density lipoprotein, and total lipid concentrations). On the other hand, they reported that dietary OA supplementation (1.0 and 1.5%) significantly increased the total serum protein and albumin concentration in laying hens. Soltan (2008) reported that the serum calcium concentration of laying hens fed a diet supplemented with OAs showed a linear increase and that their total protein and albumin concentrations also significantly increased. The OAs may have enhanced the environment of the intestinal tract by aiding the digestion and the absorption of nitrogen and calcium. Salgado *et al*. (2011) found that adding different levels of citric acid to broiler rations had no significant effect on hematocrit, serum total protein, or albumin concentrations. Dehghani and Jahanian (2012) observed that OAs (citric and butyric acid) added to the diets of broilers increased serum cholesterol in 21-day-old chicks but not in 42 day-old chicks. They also found that citric acid or butyric acid supplementation did not affect the serum triglyceride levels of 21-day-old chicks but that the supplementation decreased these levels in 42-day-old birds. Adil *et al*. (2010) reported that broiler rations supplemented

TABLE 4. The effects of experimental diets on blood parameters of hens

Treatment	Response variables									
	Alb[2]	Chol[1]	HDL[1]	TP[2]	Glu[1]	ALP[3]	AST[3]	ALT[3]	Ca[1]	P[1]
Control	1.87	133	26.3	5.30	232	106	227	22.7	31.0	6.20
OAM1	1.80	116	26.0	4.90	241	87.7	227	21.7	31.7	5.00
OAM2	1.87	145	26.5	5.20	209	144	229	19.0	31.4	5.90
OAM3	1.93	130	25.5	5.30	245	94.6	225	21.3	31.9	6.80
SEM	0.06	8.87	2.35	0.250	17.7	22.6	22.3	2.58	2.75	0.580
P value	0.487	0.225	0.436	0.688	0.518	0.359	0.988	0.783	0.996	0.240
Polynomial Analyze										
Linear	0.332	0.628	0.204	0.665	0.935	0.823	0.969	0.580	0.852	0.307
Quadratic	0.282	0.942	0.371	0.409	0.485	0.508	0.908	0.565	0.977	0.108
Cubic	0.620	0.053	0.644	0.475	0.203	0.113	0.938	0.581	0.881	0.428

OAM 1= 1.5 g/kg Organic Acid Mixture, OAM 2= 3.0 g/kg Organic Acid Mixture, OAM 3= 4.5 g/kg Organic Acid Mixture
Alb = Albumin; Chol = Cholesterol; HDL = High density lipoprotein. TP = Total protein; Glu = Glucose; ALP = Alkaline phosphatase; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase Ca = Calcium; P = Phosphorus
SEM= Standart error of mean
[1] mg/dL [2] g/dL [3] Unit/L

with OAs did not have a significant effect on serum glucose or cholesterol but increased calcium and phosphorus levels. The results obtained in the current study regarding the serum blood parameters are in agreement with those obtained by Hernandez *et al.* (2006).

Intestinal histomorphology: The effects of the levels of OAMon the villus hight VH, villus width (VW), intestinal crypt depth (CD), and tunica mucosal width (TMW) are reported in Table 5. The VH increased quadraticly due to inclusion of OAM into the basal diets, but the crypt depth was not different among the groups. The VW was increased quadratically with increasing the proportion of the OAM in the diet. Also the TMW increased linearly with increasing level of supplemental OAM (Figure 1). The OAM altered the ileal mucosa by increasing VH, VW and TMW but not CD in present study. Reducing the thickness of the intestinal wall through nutritional manipulation may facilitate the absorption of nutrients, which may lead to positive growth performance (Jamroz *et al.*, 2006) and the improvement of the feed conversion ratio (Özdogan *et al.*, 2011). The thickening of mucous layer on the intestinal mucosa contributes to the reduced digestive efficiency and nutrients absorption (Teirlynck *et al.*, 2009). Thus there were no effects of OAM on performance or egg quality parameters in this experiment. Samanta *et al.* (2010) reported that in the duodenum, villus height increased linearly with the dose of the organic acids mixture. In another study, Esmaeilipour *et al.* (2012) found that supplementation of the diets of broilers with citric acid and xylanase did not have a significant effect on the relative lengths of the small intestine. Saki *et al.* (2011) reported that the addition of 1% level of organic acid mixture significantly increased villus height and crypt depth of broiler chicken. Senköylü *et al.* (2007) found that a combination of different OAs significantly increased the VH in broilers. In the same study, the VW was increased following the addition of the OA mixtures, but the depth of the crypt was decreased. The thickness of the lamina muscularis mucosae also was not statistically significant. The results obtained in our study are in harmony with those of Sheikh *et al.* (2010) who reported an increase in the VH in the duodenum and the jejunum following the addition of OAs. They attributed this finding to the OAs reducing the growth of many pathogenic and nonpathogenic intestinal bacteria, decreasing intestinal colonization and infectious processes, and ultimately decreasing inflammatory reactions in the intestinal mucosa. In addition to increasing the VH, the OAs would have enhanced the functions of secretion, digestion, and absorption of nutrients by the mucosa. Sheikh *et al.* (2010) found that OAs did not affect the crypt depth in the duodenum and the ileum. Discrepancies between the different studies may be related not only to the amount of OA salts in the layer diets but also to the layer age (and the growth capacities of the small intestine) at the beginning of the supplementation (Kum *et al.*, 2010). Dibner and Buttin (2002) reported that lack of consistency in demonstrating an organic acid benefit is related to uncontrolled variables such as buffering capacity of dietary ingredients, presence of other antimicrobial compounds, cleanliness of the production environment, and heterogeneity of gut microbiota.

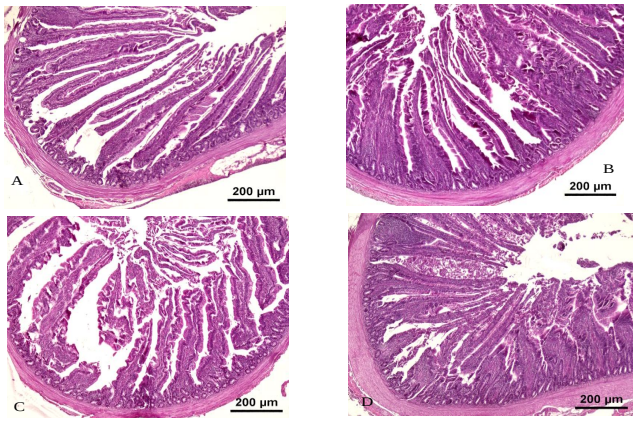

FIG 1: Photomicrographs of small intestinal villi of laying hens in control (A), OAM1 (1.5 g/kg) (B), OAM2 (3.0 g/kg) (C), OAM3 (4.5 g/kg) (D) groups.

TABLE 5. The effects of experimental diets on intestinal histomorphology of hens (mμ)

Treatment	Response variables			
	VH	VW	CD	TMW
Control	1658	162	196	160
OAM 1	1656	218	225	213
OAM 2	1772	243	222	205
OAM 3	1390	216	219	223
SEM	86.5	24.2	13.4	22.2
Polynomial Analyze				
Linear	0.023	0.004	0.078	0.000
Quadratic	0.006	0.005	0.065	0.105
Cubic	0.061	0.738	0.381	0.071

OAM 1= 1.5 g/kg Organic Acid Mixture, OAM 2= 3.0 g/kgOrganic Acid Mixture, OAM 3= 4.5 g/kgOrganic Acid Mixture
VH: Villus Height; VW: Villus Width CD: Intestinal Crypt depth; TMW: Tunica Mucosal Width
[a-b] Means within a column with different superscripts are significantly different ($p < 0.05$). SEM: Standard error of mean.

CONCLUSION

As a result of this study, supplementation of OAM (60% formic acid, 20% propionic acid, 20% soft acid) at different levels into laying hen rations did not exhibit any positive or negative effects on laying performance, egg quality criteria, and serum blood parameters. However, the OAM supplementation had a positive effect on the intestinal histomorphology except for CD. To increase understanding of the effect of OAM on metabolism and the digestion enzyme activity, the effects of OAM supplementation on nutrient utilization and their metabolism and disease resistance in laying hens should be studied properly.

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