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Effects of Organic Acids Supplement on Performance, Egg Traits, Blood Serum Biochemical Parameters and Gut Microflora in Female Japanese Quail (Coturnix coturnix japonica)

■Author(s)

Fouladi P[I]
Ebrahimnezhad Y[I]
Aghdam Shahryar H[I]
Maheri N[I]
Ahmadzadeh A[I]

[I] Department of Animal Science, Faculty of Animal Science and Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, East Azerbaijan, Iran

■Mail Address

Corresponding author e-mail address
Y. Ebrahimnezhad
Department of Animal Science, Faculty of Animal Science and Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, East Azerbaijan, Iran
Email: ebrahimnezhad@iaushab.ac.ir

■Keywords

Biochemical parameters, egg trials, gut microflora, Japanese quail, organic acids.

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ABSTRACT

This study was conducted to evaluate the effects of acetic acid, lactic acid, and butyric acid on the production performance, egg parameters (quality and quantity traits), blood and liver serum biochemical parameters and gastrointestinal tract microorganism on female Japanese quails. A total of 640 female Japanese quails aged 35-84 days were housedfor eight treatments with four replicates. They were arranged in completely randomized design (CRD) to evaluate the effects of basal diet (BD) with a supplement of acetic acid (AA), lactic acid (LA) and butyric acid (BA). The performance parameters to study this effect are feed intake, feed conversion ratio, egg traits such as egg weight, egg production, and egg mass along with specific gravity, internal quality unit (IQU), yolk and albumen indexes, and eggshell traits such as weight, thickness, and surface. It also includes blood serum biochemical parameters such as cholesterol, triglyceride, HDL, LDL, albumin, globulin, glucose, and gastrointestinal tract microflora (*E.Coli* and *Salmonella*). Eight dietary treatments were formulated by addition of organic acids such as BD without organic acids additive, BD with 124 mg/kg AA additive, BD with 104 mg/kg LA additive, BD with 113 mg/kg BA additive, BD with 63.5 mg/kg AA+53.5 mg/kg LA additive, BD with 63.5 mg/kg AA+57 mg/kg BA additive, BD with 53.5 mg/kg LA+57 mg/kg BA additive and BD with 41.6 mg/kg AA+35 mg/kg LA+37 mg/kg BA additive. The results showed that the diets containing BD+LA+BA+AA and BD+AA alone considerably increased feed intake, feed conversion ratio, egg weight, egg production and egg mass ($p<0.01$). The diets containing BD+AA and BD+LA significantly affected some quality traits such as eggshell thickness, eggshell weight ($p<0.01$), shell surface, and IQU ($p<0.05$). The diets containing BD+AA and BD+LA alone increased serum cholesterol, HDL, albumin, globulin and total protein significantly ($p<0.01$). Moreover, the groups containing BD+AA and BD+LA significantly decreased triglyceride contents in serum ($p<0.05$). The results showed that the diets containing BD+LA and BD+BA significantly decreased the *E.Coli* and *Salmonella* population in the gastrointestinal tract ($p<0.01$). Based on the experiment, it can beinferred that LA alone, or in combination with other organic acids, improved and showed positive effects on performance and egg traits in female Japanese quail.

INTRODUCTION

The removal of antibiotics growth stimulator from the feed is one of the major challenges in industrial poultry and domestic animals. Many additives can be alternatively used instead of antibiotics in poultry feed. These additives include vegetable or condensed matter such as vegetable oils essential for growth, probiotics, prebiotics and organic acids (Dibner & Richards, 2005; Cakir *et al.*, 2008). Gram-positive microbes are often very sensitive to organic acids such as lactic, butyric, acetic, etc, than gram-negative microorganism (Dibner & Buttin, 2002). Given the growing concerns of bacterial resistance against antibiotics and the incidence of some cancers and disruption of food chains, Europe Union (EU) in 2006, the use of all antibiotics in the feed of poultry and other farm animals banned. Therefore, various strategies to reduce the risk of bacterial resistance to antibiotics through food pyramid from farm livestock to humans are required. Due to the difficulties ahead, there is the need to identify new alternatives to antibiotics. Organic acids have the potential to replace antibiotics as growth promoters (Gornowicz & Dziadek, 2002; Dibner & Buttin, 2002; Dibner & Richards, 2005; Gunal *et al.*, 2006; Cakir *et al.*, 2008). In current literature is said that organic acids can be replaced by antibiotics (Langhout, 2000). Therefore, organic acids might be used as a growth-promoting instead of antibiotics. Also, the addition of organic acids to the diets of broiler chickens can reduce the toxic substances in the intestines (Langhout, 2000; Denbow, 2000; Denli *et al.*, 2003; Liu *et al.*, 2010; Fouladi *et al.*, 2014). Organic acids are likely to influence the cell membranes of bacteria and disrupt the transport of nutrients materials and impact on energy pathways, doing their antimicrobial effect (Ricke, 2003). Also, when organic acids are used, the pH of the stomach is reduced, which increases the activity of pepsin and other digestive enzymes or proteins (Ricke, 2003; Dibner & Richards, 2005). Peptides, derived from the digestion of proteins increase gastric hormones and stimulate the release of cholecystokinin which regulates the digestion and absorption of proteins (Ricke, 2003; Dibner & Richards, 2005). Organic acids additives have been reported to decrease the population of harmful bacteria and yield of poisoning metabolites, increase digestibility of peptides and mineral micronutrients like Ca, P, Mg, Zn and Mo and also serve as substrates in the intermediary metabolism (Moharrery, 2005). Several studies have been conducted to evaluate the effect of organic acid additives on growth factors of broiler chickens. Therefore, the present study aimedto evaluate the efficiency of organic acids supplement as growth promoters in Japanese quail. The present study focusedon evaluating the effects of lactic, acetic and butyric acids on the production performance, serum and liver biochemical parameters, gut microflora and egg quantity traits (such as egg weight, egg mass, egg production, yolk cholesterol) and quality traits (such as egg specific gravity, yolk index, albumen index, yolk color unit, IQU[1], eggshell thickness, eggshell weight and eggshell surface) on 50 to 84 d old female Japanese quails. The Japanese quails were considered as laboratory birds (Gunal *et al.*, 2006; Poynter *et al.*, 2009; Liu *et al.*, 2010). Earlier research has been conducted on the effect of organic acids in Japanese quail research for evaluation the production parameters, the measured amounts of the cholesterol, triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) levels, glucose, albumin, globulin contents *in serum*, and also measured liver's cholesterol, triglyceride, HDL, and LDL levels in female Japanese quail with intake of organic acids additives in diets.

MATERIALS AND METHODS

Birds and diets

The study was carried out in a poultry farm located in Tabriz, East Azarbaijan province, Iran. All bird care and use procedures were approved by the Department of Animal and Poultry Science, Islamic Azad University of Shabestar Care and Use Committee. A collection of 1150 one-day old Japanese quail's chicks of both sexes were placed in 12 pens together. From a collection of 1150 Japanese quail, only640quails of 35-84 days (at 5 to 12 weeks of age) were selectedand distributed randomly, in 32 pens (dimensions 200×80 cm, height 90 cm) with 20 birds per pen (20 female), for eight treatments with four replicates. The experimental diets were used, and feed and water were provided *adlibitum* to the Japanese quail. All parameters were evaluated from days 35 until 84.The light period from the initial until the50th day set at 65 Lux and after 62 days, it was decreased to 10 Lux. Eventually, 12 hours of light and 12 hours of darkness for the entire period was intended. The temperaturewas maintained at 21±2°C.The organic acids used in this study, include the AA (pKa=4.75, Purity=80% and Density=1.05 g/cm^3), LA (pKa=3.87, Purity=95% and Density=1.21 g/cm^3) and BA (pKa=4.83, Purity=90% and Density=0.99 g/cm^3). All diets were balanced by adding AA (80%), LA (95%) and BA (90%) on BD (corn and soybean meal). Organic acids are calculated based on weight and were added to the diet. The composition of diets were BD without organic acids, BD with 124 mg/kg AA (1% of diet), BD with 104 mg/kg LA (1% of diet), BD with 113 mg/kg BA (1% of diet), BD with 63.5 mg/

[1] Internal Quality Unit

kg AA (0.5% of diet) + 53.5 mg/kg LA (0.5% of diet), BD with 63.5 mg/kg AA (0.5% of diet) + 57 mg/kg BA (0.5% of diet), BD with 53.5 mg/kg LA (0.5% of diet) + 57 mg/kg BA (0.5% of diet) and BD with 41.6 mg/kg AA (0.33% of diet) + 35 mg/kg LA (0.33% of diet) + 37 mg/kg BA (0.34% of diet) with four replicates. The experimental diets were identical regarding energy and protein. All of the diets were balanced for the DCAB. The DCAB content was kept constant at 250 (meq/kg). The diets were balanced with a recommendation by the National Research Council (1994). Trial diets were balanced using the UFFDA[2] software. Crude protein levels, metabolizable energy, and experimental diet compositions were balanced with nutrient requirements recommended by NRC (1994) and are shown in Table 1.

Table 1 – Ingredient and chemical composition of basal diet at 35-84 d (at 8 to 12 weeks of age) (%)

Ingredients (%)	Basal diet
Corn grain	56.87
Soybean meal (44% CP)	29.98
Corn gluten	3.02
Soybean oil	2.12
Dicalcium Phosphate	1.12
Oyster shell	5.33
Sodium bicarbonate	0.72
Salt	0.19
Mineral premix[a]	0.26
Vitamin premix[b]	0.26
DL-Methionine	0.12
L-Lysine	0.01
Analyzed Composition	
ME (kcal/kg)	29.02
Crude protein (%)	20.03
Calcium (%)	2.55
Available phosphorus (%)	0.32
Available sodium (%)	0.15
Available potassium (%)	0.41
Available chlorine (%)	0.14
Methionine (%)	0.77
Methionine + Cysteine (%)	0.46
Lysine (%)	1.02
Tryptophan (%)	0.31
Threonine (%)	1.10
DCAB (meq/kg)	250.13

[a] Vitamin content of diets provided per kilogram of diet: vitamin A (5000 IU), D (400 IU), E (30 IU), K (10 mg), B1 (1.5 mg), B2 (1.7 mg), Niacin (20 mg), Choline (350 mg), Pantotenic acid (40 mg), B6 (2 mg), Biotin (0.12 mg), Folic acid (0.5), B12 (6 mg) and C (60 mg).

[b] Composition of mineral premix provided as follows per kilogram of premix: Mn, 120,000 mg; Zn, 80,000 mg; Fe, 90,000 mg; Cu, 15,000 mg; I, 1,600 mg; Se, 500 mg; Co, 600 mg.

Data collection

Feed intake was determined daily. Data on feed intake and egg mass were used to calculate feed conversion ratio (FCR, feed intake/egg mass, g/g) from age 35 to 84 days. In the last two days, every week (48 hours per week), eggs laid per replicate were collected and weighed. In the last 48 hours each week for 2 hours, the entire feed was collected from each replicate, and 5 males were caged per cage. After an hour, isolate the males and collect eggs for fertility measurement for up to 48 hours. From the total eggs laid in each replicate, seven eggs were selected, which weighed nearly the average weight of each replicate. After that, traits related to eggs weight, egg production, egg mass, egg specific gravity, eggshell weight, eggshell thickness, IQU[3], albumen index, yolk index, and yolk color were measured on these seven eggs from each replicate (35 eggs per treatment). The procedure continued until the end of the experiment. The egg mass per bird was calculated as the number of eggs produced in a replicate × average egg weight. Eggs collected at 4°C were sent to the lab to be evaluated on other egg traits. At the end of the experiment, for 48 hours, 12 eggs laid per replicate were collected and stored in the condition of 14°C with approximately 70% moisture content. Then, all eggs were transferred to the incubation machine to determine the hatchability. At the end of the experimental period (at 84 d), six birds were selected from each replicate, and before slaughter, blood samples (5 mL) were collected using a tiny syringe and blood was taken from the jugular vein in the neck. After selecting the birds and collection of blood samples, the birds were slaughtered using the Islamic Halal method in compliance with Shabestar Branch-Islamic Azad University Protocol (SBAUP). The blood samples were drawn and allowed to clot at normal room temperature (21°C) up to 2.5 hours before serum collection. The blood samples were collected after bleeding for serum separation and then centrifuged (Autolab, BT 3500, at -20°C, rpm=3000, time=15 min). The serum samples were collected in vials and were transferred to the biochemistry lab for assessing cholesterol, triglyceride, HDL, LDL, glucose, albumin, globulin, and total protein content. The liver and gastrointestinal tracts were flushed using diluted sodium chloride (155 m/mol) to prevent infection,. After washing the liver sample, 10 g of the liver tissue was cut and collected in vials. Formalin was added into the vial for fixation of the tissue, and the tissue

[2] User-Friendly Feed Formulation Done Again

[3] Internal Quality Unit

was then frozen at -21°C. After that, the liver samples were transferred to the biochemistry lab for assessing cholesterol, triglyceride, HDL and LDL contents. Using a pH meter (model AZ. 86502), the pH of different parts of the gastrointestinal tract such as crop, gizzard, duodenum, jejunum, ileum, ceca and cloaca, was measured and recorded. After measuring the pH of different parts, samples of fresh ileal digesta (0.5g) were diluted in physiological serum (saline) to initial 10^{-1} dilution. The ileum contents of each bird were pooled for serial dilution. Then all ileal samples were immediately sent to the microbiology laboratory to determine the amount of *E.Coli* and *salmonella* population present.

Data analysis

In the biochemistry lab, one gram of liver tissue was cut and homogenized in Triss dilution. After homogenization of the liver samples,5 mL of the homogenized solution was centrifuged (Autolab, BT 3500, at -20°C, rpm=3000, time=15 min). After the procedure, 3 mL of the centrifuged liver and blood serum samples were removed using a pipette sampler and transferred to an autoanalyzer system (BACKMAN 2300, Germany) for determining the cholesterol, triglyceride, HDL, LDL,glucose, albumin, globulin for serum, and cholesterol, triglyceride, HDL and LDL contents for liver. These parameters were measured using commercially available kits (Zist Chem. Co., Tehran, Iran) and after ward, thetotal protein content was calculated for serum. Before the measurement of samples, the autoanalyzer system was calibrated. Egg specific gravity was determined using 11 gradient saline solutions varying in specific gravity from 1.060 to 1.100 at 0.005-unit increments (Holder & Bradford, 1979). Eggs were broken on a flat surface and yolks were separated from the albumen, to assess the egg quality; then, after breaking open the eggs, the albumen and egg yolk were measured by caliper to the nearest 0.05 mm. Albumen and yolk heights were measured with a tripod micrometer to the nearest 0.01 mm. Eggshells were carefully washed with warm water and dried at 55°C for 48 hours in an oven and then weighted. The eggshell thickness was measured using a micrometer caliper with an accuracy of 1 ± µm (series 500, Mitutoyo, Tokyo, Japan) and the average was calculated (Tahmasbi *et al.*, 2012). The surfaces of eggs were calculated as indicated by Ousterhout (1980). Albumen height was determinedby using a spherometer. The yolkwas separated using an egg separator and then weighted. Albumen weight was calculated by subtracting, the yolk and eggshell weight from the total egg weight. The yolk index was determined as the ratio of the yolk height to the yolk width and yolk color, which ranges from a pale yellow (score 1) to dark orange (score 15) (Vuilleumier, 1969). The length and width of the eggs were measured by a caliper to the nearest 0.05 mm. Internal quality unit (IQU) was calculated according to the equation derived by Kondahia *et al.* (1983) as follows: $IQU=100 \log (H+4.18–0.8989\times W^{0.6674})$, where H=albumen height in mm and W=egg weight in g. Seven random samples of eggs from each replicate were obtained for egg yolk cholesterol analyses at the end of the experiment. Separated egg yolks were used for the determination of egg cholesterol. After homogenization, one gram of yolk sample was extracted with isopropanol, followed by centrifugation at 1200×g at 4°C for 15 min. Egg cholesterol content was determined by a UV spectrophotometer (Apel, PD-303S, Japan), and absorbance was read at 500 nm (Boehringer Mannheim, 1989). For determining the gut microflora, the ileum contents of each bird were pooled for serial dilution. Microbial anaerobic dilution was undertaken, before inoculation onto Petridishes of sterile agar, for calculating the *E.Coli* and *Salmonella* population.

Statistical analyses

All data collected were analyzed together for the effect of treatment on each variable (egg weight, egg production, egg mass, egg specific gravity, eggshell weight, eggshell thickness, IQU, albumen index, yolk index, and yolk color) at 35 to 84 days. Other parameters, except the egg parameter traits mentioned above, were compared between different treatment groups using one-way ANOVA. The statistical design used in the data analysis was complete randomized design (CRD). The general liner method (GLM) was also used with SAS software version 12, for testing. The difference between the treatment means were measured by Duncan's multiple ranges test and was considered significant at $p<0.05$, $p<0.01$(SAS Inst. Inc. Cary, NC, 2000; Duncan, 1955).

RESULTS

Performance parameters

The results of production performance parameters in Japanese quails for 35-84 d old are given in Table 2. The results of this study showed that diets containing BD+LA, BD+AA+LA+BA, BD+LA+BA and BD+BA respectively had the most significant effect on feed intake (FI) as they as significantly increased FI compared with other treatments in female Japanese quail (*p*<0.01). Also, diets containing BD+LA, BD+AA, and BD+BA, respectively had the most effect on FCR as they significantly decrease FCR as compared with another diet in female Japanese quail (*p*<0.01). Furthermore, the results of the present study showed that the use of organic acid supplements in experimental diet caused significant increase in FI from 23.66 (g/bird/day) for basal diet without organic acids supplement to 24.09, 23.86, 23.84, and 23.79 (g/bird/day), respectively, for diets containing BD+LA, BD+AA+LA+BA, BD+LA+BA and BD+BA (*p*<0.01). Also, diets containing BD+LA, BD+AA and BD+BA respectively decreased FCR from 3.18 for a diet without organic acids supplement to 2.61 and 2.72, respectively, for diets BD+LA, BD+AA, and BD+BA (*p*<0.01). Diets containing BD+AA and BD+LA, respectively, had the most effect on egg weight, egg production, and egg mass, as they increased in comparison with other diets in female Japanese quail (*p*<0.01). However, the use of organic acid supplements in experimental diet caused a significant increase in egg weight from 10.78 g for diets without organic acids to 12.32 and 12.03 g respectively for diets containing BD+AA and BD+LA (*p*<0.01). Also, the use of organic acid supplements in experimental diet resulted in significant increase in egg production from 73.10 (%) for diets without organic acids to 83.58 and 80.80 (%) respectively, for diets containing BD+AA and BD+LA (*p*<0.01). The use of organic acid supplements in experimental diet caused a significant increase in egg mass from 7.88 g for without organic acids to 10.29 and 9.72 g respectively, for diets containing BD+AA and BD+LA (*p*<0.01). The improvement of production performance parameters traits in Japanese quails at 35-84 d with LA and AA diets in this research is notable.

Table 2 – Effects of dietary organic acids supplement on production performance in female Japanese quails at 35-84 d

	Experimental diets									
Traits	BD	BD+AA	BD+LA	BD+BA	BD+AA+LA	BD+AA+BA	BD+LA+BA	BD+AA+LA+BA	SEM	*p*-Value
Feed Intake (g/bird/day)	23.66^{cd}	23.61^{d}	24.09^{a}	23.79^{bc}	23.63^{e}	23.65^{cd}	23.84^{b}	23.86^{b}	1.70	0.0001
Feed conversion ratio	3.18^{a}	2.72^{cd}	2.61^{d}	2.72^{cd}	2.88^{bc}	2.86^{bc}	2.80^{bcd}	2.94^{b}	0.03	0.0001
Egg weight (g)	10.78^{f}	12.32^{a}	12.03^{b}	11.89^{b}	11.16^{de}	11.25^{d}	11.55^{c}	11.03^{e}	0.05	0.0001
Egg production (%)	73.10^{d}	83.58^{a}	80.80^{b}	78.78^{bc}	76.47^{c}	79.37^{bc}	77.02^{c}	77.85^{c}	0.06	0.0001
Egg mass (g/bird/day)	7.88^{e}	10.29^{a}	9.72^{b}	9.36^{b}	8.53^{d}	8.92^{c}	8.89^{c}	8.58^{d}	0.05	0.0001

$^{a-g}$: Means in rows with no common superscript differ significantly (*p*<0.05).

BD without organic acids, BD+124 mg/kg AA, BD+104 mg/kg LA, BD+113 mg/kg BA, BD+63.5 mg/kg AA+52.5 mg/kg LA, BD+63.5 mg/kg AA+57 mg/kg BA, BD+53.5 mg/kg LA+57 mg/kg BA and BD+41.6 mg/kg AA+35 mg/kg LA+37 mg/kg BA.

Egg quality traits

The results of egg quality parameters Japanese quails for 35-84 d old are given in Table 3. The results showed that specific gravity is no significantly changed in all diets. Furthermore, diets containing BD+LA, BD+AA, BD+LA+BA, BD+AA+BA and BD+AA+LA+BA respectively recorded the highest significant effect and increased the IQU (*p*<0.05), and diets containing BD+LA, BD+AA, BD+BA, BD+LA+BA and BD+AA+LA+BA respectively recorded the highest significant effect and increased the eggshell weight, eggshell thickness, and eggshell surface (*p*<0.01). The use of organic acid supplements in experimental diet resulted in significant increase in IQU from 44.25 for a diet without organic acids supplement to 46.61, 46.44, 46.29,46.33 and 46.23 respectively. Also, the use of organic acid supplements in experimental diet resulted in significant increase in eggshell weight from 0.95 (%) for diet without organic acids supplement to 1.15 (BD+LA), 1.05 (BD+AA and BD+BA) and 1.04 (%) (BD+AA+LA+BA) respectively and eggshell thickness significantly increased from 0.21 mm for diet without organic acids supplement to 0.26 (BD+LA), 0.25 (BD+AA) and 0.24 mm (BD+BA, BD+LA+BA, and BD+AA+LA+BA), respectively. To, the eggshell surface significantly increased from 30.25 cm^2 for a diet without organic acids supplement to 34.58, 33.76, 33.37 and 32.42 cm^2 respectively for diets containing BD+LA, BD+AA, BD+BA, and BD+AA+LA+BA. On the other hand, egg specific gravity, yolk index, albumen index and yolk color was not affected in all diets containing organic acid in these trials. In general, in this study, the use of organic acids especially LA and AA improved the traits quantity of eggs. The addition of organic acid supplements to the experimental diet resulted in significant increase in hatchability from 68.75% for treatment without organic acids to 80.50 and 78.25 respectively for BD+AA and BD+LA (*p*<0.01). The improvement of production performance parameters traits with LA and AA treatments in this research is notable.

Table 3 – Effects of dietary organic acids supplement on egg quality traits on female Japanese quails at 35-84 d

	Experimental diets									
Traits	BD	BD+AA	BD+LA	BD+BA	BD+AA+LA	BD+AA+BA	BD+LA+BA	BD+AA+LA+BA	SEM	*p*-Value
Specific gravity (g/cm^3)	1.10	1.11	1.10	1.11	1.12	1.11	1.12	1.11	0.10	0.4553
IQU	44.25^{ab}	46.44^{a}	46.61^{a}	44.22^{ab}	42.12^{b}	46.29^{a}	46.33^{a}	46.23^{a}	0.02	0.0256
Eggshell weight (%)	0.98^{c}	1.05^{ab}	1.15^{a}	1.05^{ab}	1.03^{b}	1.03^{b}	1.04^{ab}	1.04^{ab}	0.01	0.0001
Eggshell thickness (mm)	0.21^{c}	0.25^{ab}	0.26^{a}	0.24^{b}	0.23^{bc}	0.21^{c}	0.24^{b}	0.24^{b}	0.006	0.0002
Eggshell surface (cm^2)	30.25^{cd}	33.76^{ab}	34.58^{a}	33.37^{ab}	31.32^{bc}	31.57^{bc}	32.42^{b}	30.96^{c}	0.06	0.0181
Yolk index (%)	45.14	45.03	45.40	45.50	45.50	45.42	45.62	45.42	0.22	0.0993
Albumen index (%)	5.12	5.14	5.15	5.14	5.16	5.13	5.14	5.15	0.07	0.3742
Roch·s yolk color	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	0.006	0.2549
Hatchability	68.75^{g}	80.50^{a}	78.25^{b}	73.00^{e}	73.75^{de}	76.50^{bc}	75.50^{cd}	70.75^{f}	0.66	0.0001

$^{a-d}$: Means in rows with no common superscript differ significantly (p<0.05).

BD without organic acids,BD+124 mg/kg AA, BD+104 mg/kg LA, BD+113 mg/kg BA, BD+63.5 mg/kg AA+52.5 mg/kg LA, BD+63.5 mg/kg AA+57 mg/kg BA, BD+53.5 mg/kg LA+57 mg/kg BA and BD+41.6 mg/kg AA+35 mg/kg LA+37 mg/kg BA.

Serum metabolites

The results of serum biochemical parameters are given in Table 4. In the present study, diets containing BD+AA and BD+LA, respectively, had the most effect on serum cholesterol. Diets containing BD+AA and BD+LA significantly increased serum cholesterol as compared with other diets in female Japanese quail (p<0.5). The use of organic acid supplements in experimental diet resulted in significant increase in serum cholesterol from 224 mg/dl for a diet without organic acids supplement to 275.75 mg/dl and 270.50 mg/dl respectively, for diets containing BD+AA and BD+LA. Diets containing BD+AA+LA+BA,BD+BA, BD+LA+BA, and BD+AA+BA had the most significant effect on the serum triglyceride, as compared with other diets containing organic acids in female Japanese quail (p<0.0026). The result showed that the use of organic acid supplements in an experimental diet expressively decreased serum triglyceride from 258.25 mg/dl for adiet without organic acids supplement to 255, 253.25, 241 and 240.25 mg/dl for the diets containing BD+AA+LA+BA, BD+BA, BD+LA+BA, and BD+AA+BA, respectively. Moreover, it seems that there was no interaction effect between various diets containing organic acids. In this study, diets containing AA had significant effects on performance and blood biochemical parameters, in female Japanese quail. The addition of organic acids supplements to experimental diets significantly increased serum HDL from 148.75 mg/dl for a diet without organic acids supplement to 204.25, 200.75 and 176.25 mg/dl, respectively for diets containing BD+AA, BD+LA, and BD+AA+LA. Also, this study showed that the use of supplements of organic acids in diets had no significant difference in serum LDL, but diets consisting BD+AA increased serum LDL numerically. On the other hand, a result has been showed the treatment containing BD+AA and BD+LA had the most significant effects on albumin, globulin and total serum proteins (p<0.01). The use of organic acid supplements in experimental diet, significantly increased serum albumin from 2.23 g/dl for a diet without organic acids supplement to

Table 4 – Effects of dietary organic acids supplement on blood biochemical parameters and egg yolk cholesterol in female Japanese quails at 84 d

	Experimental diets									
Traits	BD	BD+AA	BD+LA	BD+BA	BD+AA+LA	BD+AA+BA	BD+LA+BA	BD+AA+LA+BA	SEM	*p*-Value
Cholesterol (mg/dl)	224.00^{d}	275.75^{a}	270.50^{a}	245.10^{bc}	246.00^{bc}	235.75^{c}	235.50^{c}	259.25^{b}	14.75	0.0052
Triglyceride (mg/dl)	258.25^{a}	229.50^{c}	230.00^{c}	253.25^{ab}	228.50^{c}	240.25^{bc}	241.00^{bc}	255^{ab}	5.11	0.0026
HDL (mg/dl)	148.75^{d}	204.25^{a}	200.75^{ab}	170.50^{c}	176.25^{c}	163.75^{cd}	163.25^{cd}	184.50^{b}	4.26	0.0001
LDL (mg/dl)	24.07	24.10	24.08	24.07	24.08	24.08	24.07	24.09	1.99	0.0981
Albumin (g/dl)	2.23^{e}	4.30^{a}	4.00^{ab}	3.54^{c}	2.55^{e}	3.48^{c}	3.67^{bc}	3.00^{d}	0.12	0.0001
Globulin (g/dl)	1.66^{d}	2.49^{a}	2.09^{b}	1.69^{d}	1.64^{d}	1.86^{cd}	1.96^{bc}	1.67^{d}	0.07	0.0001
Total protein (g/dl)	3.89^{e}	6.80^{a}	6.09^{b}	5.23^{c}	4.19^{e}	5.34^{c}	5.63^{c}	4.68^{d}	0.15	0.0001
Glucose (mg/dl)	231.50^{c}	245.75^{abc}	258.00^{ab}	258.50^{ab}	238.75^{bc}	239.25^{bc}	263.00^{a}	247.70^{abc}	6.06	0.0015
Yolk cholesterol (mg/g/yolk)	13.25	13.50	13.25	13.50	13.25	13.75	13.00	13.00	1.63	0.5233

$^{a-e}$: Means in rows with no common superscript differ significantly (p<0.05).

BD without organic acids,BD+124 mg/kg AA, BD+104 mg/kg LA, BD+113 mg/kg BA, BD+63.5 mg/kg AA+52.5 mg/kg LA, BD+63.5 mg/kg AA+57 mg/kg BA, BD+53.5 mg/kg LA+57 mg/kg BA and BD+41.6 mg/kg AA+35 mg/kg LA+37 mg/kg BA.

4.30, 4.00 and 3.67 g/dl for diets containing BD+AA, BD+LA, and BD+LA+BA respectively. Furthermore, diets containing BD+LA+BA had the most effects on serum glucose. Among all diets, the use of organic acid supplements, significantly increased serum glucose from 231.50 mg/dl for a diet without organic acids supplement to 263, 258.50 and 258.50 mg/dl for diets containing BD+LA+BA, BD+BA, and BD+LA, respectively. The results showed that diets containing BD+AA and BD+BA values decreased yolk cholesterol contents.

Liver biochemical parameters

The results of liver biochemical parameters are given in Table 5. Results showed that the diets containing organic acids did not present effects on the liver cholesterol, HDL, and LDL. However, diets containing BD+AA, BD+LA, and BD+BA significantly increased liver triglyceride among the other diets in female Japanese quail ($p<0.05$).

The addition of organic acid supplements to experimental diet significantly increased liver triglyceride from 288 mg/dl for a diet without organic acids supplement and 278.75 mg/dl for diets containing BD+AA+L to 320 mg/dl (BD+AA), 315.25 mg/dl (BD+LA and BD+BA) respectively.

Table 5 – Effects of dietary organic acids supplement on liver biochemical parameters in female Japanese quails at 84 d

	Experimental diets									
Traits	BD	BD+AA	BD+LA	BD+BA	BD+AA+LA	BD+AA+BA	BD+LA+BA	BD+AA+LA+BA	SEM	*p*-Value
Cholesterol (mg/dl)	75.00	77.50	76.50	75.25	77.75	77.00	76.75	77.75	1.27	0.2938
Triglyceride (mg/dl)	288.00^{c}	320.00^{a}	315.25^{a}	315.25^{a}	278.75^{d}	288.25^{c}	305.50^{b}	276.00^{d}	2.33	0.0003
HDL (mg/dl)	34.00	34.00	34.00	33.25	34.00	34.50	33.75	34.75	0.71	0.1601
LDL (mg/dl)	42.00	43.50	42.75	42.50	43.00	43.00	43.50	43.25	0.90	0.2809

$^{a-d}$: Means in rows with no common superscript differ significantly ($p<0.05$).

BD without organic acids,BD+124 mg/kg AA, BD+104 mg/kg LA, BD+113 mg/kg BA, BD+63.5 mg/kg AA+52.5 mg/kg LA, BD+63.5 mg/kg AA+57 mg/kg BA, BD+53.5 mg/kg LA+57 mg/kg BA and BD+41.6 mg/kg AA+35 mg/kg LA+37 mg/kg BA.

Gastrointestinal tract pH

The results of different parts of gastrointestinal tract pH are given in Table 6. The results showed that all diets containing organic acids compared with the control group reduces the pH levels in the crop, gizzard, duodenum, jejunum, ileum, and cecum ($p<0.01$). Among diets containing organic acids, little change was observed, but these traits were significant as compared to the control diet (BD). Among all groups, there was no significant change in cloaca pH.

Table 6 – Effects of dietary organic acids supplement on different parts of intestinal tract pH in female Japanese quails at 84 d

	Experimental diets									
Traits	BD	BD+AA	BD+LA	BD+BA	BD+AA+LA	BD+AA+BA	BD+LA+BA	BD+AA+LA+BA	SEM	*p*-Value
Crop	5.83^{a}	4.86^{b}	4.77^{b}	4.86^{b}	4.83^{b}	4.87^{b}	4.87^{b}	4.86^{b}	0.02	0.0001
Gizzard	2.02^{a}	1.93^{ab}	1.76^{b}	1.92^{ab}	1.91^{ab}	1.92^{ab}	1.93^{ab}	1.91^{ab}	0.008	0.0001
Duodenum	6.02^{a}	5.56^{ab}	5.32^{b}	5.56^{ab}	5.55^{ab}	5.55^{ab}	5.56^{ab}	5.55^{ab}	0.01	0.0001
Jejunum	6.42^{a}	5.95^{ab}	5.44^{b}	5.95^{ab}	5.95^{ab}	5.95^{ab}	5.95^{ab}	5.95^{ab}	0.006	0.0001
Ileum	6.83^{a}	5.24^{ab}	5.00^{b}	5.23^{ab}	5.23^{ab}	5.23^{ab}	5.22^{ab}	5.24^{ab}	0.01	0.0001
Cecum	5.60^{a}	5.13^{b}	5.49^{ab}	5.45^{ab}	5.42^{ab}	5.42^{ab}	5.44^{ab}	5.43^{ab}	0.008	0.0012
Cloaca	6.88	6.88	6.88	6.88	6.88	6.89	6.88	6.88	0.01	0.0812

$^{a-b}$: Means in rows with no common superscript differ significantly ($p<0.05$).
BD without organic acids,BD+124 mg/kg AA, BD+104 mg/kg LA, BD+113 mg/kg BA, BD+63.5 mg/kg AA+52.5 mg/kg LA, BD+63.5 mg/kg AA+57 mg/kg BA, BD+53.5 mg/kg LA+57 mg/kg BA and BD+41.6 mg/kg AA+35 mg/kg LA+37 mg/kg BA.

Gut microflora

The results of gut microflora are given in Table 7. Results showed that the diets containing organic acids presented significant effects on the *E.Coli* and *Salmonella* population in ileum compared with the control group in female Japanese quail ($p<0.01$). Diets containing BD+BA, BD+LA, and BD+AA+LA+BA had the highest effect on decrease the *E.Coli* and *Salmonella* population in the ileum, respectively.

Results showed that with use oforganic acid supplements in the experimental diet the *E.Coli* population decreased, and 7.31 CFU/g for a diet without organic acids supplement reached to 3.28, 3.45 and 3.68 CFU/g for diets containing BD+BA,

Table 7 – Effects of organic acids supplement on gut microflora (CFU/g) in female Japanese quails at 84 d

	Experimental diets									
Traits	BD	BD+AA	BD+LA	BD+BA	BD+AA+LA	BD+AA+BA	BD+LA+BA	BD+AA+LA+BA	SEM	*p*-Value
E.Coli (CFU/g)	7.31[a]	4.28[c]	3.45[f]	3.28[f]	4.39[c]	4.01[d]	5.24[b]	3.68[e]	0.07	0.0001
Salmonella (CFU/g)	5.31[a]	3.41[c]	2.09[e]	2.08[e]	3.29[c]	2.78[d]	4.00[b]	2.70[d]	0.15	0.0001

[a-f]: Means in rows with no common superscript differ significantly ($p<0.05$).

BD without organic acids,BD+124 mg/kg AA, BD+104 mg/kg LA, BD+113 mg/kg BA, BD+63.5 mg/kg AA+52.5 mg/kg LA, BD+63.5 mg/kg AA+57 mg/kg BA, BD+53.5 mg/kg LA+57 mg/kg BA and BD+41.6 mg/kg AA+35 mg/kg LA+37 mg/kg BA.

BD+LA, and BD+AA+LA+BA respectively. The results also point out that diets containing BD+BA, BD+LA, and BD+AA+LA+BA havethe highest effect on the *salmonella* population in female Japanese quail as significantly ($p<0.01$) than other diets containing the organic acids. Where as the result showed that with use oforganic acid supplements in experimental diet, the *salmonella* population significantly decreased from 5.31 CFU/g for a diet without organic acids supplement reached to 2.08, 2.09 CFU/g and 2.70 CFU/g respectively for diets contains BD+BA, BD+LA, and BD+AA+LA+BA.

DISCUSSION

In a previous study, it was reported that organic acids did not affect the performance parameters (Samanta & Ghosh, 2008). In contrast, the results of other studies showed that some organic acids had significant effects on performance parameters in Japanese quail (Fushimi *et al.*, 2001; Denli *et al.*, 2003; Arsalan & Saatci, 2004; Daskirn *et al.*, 2004; Moharrery, 2005; Lesson *et al.*, 2005; Abdel-Fattah *et al.*, 2008; Ao *et al.*, 2009; Mohamed & Bahnas, 2009; Nuhocak *et al.*, 2009; Sheikh *et al.*, 2010; Adil *et al.*, 2011). Also, it has been reported that lactic acid has the greatest impact on improving FI and FCR in female Japanese quail of 35-84 d. In general, diets containing organic acids improved all performance parameters. The results in the current study were consistent with the findings of some researchers (Fushimi *et al.*, 2001; Dibner & Buttin, 2002; Arsalan & Saatci, 2004; Daskirn *et al.*, 2004; Sheikh *et al.*, 2010). Between acetic (pKa = 4.76) and butyric acid (pKa = 4.82), the lactic acid pKait's lowest (pKa = 3.86) (Garcia, 2007). Therefore, the acidic power of LA in the present trial can be compared with other organic acids used in the experiment (Garcia, 2007). It seems thatLA is more effective in reducing the population of pathogenic microorganisms in feed and GIT (Dibner & Buttin, 2002; Apajalahati *et al.*, 2004; Garrido *et al.*, 2004; Bourassa *et al.*, 2005; Harris *et al.*, 2006; Samanta & Ghosh, 2008; Laury *et al.*, 2009; Hassan *et al.*, 2010).

As the present results show, diets containing BD+LA had the greatest impact on reducing pathogenic microorganisms such as *E.Coli* and *Salmonella*. Also, it seems that the reduction of pathogenic bacteria in the crop by LA resulted in reduced competition for nutrients between birds and microorganisms. Eventually, with decrease in competition for nutrient between birds and microorganisms, the digestibility of feeds and the absorption of nutrients in the gastrointestinal tract improved (Dibner & Buttin, 2002; Apajalahati *et al.*, 2004; Garrido *et al.*, 2004; Bourassa *et al.*, 2005; Harris *et al.*, 2006; Samanta & Ghosh, 2008; Laury *et al.*, 2009; Hassan *et al.*, 2010).In other studies, it has been reported that LA (for poultry) stimulates increased FI with increasing body weight changes (Dibner & Buttin, 2002; Apajalahati *et al.*, 2004; Garrido *et al.*, 2004; Bourassa *et al.*, 2005; Harris *et al.*, 2006; Samanta & Ghosh, 2008; Laury *et al.*, 2009; Hassan *et al.*, 2010). The use of diets containing LA resulted in the improvement of FI, as well as FCR. In the case of eggs parameters, diets containing BD+AA and BD+LA respectively had the greatest impact on egg weight, egg mass, and egg production. In the current study, performance results showed that diets containing BD+AA and BD+LA had the highest increase in FI and body weight. It appears that, with increased FI and increasing consumption of vitamins and minerals, egg weight increased. Given that a very high percentage of embryos inside the eggs die due to lack of vitamins and minerals during the incubation period, it is understandable that with increased nutrients in the feed for breeders, eggs richer in vitamins and minerals are produced resulting in higher fertility rate. This particular effect might be because diets containing BD+AA and BD+LA, increase fertility in eggs (Chan *et al.*, 1994; Gama *et al.*, 2000; Zanaty *et al.*, 2001; Yakout *et al.*, 2004; Garcia *et al.*, 2005; Mohamed & Bahnas, 2009). However, many studies have shown a direct correlation between live weight and sexual maturity there (Chan *et al.*, 1994). On the other hand, with an increase in egg weight, egg mass and percentage of laying both impressed and recover. The results of this study also showed that diets containing

BD+AA and BD+LA had the most impact on the increase in albumin, globulin and total serum protein. Therefore, it can be said that increase in serum levels of these proteins, increased the level of protein secreted to the eggs by the reproductive system of the female Japanese quail resulting in high egg weight that could have high fertility effect (Chan *et al.*, 1994; Gama *et al.*, 2000; Zanaty *et al.*, 2001; Yakout *et al.*, 2004; Garcia *et al.*, 2005; Mohamed & Bahnas, 2009). However, diets containing LA decreased bacteria population in the gut and stool. It can be guessed fertility eggs have been associated with reducing environmental pollution (Chan *et al.*, 1994). Another results of the addition of organic acids on the microorganism was observed in the diets containing the LAthat effectively decrease the *E.Coli*and *Salmonella* population in the GIT in female Japanese quail (Dibner & Buttin, 2002; Apajalahati *et al.*, 2004; Garrido *et al.*, 2004; Dibner & Richards, 2005; Bourassa *et al.*, 2005; Harris *et al.*, 2006; Samanta &Ghosh, 2008; Laury *et al.*, 2009; Abdel-Hakim & Cherian, 2009; Hassan *et al.*, 2010).It seems that diets containing LA reduced the population of *E.Coli* and *Salmonella* in the GIT, resulting in an improvement of the digestive tract health. Improving the health of the digestive system resulted in enhanced digestion and absorption of nutrients, and all other factors that could improve digestion and absorption such as, FI and FCR in female Japanese quails within 35-84 d. LA can be produced anaerobic metabolism of carbohydrates in the body of organic animals (Dibner & Buttin, 2002; Apajalahati *et al.*, 2004; Garrido *et al.*, 2004; Bourassa *et al.*, 2005; Harris *et al.*, 2006; Samanta & Ghosh, 2008; Laury *et al.*, 2009; Hassan *et al.*, 2010). The LA in muscle, kidney, liver, and heart remain in the tissue turned and converted into glycogen and amino acids. As a result, diets containing LA might cause the accumulation of glycogen in the muscle, resulting in increase in carcass, breast and thigh weight (Hassan *et al.*, 2010). The LA accumulation in the tissues increases the need for water. It turns out that birds excrete large amounts of LA, water consumption increases, and water trapped in the muscle tissue and different parts of carcass weight has increased (Hassan *et al.*, 2010). On the other hand, by increasing the palatability of feed under the influence of diets containing LA, increased FI and ultimately lead to improved synthesis of proteins (Dibner & Buttin, 2002; Apajalahati *et al.*, 2004; Garrido *et al.*, 2004; Dibner & Richards, 2005; Bourassa *et al.*, 2005; Harris *et al.*, 2006; Samanta & Ghosh, 2008; Laury *et al.*, 2009; Abdel-Hakim & Cherian, 2009; Hassan *et al.*, 2010). In addition, studies show that organic acids to reduce the feed pH causing the breakdown of the structure of proteins and carbohydrates in food, and a combination of digestive activity before they happen, however, the LA to reduce the pH content of the digestive system, increases the activity of digestive enzymes pepsinogen and other zymogen, and subsequent levels of pepsin, trypsin and chymotrypsin increases (Canibe *et al.*, 2001). By increasing the breakdown of proteins and peptides accumulation, digestive hormones such as gastrin and cholecystokinin increased. Finally, under the impact of the mechanism of protein digestion and absorption in the gastrointestinal tract increased efficiency and production in the breast, hip and whole body increased (Canibe *et al.*, 2001). It causes muscle hypertrophy and water retention in the muscles of the breast and thigh, and increase the body weight gain and weight of different part of carcass (Canibe *et al.*, 2001). In additional, diets containing BD+AA and BD+LA, significantly increased albumin, globulin and total serum protein in female Japanese quail at 84 d. The diets contain BD+AA and BD+LA showed a significantly increased cholesterol and HDL, and reduced the triglyceride content of the serum. It seems that LA and AA, with increased protein synthesis, increased the synthesis of lipoprotein in liver cells and transferred triglycerides from the blood into the liver cells (Fushimi *et al.*, 2001; Dibner & Buttin, 2002; Arsalan & Saatci, 2004; Daskirn *et al.*, 2004; Sheikh *et al.*, 2010). On the other hands, some studies finding showed, the organic acids such as BA, LA and AA significantly increased the intestine villi height and crypt depth in male Japanese quail in 35 d (Fouladi *et al.*, 2014). Then, it has seem these organic acids effects lead to improved digestion and absorption of the energy and protein, and all of the digestion materials in the intestine and lipoprotein synthesis have improved (Fouladi *et al.*, 2014). With the increase the lipoprotein anabolism reaction in the liver's cells lead to transfer the cholesterol and triglyceride in blood serum to liver cells (Canibe *et al.*, 2001; Fouladi *et al.*, 2014). The organic acids such as LA and AA seems to improve microflora and also improves digestion and absorption of proteins and amino acids, thereby increasing protein anabolism increases lipoprotein synthesis and process of transfer of serum triglyceride and cholesterol increased blood to the liver (Fushimi *et al.*, 2001; Dibner & Buttin, 2002; Arsalan & Saatci, 2004; Daskirn *et al.*, 2004; Sheikh *et al.*, 2010). In the case of measured serum glucose in the experiment, it seems, LA and BA in combination with each other decreased stimulate insulin secretion from the pancreas, as a result in increased blood glucose levels. Insulin hormones according to what is described above, as causes increased body metabolism and protein synthesis (Fushimi *et al.*, 2001; Dibner & Buttin, 2002; Arsalan & Saatci, 2004; Daskirn *et al.*, 2004; Sheikh *et al.*, 2010). Due to the consistent increase in serum glucose levels with increased FI, appears to cause an increase in the blood sugar, as FI increases (Canibe *et al.*, 2001; Fouladi *et al.*, 2014). In the current study, it was shown that all diets containing AA reduced the level of blood glucose. However, studies have shown that AA derivative of acetyl groups to the biochemistry of virtually all species is essential (Fushimi *et al.*, 2001; Dibner & Buttin, 2002; Arsalan & Saatci, 2004; Daskirn *et al.*, 2004; Sheikh *et al.*, 2010). The acetyl group in the presence of coenzyme-A, which is converted to acetyl coenzyme-A is the most important factor influencing the metabolism of carbohydrates and fats. Most likely, increasing amounts of acetyl coenzyme-A and enter the cycle of glycolysis, the citric acid cycle and decarboxylation chain to speed up the metabolism of carbohydrates in the body and the serum glucose level is reduced (Dibner & Buttin, 2002; Arsalan & Saatci, 2004; Daskirn *et al.*, 2004; Sheikh *et al.*, 2010). All diets containing organic acids had lower pH in the GIT in this trial. However, all diets containing organic acids reduced the levels of *E.Coli* and *Salmonella* population in the GIT; diets containing BD+LA, and BD+BA had the greatest effect. The LA is the strongest acid among other organic acids. Some studies reports have shown that the strength of BA is maintained until the end of the digestion process. As a result, reduce the *E.Coli* and *Salmonella* population in GIT in this experiment by the organic acid to lower the pH by organic acids is concerned (Van Immerseel *et al.*, 2005; Dhawale, 2005; Moharrery & Mahzonieh, 2005; Cabuk *et al.*, 2006; Wolfenden *et al.*, 2007; Bin jasass, 2008; Mohamed & Bahnas, 2009; Hassan *et al.*, 2010). Regarding the eggs quality traits, diets containing BD+LA and BD+AA respectively had the greatest impact on IQU, egg shell thickness, egg shell weight and egg shell surface. Many researchers have reported FI is associated with IQU, egg shell thickness, egg shell weight and egg shell surface (Chan *et al.*, 1994; Gama *et al.*, 2000; Zanaty *et al.*, 2001; Yakout *et al.*, 2004; Garcia *et al.*, 2005; Mohamed & Bahnas, 2009). Given that the diets containing BD+LA and BD+AA increased FI, it seems that an increase in FI increased calcium intake and egg shell thickness; this process resulted in body weight changes of shell and shell surface. It is not entirely clear whether calcium increases FI or increase in FI increased calcium intake by female Japanese quails. Studies show that the low pH resulting from the use of organic acids, led to an increase in the gut microbial phytase activity as well as phytic acid dissolution. Following this process, the digestion and absorption of calcium and phosphorus were improved (Chan *et al.*, 1994). In addition, LA with the highest acid strength decreased the pH values in ovarian contents. On the other hand, the separation of calcium ions of calmodulin protein in an acidic environment facilitated carriers (Chan *et al.*, 1994). All these mechanisms increases the deposition of calcium in the egg shell, which subsequently increase the weight and thickness of the egg shell (Chan *et al.*, 1994; Gama *et al.*, 2000; Zanaty *et al.*, 2001; Yakout *et al.*, 2004; Garcia *et al.*, 2005; Mohamed & Bahnas, 2009).

CONCLUSIONS

In this study, it was shown that supplements of organic acids have positive effects on the performance parameters in laying Japanese quails. Amongst the organic acids used in this study, the diets with LA alone have the most impact on performance parameters. So that, LA with an improvement in other parameters, such as decline the pathogenic population bacteria and improve blood and liver biochemical parameters, other parameters yield and also improves the quality and quantity of eggs traits. However, the LA and BA in combination with other organic acids, organic acids efficiency is improved. Therefore, in this study, it seems that organic acids such as AA, LA and BA, especially LA in the diet of laying Japanese quail have been useful. The use of organic acids, especially LA in laying Japanese quail is recommended for production efficiency.

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