PAPER

Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of acetic acid

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Abstract

In order to evaluate the effect of acetic acid on growing and laying performance of Japanese quail (JQ), 180 15-day-old JQ were divided into 4 groups. During the growing (15-42 days of age) and laying (43-84 days of age) periods, the groups fed the same basal diets supplemented with 0, 1.5, 3 and 6% of acetic acid. Each diet was fed to five replicates of 9 JQ (3 males:6 females) during the growing period. During the laying period, 128 birds were housed in 32 cages (4 birds per cage, 1 male and 3 females, 8 replicates per treatment). Birds were housed in wire cages (46L×43W× 20H cm) in an open room. Acetic acid supplementation at 3% in the diets significantly increased the growth and laying rate and the Haugh unit score. The liver percentage significantly decreased with acetic acid at 6%. Acetic acid at 3% significantly increased hemoglobin concentrations at 6 weeks of age and increased weight of day old chicks hatched. Acetic acid affected the immune system as manifested by an excess of cellular reactions in the intestine as well as lymphoid hyperplasia in the spleen tissue. Degenerative changes in the covering epithelium of the intestinal villi were noted at the 6% concentration of acetic acid. Hepatocyte vacuolation and fatty changes were also observed at this concentration of treatment. In conclusion, 3% acetic acid may be used as a feed supplement for JQ during the growing and laying period to improve the productive performance.

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Introduction

The ban on use of antibiotics as growth promoters in EU since 2006 endorsed the finding of alternative to antibiotics in farm animal nutrition (Attia *et al*., 2006; 2012; El-Deek *et al*., 2011). Thus, search for effective alternatives to antibiotics in intensive animal production is a very important topic, considering not only the impact in animal welfare but also in human health. Antibiotic-resistant bacteria (whose number is increasing year by year) are considered also a social problem with a high economical impact due to the increasing number of hospitalizations. The finding of natural molecule as an alternative to antibiotics could help to improve welfare both in animals and humans.

Organic acids are a group of feed additives used to reduce the proliferation of *Salmonell*a and *E. coli* in the digestive tract of birds by decreasing the pH. They can be classified as growth promoters, acidifiers or bacterial inhibitors. The organic acids are globally used to inhibit pathogens like *Salmonella* in both raw materials and finished feed (Attia *et al*., 2012). The organic acids in their undissociated forms are able to pass through the cell membrane of bacteria. Once inside the cell, the acid dissociates to produce H+ ions, which reduces the pH of the cell causing the organism to use its energy trying to restore the normal balance, whereas the RCOO- anions produced from the acid can disrupt DNA and protein synthesis, putting the organism under stress and unable to replicate.

In poultry, pathogenic bacteria, such as *Salmonella,* enter the gastrointestinal tract via the crop. The environment of the crop with respect to microbial composition and pH seems to be very important in relation to the resistance to pathogens. In addition, the antibacterial effect of dietary organic acids in chickens is believed to take place mainly in the upper part of the digestive tract (crop and gizzard). Following addition of a combination of formic and propionic acid at high concentrations could only be recovered from crop and gizzard (Thompson and Hinton, 1997). Little amounts of dietary propionic acid reach the lower digestive tract and the caeca (Hume *et al.*, 1993). A part the productive performance, another ways to better evaluate the effects of dietary treatments on animal health is the study of blood profile (Moniello *et al*., 2005) and on intestinal histopatology of animals (Incharoen *et al*., 2010). This study aimed to investigate the effect of different concentrations of acetic acid on productive performance, egg quality, physiological traits, biochemical constituents of blood plasma and histopathology traits of the intestine, liver and the spleen of the Japanese quail (JQ).

Materials and methods

Experimental design, diets and husbandry

A straight run experimental design was used in which dietary acetic acid at concentrations of 0, 1.5, 3 and 6 g/100 g of diet were tested from 15 to 84 days of age. The 6% diet was prepared by adding 6 g of acetic acid to 100 g of the control diet. The 3% and 1.5% diets were prepared by dilution of the 6 % diet with control feed. The diets were fed during the growing (15-42 days of age; Table 1) and the laying periods (43- 84 days of age; Table 2). Each diet was fed to five replicates of 9 birds each (3 males-6 females) during the growing period. During the laying period, the extra birds were eliminated to obtain a total of 128 birds and the mating ratio was 1 male per 3 females. During the laying period, 1 male and 3 females were housed in each of 32 separate cages and treated as replicates with 8 replicates per treatment. The chickens were housed in wire cages (46 cm L x 43 cm W x 20 H cm). The experimental diets were formulated according to NRC (1994) to meet the nutrient requirements of JQ. Feed in mash form and water were available *ad libitum*. Chicks were housed in battery brooders with the room temperature maintained at 33°C during the 1st week, and reduced by 3°C/wk until 24°C. A photoperiod of 23L:1D was provided up to 42 days of age then gradually adjusted to reach a photoperiod of 16L:8D at 9 weeks of age.

Productive performance

Mortality rate was recorded daily. Body weight and feed intake were recorded weekly and feed conversion ratio during the growing and the laying period was calculated as the unit of feed required to produce one unit of weight gain (g feed/g gain) or one unit of egg mass (g feed/g egg mass), respectively. Age at 30% production was calculated per replicates according to Attia *et al*. (1995). Eggs were individually weighed to the nearest 0.1 g. Percent hen-day egg production was determined. Daily egg mass (laying rate/average egg weight) was calculated.

Egg quality traits

Fifteen eggs per treatment were collected at 70, 77 and 84 d of age and used to determine egg quality according to Attia *et al*. (1994).

Fertility, hatchability and weight of day old chicks

At 70, 77 and 84 d of age all the eggs from each group were collected and stored for one week at room temperature. Eggs were incubated and hatched at 37°C and 65% RH. All unhatched eggs were inspected to differentiate infertile and dead embryos eggs. Hatched chicks were counted and weighed. Fertility and hatchability of fertile eggs were calculated according to Attia *et al*. (1995).

Carcass traits

At 42 and 84 d of age, 6 quails (sex ratio 1:1) per group were slaughtered to determine the carcass traits. The birds were starved overnight then weighed, slaughtered by the Islamic method and plucked. After removal of the head, viscera, shanks, digestive tract, liver, heart, spleen and abdominal fat, the rest of the body was weighed to determine the dressed weight. Dressing percentage (dressed weight/live weight $\times$ 100) was calculated. The heart, empty gizzard, liver and abdominal fat (from the proventriculus surrounding the gizzard down to the cloaclal) from each bird were weighed and calculated as a percentage of live body weight.

Histopathology

Tissue specimens were collected from the intestine, liver and spleen of three birds per treatment at 56 d of age and fixed in 10% neutral buffered formalin solution for at least 24 h. After fixation the specimens were washed in tap water and then passed through the routine paraffin embedding procedures (dehydration in ascending grades of the ethyl alcohol, clearing in a series of xylene baths and then infiltrated with melted paraffin wax, embedded and put in paraffin blocks). Sections, 3-5 microns thick were stained with hematoxylin and eosin (Bancroft and Marilyn, 2002) for histological examination.

Hematological and biochemical traits

Blood samples per treatment were collected from live birds at 42, 70 and 84 d of age (n=6/treatment/time). The heparinized blood was centrifuged at 1500x g for 20 min to obtain plasma. Samples were also centrifuged for 20 min at 2000x g to determine packed cell volume (PCV %) using Wintrobe hematocrit tubes (Jiangdu Sunflower Glass Instrument Factory, Jiangdu, China). Hemoglobin (Hgb) concentration was determined by the cyanomethemoglobin method (Eilers, 1967). Total plasma protein, and albumin concentration were determined using the methods of Armstrong and Carr (1964) and Doumas *et al.* (1977), respectively. Globulin concentration was estimated by subtraction of albumin concentration from serum total protein according to Coles (1974). The activities of alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L) and creatinine concentration (mg/dL) were also assayed by the method of Reitman and Frankel (1957).

Statistical analysis

Data were tested for significance by ANOVA using General Linear Models (GLM) procedure of the SAS (2000). Growth performance and

Table 1. Ingredients and estimated or determined chemical-nutritional characteristics of the growing diets.

	Acetic acid, %			
	0	1.5	3	6
Ingredients, %				
Yellow corn	45.00	45.00	45.00	45.00
Soybean meal, 44% CP	47.20	47.20	47.20	47.20
Rice bran	1.40	1.40	1.40	1.40
Dicalcium phosphate	2.05	2.05	2.05	2.05
Limestone	0.45	0.45	0.45	0.45
Vit+Min mix°	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30
Vegetable oils	3.00	3.00	3.00	3.00
Acetic acid	0.00	1.50	3.00	6.00
Total	100.0	101.5	103.0	106.0
Estimated values as fed				
ME, MJ/kg	12.14	11.96	11.79	11.45
Calcium, %	1.06	1.04	1.02	1.00
Phosphorus available, %	0.50	0.49	0.48	0.47
Methionine, %	0.67	0.66	0.65	0.63
Total sulfur amino acids, %	1.06	1.04	1.03	1.00
Lysine, %	1.40	1.38	1.36	1.32
Determined values				
Dry matter, %	92.11	91.05	89.50	87.80
Crude protein, % as fed	24.50	24.10	23.70	23.00
Ether extract, % as fed	3.42	3.26	3.18	3.06
Crude fibre, % as fed	3.19	3.00	2.92	2.80
Ash, % as fed	7.65	7.92	8.18	8.59

CP, crude protein; ME, metabolizable energy. °Vitamin and mineral mixture provides per kg of diet: retinol acetate, 1800 g; DL-alpha-tocopherol acetate, 10 mg; menadione sodium bisulphate, 2.5 mg; cholecalciferol, 50 g; riboflavin, 2.5 mg; Ca-D-pantothenate, 10 mg; nicotinic acid, 12 mg; choline chloride, 50 mg; cyanocobalamin, 4 mg; pyridoxine hydrochloride, 5 mg; thiamine mononitrate, 3 mg; folic acid, 1.0 mg; d-biotin, 0.2 mg. Trace mineral (milligrams per kilogram of diet): Mn, 80; Zn, 60; Fe, 35; Cu, 8; Se, 0.02.

egg production traits were processed by one-way model in which the concentration of acetic acid was the fixed effect. Data on carcass and organs traits, egg quality traits, fertility, hatchability, weight of chicks at one day, blood and plasma biochemical traits were analyzed by a two-way model in which the main effects were the concentration of acetic acid and birth age; the interaction between the two effects was also included in the model. Mean differences were tested using Student-Newman-Keuls test. All the percentages were converted as $\log_{10}$ to normalize data distribution. Mortality rate was tested using Chi-square analysis during the growing period.

Results

Growth performance

Data for growth performance are presented in Table 3. Body weight at 21 d of age was significantly decreased by 6% acetic acid in the diet in comparison to the control group. At 63 d of age birds fed the 3% acetic acid diet were significantly heavier than the control and 6% acetic acid groups, respectively.

Feed intake from 15 to 42 days of age decreased (P<0.01) with the addition of acetic acid and the lowest value of feed intake was reached at 3.0% of acetic acid. Acetic acid at 1.5, 3 and 6% significantly improved FCR compared to the control group.

Mortality rate during the growing period was not affected by acetic acid supplementation. Values ranged from 2.22 to 8.88% for groups supplemented with 3% acetic acid and control group respectively.

Egg production traits

Results for egg production traits are displayed in Table 4. Inclusion of 1.5 and 6% acetic acid significantly increased the age at 30% production compared to the control and 3% acetic acid groups. There was a significant positive effect due to 3% acetic acid supplementation on the laying rate and the egg mass compared to all the other groups.

However, 1.5 and 6% acetic acid significantly decreased the laying rate and egg mass compared to the other groups group. Feed intake showed a significant linear decrease with increasing acetic acid. The FCR of pullets during the early stage of egg production was significantly improved with 3% acetic acid supplementation compared with the other concentrations and with the control group.

Egg quality traits

Egg index and shell weight were unaffected by dietary treatment (Table 5). Yolk index was significantly increased by 1.5% acetic acid supplementation compared to the other groups (Table 6). All acetic acid concentrations significantly increased the Haugh unit score compared to the control group (Table 6).

Carcass characteristics and inner organs

Results for carcass and inner organs traits are shown in Table 7. The percentage of abdominal fat was higher (P<0.05) in the carcass of birds supplemented with 1.5 and 3% acetic acid and was minimized due to the inclusion of 6% acetic acid in the diet. The liver percentage was reduced (P<0.05) in the birds fed 6% acetic acid in comparison to the control group. Acetic acid did not affect dressing percentage or other body organs.

Fertility, hatchability of fertile eggs and weight of day old chicks

Data for reproductive traits are displayed in Table 8. Fertility and hatchability were not affected by acetic acid supplementation. Supplementation of 3% acetic acid significantly increased weight of one day old chicks compared to only 1.5% acetic acids.

Hematology and biochemical constituents of blood plasma

Results for hematology traits are shown in Table 9. The PCV was significantly affected by different concentrations of acetic acid at 42 and 84 d of age. At 42 d of age, all the concentrations of acetic acid increased (P<0.01) PCV compared to the control group. At 84 d of age, 3% acetic acid significantly decreased the PCV compared to 1.5 and 6% acetic acid. Hgb was significantly affected by different concentrations of acetic acid at 42 and 70 d of age: 3.0%

Table 2. Ingredients and estimated or determined chemical-nutritional characteristics of the laying diets.

	Acetic acid, %			
	0	1.5	3	6
Ingredients, %				
Yellow corn	52.00	52.00	52.00	52.00
Soybean meal, 44 % CP	33.65	33.65	33.65	33.65
Wheat bran	2.40	2.40	2.40	2.40
Rice bran	2.40	2.40	2.40	2.40
Dicalcium phosphate	1.50	1.50	1.50	1.50
Limestone	6.10	6.10	6.10	6.10
Vit+Min mix°	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30
Methionine	0.15	0.15	0.15	0.15
Vegetable oils	1.20	1.20	1.20	1.20
Acetic acid	0.00	1.50	3.00	6.00
Total	100.0	101.5	103.0	106.0
Estimated values, as fed				
ME, MJ/kg	11.23	11.06	10.90	10.59
Calcium, %	2.99	2.95	2.90	2.82
Phosphorus available, %	0.39	0.38	0.38	0.36
Methionine, %	0.45	0.44	0.44	0.42
Total sulfur amino acids, %	0.77	0.76	0.75	0.73
Lysine, %	1.07	1.05	1.04	1.01
Determined values				
Dry matter, %	93.30	92.90	91.40	90.10
Crude protein, % as fed	19.60	19.10	18.90	18.50
Ether extract, % as fed	3.12	3.06	2.88	2.56
Crude fibre, % as fed	3.99	3.85	3.69	3.73
Ash, % as fed	14.30	14.40	14.90	15.20

CP, crude protein; ME, metabolizable energy. °Vitamin and mineral mixture provides per kg of diet: retinol acetate, 1600 g; DL-alpha-tocopherol acetate, 12 mg; menadione sodium bisulphate, 2.5 mg; cholecalciferol, 50 g; riboflavin, 2.5 mg; Ca-D-pantothenate, 10 mg; nicotinic acid, 12 mg; choline chloride, 300 mg; cyanocobalamin, 4 mg; pyridoxine hydrochloride, 5 mg; thiamine mononitrate, 3 mg; folic acid, 0.5 mg; D-biotin, 0.2 mg. Trace mineral (milligrams per kilogram of diet): Mn, 40; Zn, 40; Fe, 40, Cu, 4; Se, 0.02; iodine, 0.5.

Table 3. Effect of different dietary concentrations of acetic acid on body weight and body weight gain, feed intake, feed conversion ratio, and mortality rate of Japanese quail from 15 to 42 d of age and body weight at 63 d of age (mean ±SE).

Acetic acid, %	Body weight at 21 d of age, g	Body weight at 42 d of age, g	Body weight gain, g	Feed intake, g /bird/period	Feed conversion ratio, g feed/g gain	Mortality 15-42 d of age, %	Body weight at 63 d of age, g
0	88.7±2.1[a]	157.3±4.2	68.6±3.4	384.3±0.42[a]	5.68±0.11[a]	8.88±1.13	179.9±5.3[b]
1.5	86.6±2.1[ab]	165.5±4.4	78.9±4.5	356.0±0.63[b]	4.50±0.06[b]	6.66±1.24	186.5±3.8[ab]
3	87.7±2.2[ab]	168.1±4.1	80.4±5.1	326.2±0.93[d]	4.10±0.07[b]	2.22±0.09	204.3±4.8[a]
6	80.7±2.1[b]	158.1±3.2	77.4±3.9	338.5±1.61[c]	4.50±0.09[b]	4.44±2.10	175.3±4.1[b]
Significance	0.045	ns	ns	0.010	0.047	ns	0.009

[a-d]Means within a column not sharing a common superscript are significantly different. ns, not significant.

Table 4. Effect of different dietary concentrations of acetic acid on egg production traits of Japanese quail hens from 43 to 84 days of age (mean ±SE).

Acetic acid, %	Age at 30% production, days	Laying rate, %	Egg weight, g	Egg mass, g/h/d	Feed intake, g/h/d	Feed conversion ratio, g feed/g egg
0	56.3±0.25[c]	32.3±1.03[b]	10.4±0.15	3.36±0.11[b]	19.0±0.18[a]	5.67±0.18[a]
1.5	61.8±0.56[b]	26.3±0.63[c]	10.0±0.11	2.63±0.06[c]	17.8±0.03[b]	6.78±0.16[a]
3	52.8±0.46[c]	44.8±0.48[a]	10.3±0.18	4.62±0.05[a]	17.2±0.30[b]	3.73±0.04[b]
6	65.0±1.13[a]	23.0±2.48[c]	9.90±0.11	2.20±0.24[c]	16.3±0.28[c]	6.03±0.58[a]
Significance	0.001	0.009	ns	0.008	0.010	0.044

[a,b,c]Means within a column not sharing a common superscript are significantly different. ns, not significant.

Table 5. Effect of different dietary concentrations of acetic acid on shell quality criteria of Japanese quail from 43 to 84 day of age (mean ±SE).

Acetic acid, %	Egg index	Shell weight, g	Shell weight, %	SWUSA, mg/cm^2
0	70.0±0.16	1.39±0.04	13.5±0.39	68.8±1.9
1.5	70.4±0.89	1.43±0.03	14.4±0.34	72.7±1.5
3	69.8±1.2	1.40±0.02	13.9±0.32	71.4±1.3
6	71.5±1.07	1.39±0.04	14.1±0.31	71.1±1.6
Significance	ns	ns	ns	ns

SWUSA, Shell weight per unit of surface area. ns, not significant.

Table 6. Effect of different dietary concentrations of acetic acid on egg quality criteria of Japanese quail from 43 to 84 day of age (mean ±SE).

Acetic acid, %	Yolk weight at 43 d of age, g	Yolk weight at 84 d of age, %	Yolk index	Albumin weight at 43 d of age, g	Albumin weight at 84 d of age, %	Haugh unit score
0	3.36±0.9	32.3±0.7	47.5±1.9[b]	5.63±0.14	54.0±0.8	82.18±1.4[b]
1.5	3.50±0.1	35.1±0.9	56.2±1.1[a]	5.12±0.18	51.0±1.3	86.90±1.2[a]
3	3.49±0.1	33.5±0.9	51.2±1.1[b]	5.35±0.18	51.7±0.7	91.20±0.8[a]
6	3.18±0.1	32.1±0.7	49.8±1.3[b]	5.32±0.1	53.7±0.8	87.90±1.6[a]
Significance	ns	ns	0.009	ns	ns	0.008

[a,b]Means within a column not sharing a common superscript are significantly different. ns, not significant.

Table 7. Effect of different dietary concentrations of acetic acid on dressing, abdominal fat and organs as relative to live body weight of Japanese quail (mean ± SE).

Acetic acid, %	Dressing, %	Abdominal fat, %	Liver, %	Hear, %	Pancreas, %	Spleen, %	Gizzard, %	Ovary, %	Intestine, %
0	70.8±0.40	0.13±0.30[b]	2.59±0.07[a]	0.84±0.02	0.26±0.02	0.04±0.01	2.27±0.08	2.07±0.11	3.14±0.15
1.5	70.7±0.79	0.17±0.31[a]	2.11±0.11[ab]	0.79±0.03	0.33±0.06	0.04±0.01	2.18±0.06	1.48±0.11	3.12±0.11
3	69.2±0.43	0.17±0.46[a]	2.18±0.13[ab]	0.75±0.02	0.24±0.02	0.03±0.01	2.08±0.11	2.14±0.09	3.20±0.09
6	67.9±0.42	0.00±0.00[c]	1.91±0.03[b]	0.72±0.03	0.39±0.07	0.04±.0.01	2.33±0.07	1.93±0.16	3.12±0.08
Significance	ns	0.046	0.047	ns	ns	ns	ns	ns	ns

[a,b,c]Means within a column not sharing a common superscript are significantly different. ns, not significant.

acetic acid significantly increased the Hgb concentration compared with the control and the other concentrations of acetic acid. However, at 70 d of age, all the concentrations of acetic acid significantly decreased Hgb compared to the control. The albumin concentration was significantly affected by acetic acid concentration at only 70 d of age. Acetic acid at 3% significantly increased the plasma albumin compared to 1.5 and 6% acetic acid concentration. At 6% of inclusion in the diet, acetic acid significantly ($P<0.05$) reduced albumin in comparison to the control group. Plasma total protein, globulin, AST and ALT measured at different ages were not significantly affected by different concentrations of acetic acids.

Microscopic findings in the intestine

Histological examination of the intestine in the control group revealed normal structure of the villi and crypts of the glands (Figure 1). The villi base showed some cellular reactions, while the epithelium cells appeared intact but with small or mild changes at 1.5% concentration (Figure 2). The medium concentration (3%) of acetic acid was manifested by hyperplastic and vacuolar degeneration in the covering epithelium with an excess of cellular reactions at the base between the crypts (Figure 3). The high concentration (6%) of acetic acid induced a severe degeneration and necrosis in the epithelium of the glandular crypts that appeared to be widely separated by an excess of mononuclear cell infiltrations (Figure 4).

Microscopic findings in the liver

Microscopic examination of the liver in the control group revealed normal histological structure of the tissue and normal and intact acini (Figure 5). The low concentration (1.5%) of acetic acid induced edema with hepatocytic degenerations in the form of variable sized vacuolations (Figure 6). The administration of 3% acetic acid induced variable sized, sharply edged fatty vacuolations in the liver (Figure 7), while the high concentration of acetic acid (6%) led to diffuse, extensive and severe fatty vacuolations (Figure 8).

Microscopic findings in the spleen

Microscopic examination of the spleen in the control group revealed the presence of normal histological structure. Active trabecular and lymphoid germinal centers in addition to dispersed lymphocytic elements (Figure 9) were observed. Thick walled arterioles with congestion and dispersion of the lymphocytic

Table 8. Effect of different dietary concentrations of acetic acid on percentage fertility, hatchability of fertile eggs and weight of day old chick of Japanese quail from 43-84 day of age (mean ±SE).

Acetic acid, %	Fertility, %	Hatchability, %	Chick weight at one day, G
0	84.2±2.96	54.4±13.7	5.73±0.15[ab]
1.5	87.0±8.50	55.3±14.2	5.51±0.16[b]
3	97.0±2.50	67.9±4.51	7.09±0.23[a]
6	83.3±9.50	64.2±8.20	6.12±0.12[ab]
Significance	ns	ns	0.046

[a,b]Means within a column not sharing a common superscript are significantly different. ns, not significant.

Table 9. Effect of different dietary concentrations of acetic acid on blood hematological and plasma biochemical traits of Japanese quail at different ages (mean ±SE).

Acetic acid, %	PCV, %	Hemoglobin, g/dL	Protein, g/dL	Albumin, g/dL	Globulin, g/dL	ALT, U/L	AST, U/L
42-day-old							
0	44.3±2.40[b]	18.8±0.77[b]	5.38±0.24	3.43±0.18	1.95±0.23	45.6±1.50	10.4±1.22
1.5	50.7±3.18[a]	18.4±0.78[b]	4.99±0.24	3.00±0.08	1.98±0.18	45.9±1.13	10.2±0.4
3	50.3±0.88[a]	21.7±0.94[a]	4.97±0.21	3.06±0.15	1.91±0.14	40.9±1.57	10.9±2.27
6	50.7±1.45[a]	19.3±0.64[b]	4.99±0.18	2.86±0.11	2.14±0.10	40.9±1.43	7.30±1.50
Significance	0.010	0.001	ns	ns	ns	ns	ns
70-day-old							
0	48.0±2.52	30.2±1.09[a]	6.69±0.89	3.29±2.23[ab]	3.05±0.75	nd	nd
1.5	48.8±4.60	21.0±1.00[b]	6.69±0.89	2.52±0.47[bc]	3.26±0.55	nd	nd
3	47.5±1.32	22.8±2.10[b]	6.82±0.37	3.64±0.53[a]	3.55±1.25	nd	nd
6	49.7±1.76	24.4±0.54[b]	5.71±0.99	2.23±0.31[c]	3.48±0.70	nd	nd
Significance	ns	0.009	ns	0.047	ns	nd	nd
84-day-old							
0	36.0±2.19[ab]	15.3±1.28	5.90±0.53	2.09±0.25	3.31±0.39	47.9±1.79	8.21±1.46
1.5	41.0±2.73[a]	15.3±1.10	6.05±0.40	2.02±0.21	4.03±0.31	51.7±0.98	9.12±0.75
3	29.0±3.38[b]	17.0±1.32	5.60±0.44	2.15±0.15	3.45±0.30	58.6±4.39	13.23±1.05
6	45.0±2.19[a]	17.5±034	5.13±0.26	1.99±0.19	3.14±0.23	55.7±1.93	11.5±2.07
Significance	0.045	ns	ns	ns	ns	ns	ns

[a,b,c]Means within a column not sharing a common superscript are significantly different. ns, not significant; nd, not done.

elements were detected in birds fed the 1.5% concentration of acetic acid (Figure 10). Changes in the spleen of birds fed 3% acetic acid were manifested by the presence of a well developed lymph follicle surrounded by an excess of newly formed trabecular vessels (Figure 11), while spleens of birds fed the high concentration (6%) of acetic acid showed presence of a large numbers of a well developed and active lymph follicles and few trabecular elements (Figure 12), suggesting the development of a hyper immune defensive mechanism.

Discussion

The enhancements in feed conversion ratio due to the use of acetic acid at the different concentrations could be attributed to the antimicrobial and buffering capacity of acetic acid (Christian *et al*., 2004), however the best effects on feed intake reduction and growth rate increase at 63 days of age were supplied by 3% of acetic acid inclusion. Several authors found that growth rate and FCR of broilers and ducks were improved due to supplementation of organic acid salts such as formic acid (Vogt *et al*., 1981), fumaric acid (Kirchgessner *et al*., 1991), both formate and propionate (Paul *et al*., 2007; Koley *et al*., 2008) and acetic acid (Attia *et al*., 2012). Christian *et al*. (2004) found that organic acid blends did not significantly affect the FCR of broiler chickens. Thus, the effectiveness of the organic acids in poultry may also depend on the composition of the diet and its buffering capacity (Çelik *et al*., 2003).

The increase in egg production observed at 3% acetic acid could be attributed to the decrease in age at 30% production and thus increasing length of laying period. The decrease in age at 30% production might indicate a higher nutrient availability as suggested by Garcia *et al*. (2007) who reported that incorporation of 0.5% acetic, lactic or formic acids in the drinking water increases gastric proteolysis, improving protein and amino acid digestibility. Furthermore, the acid-anion has been shown to complex with Ca, P, Mg and Zn, which results in an improved digestibility of these minerals. Moreover, organic acids serve as substrates in intermediary metabolism (Kirchgessner and Roth, 1988). Abdel-Fattah *et al*. (2008) suggested that incorporation of some organic acids in drinking water might reduce *Salmonella* contamination in crops and broiler carcasses at processing, showing a beneficial effect on animal health and carcasses quality. Furthermore, organic acid may decrease the chyme pH and influence the intestinal cell morphology and the electrolyte

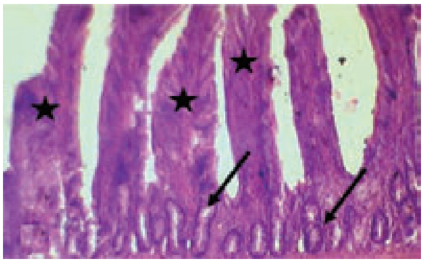

Figure 1. Intestine of the control group (0% acetic acid) showing intact tall villi (black asterisks) with intact underlying crypts of the intestinal glands (arrows). Haematoxylin and eosin stain using 160x magnification power.

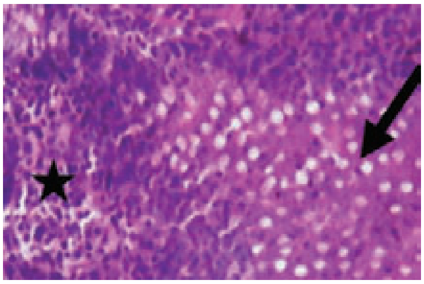

Figure 2. Intestine of the group fed 1.5% acetic acid showing thick base of the villi (black asterisk) with intact mildly vacuolated covering epithelium (arrow). Haematoxylin and eosin stain using 400x magnification power.

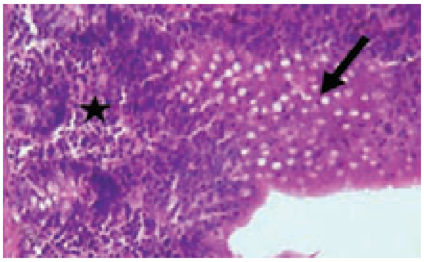

Figure 3. Intestine of the group fed 3% acetic acid showing hyperplastic and vacuolar degenerated covering the epithelium (arrow) with an excess of cellular reactions at the base of the crypts (black asterisk). Haematoxylin and eosin stain using 400x magnification power.

Figure 4. Intestine of the group fed 6% acetic acid showing degenerated epithelium of the crypts that are widely separated by an excess of mononuclear cell infiltrations (white asterisks). Haematoxylin and eosin stain using 400x magnification power.

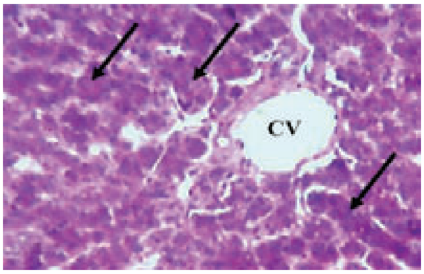

Figure 5. Liver of the control group (0% acetic acid) showing normal hepatocytic acini (arrows) and dilated central vein (CV). Haematoxylin and eosin stain using 400x magnification power.

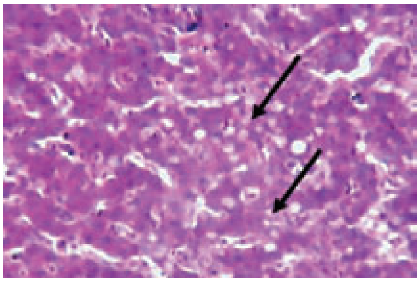

Figure 6. Liver of the group fed 1.5% acetic acid showing edema with variable sized hepatocytic vacuolations (arrows). Haematoxylin and eosin stain using 400x magnification power.

balance in the feed and intestine.

The decrease of feed intake of acetic acid supplemented groups in comparison to the control one could be due to the negative effect of acetic acid on bird's appetite even if at 6% of inclusion, the feed intake level was significantly higher than at 3%. However, at the highest level of acetic acid inclusion, probably the dilution effect on dietary nutrient concentrations affected our results, as the reduction in energy concentration induce a compensative high feed intake.

The improvement in Haugh unit score of groups fed diets supplemented with different concentrations of acetic acid may be due to decrease bacterial contamination of eggs (Kim and Marshall, 2000; Gunal *et al*., 2006; Paul *et al*., 2007) and thus improving keeping quality after harvest. The increase in yolk weight at 1.5 and 3% acetic acids may be due to the increase in the nutrients availability for yolk formation. Farran *et al*. (2001a, 2001b) reported that acetic acid significantly increased the egg weight of laying hens. The increase in the egg weight could be partly explained by the increase in the yolk weight. The effect of probiotic supplementations on egg quality was also reported by Çelik *et al*. (2008) who found that prebiotics significantly increased the albumen weight, shell weight and the shell thickness, while decreasing the yolk weight in a dose related manner, suggesting that providing probiotics at 2% could have a potential to increase egg shell quality under heat stress condition. On the other hand, the yolk index, albumen index, albumen height, yolk color and the shape index were not significantly affected by different doses of prebiotics.

Acetic acid had no harmful effect on fertility and hatchability although there was a unexplained significant positive effect due to supplementation of 3% acetic acid on weight of one-day-old chicks compared to 1.5% acetic acids however, acetic acid might have increased the availability of nutrient during the embryonic period.

The progressive decrease in abdominal fat with increasing acetic acid concentration could indicate that acetic acid acts as a lipolytic agent. The decreases were 26.3 and 100%, respectively with 6% acetic acid as compared to the control group. There was also a significant decrease in abdominal fat at other concentrations of acetic acid compared to the control group. In this regard, Denli *et al*. (2003) found that broilers given diets supplemented with flavomycin with 0.2% mineral salts exhibited higher carcass weight. Yousefi *et al.* (2008) reported that the organic acids and/or probiotic significantly increased the carcass yield.

The blood biochemical constituents found herein are in normal physiological range reported by Attia *et al*. (2006), El-Hommosany

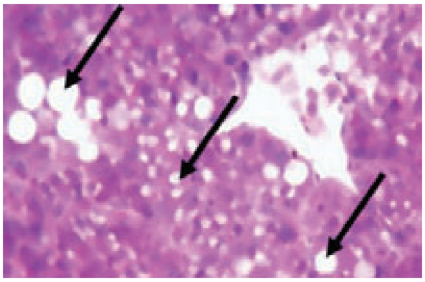

Figure 7. Liver of the group fed 3% acetic acid showing excess of the variable sized, sharply edged fatty vacuolations (arrows). Haematoxylin and eosin stain using 400x magnification power.

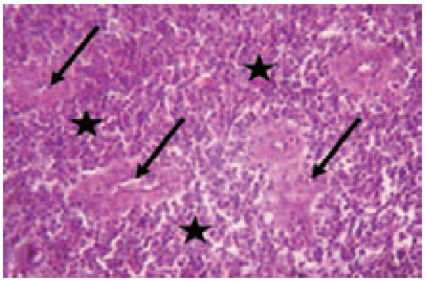

Figure 9. Spleen of the control group (0% acetic acid) showing normal splenic tissue with active trabeculae and germinal centers (arrows) in addition to the dispersed lymphocytic elements (black asterisk). Haematoxylin and eosin stain using 400x magnification power.

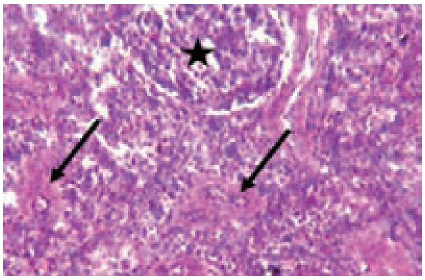

Figure 11. Spleen of the group fed 3% acetic acid showing one of the well developed lymph follicle (black asterisk) surrounded by excess of newly formed trabecular vasculatures (arrows). Haematoxylin and eosin stain using 400x magnification power.

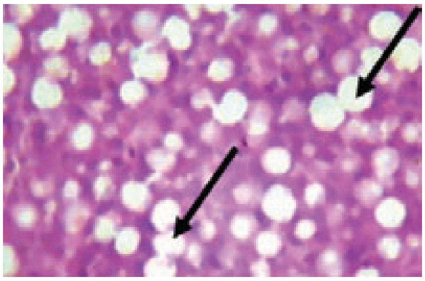

Figure 8. Liver of the group fed 6% acetic acid showing diffuse areas of extensive and severe fatty vacuolation (arrows). Haematoxylin and eosin stain using 160x magnification power.

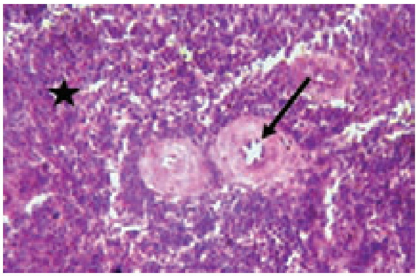

Figure 10. Spleen of the group fed 1.5% acetic acid showing thick walled arterioles (arrow) with congestion and dispersion of the lymphocytic elements (black asterisk). Haematoxylin and eosin stain using 400x magnification power.

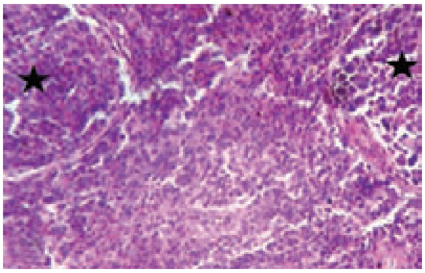

Figure 12. Spleen of the group fed 6% acetic acid showing 2 large well developed and active lymph follicles (black asterisks) and few reabecular elements. Haematoxylin and eosin stain using 400x magnification power.

(2008) and Scholtz *et al*. (2009). However, the latter authors indicated that clinical chemistry reference values are often not fully comparable between avian genera and species because the analyzed parameters are mostly bird species-specific. They added that the reference values provided are relevant in particular for the use of quail as laboratory animals when responses to specific treatments have to be monitored and appraised. Similar to the present results, Abdel-Fattah *et al.* (2008) found that the liver functions as measured by AST and ALT enzymes, total protein, albumin and globulin were not adversely affected by acetic, citric and lactic acids at 1.5 and 3% in broiler chick diets. On the other hand, El-Afifi *et al*. (2001) reported that the blood total protein was significantly decreased due to addition of citric acid up to 0.8% in broiler diets. Further study by El-Afifi (2003) indicated that the plasma total protein, albumin and globulin were not significantly affected by dietary acidifiers.

Obviously, acetic acid showed some stimulatory effect on the immune system of the body manifested with excess of the cellular reactions in the intestine as well as the lymphoid hyperplasia in splenic tissue. On the other hand, the adverse effects of acetic acid at 6% induced degenerative changes in the covering epithelium of the intestinal villi, as well as the hepatocytic vacuolar and fatty change. Moreover, increasing the acetic acid concentration to 6% severely affected the intestine inducing diffuse, extensive and severe fatty vacuolations in hepatic tissues. This might have been reflected in JQ productive performance fed 6% acetic acid. Spleens of the group supplemented with 3% acetic acid showed well developed lymph follicles surrounded by an excess of newly formed trabecular vessels. Spleens from birds fed 6% acetic acid showed a large number of well developed and active lymph follicles and few trabecular elements, suggesting the development of a hyperimmune defensive mechanism. The present results were similar to those reported by Gunal *et al.* (2006) who found that probiotics and/or organic acids mixtures numerically reduced *muscularis* thickness in the jejunum and ileum at 21 and 42 d of age and relative intestine weight compared to the control group. Kim and Marshall (2000) reported that acetic acid had greater anti-microbial activity than lactic or citric acids. In addition, Zyla *et al.* (2002) found that citric acid supplementation to a low phosphorus diet improved intestinal viscosity over adequate available phosphorus diet. However, El-Afifi *et al*. (2001, 2003) reported that 0.8% citric acid in broiler diets had no significant effect on small intestine thickness, or relative weights of the spleen or bursa. In the present study, 6% acetic acid significantly decreased relative weight of the liver and abdominal fat and had no effect on the plasma AST or ALT. In this regard, we can consider that AST is considered less specific indicator of liver function than other enzymes since it can be also found in many peripheral tissues (in particular the muscles) and hence has a very wide variability (Bovera *et al*., 2007). Recently, Hernandez *et al.* (2006) showed that dietary formic acid did not have a clear effect on intestinal histomorphology of broiler chicks raised under good hygienic conditions. On the other hand, Paul *et al.* (2007) reported that ammonium formate or calcium propionate significantly increased *villi* height in different segments of the small intestine compared to controls. This may be due to a reduction in the intestinal colonization by pathogenic and non-pathogenic bacteria which support the function of the small intestine.

Conclusions

The addition of 3% acetic acid may be used as a feed supplement for Japanese quail during the growing and laying period with expected improvement in productive performance.

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