ORIGINAL RESEARCH

THE EFFECT OF ORGANIC ACIDS ADDITION IN LAYING HENS DIETS ON EGG PRODUCTION, EGG QUALITY, SHELL CHARACTERISTICS AND SOME BLOOD CONSTITUENTS DURING THE LAST STAGE OF PRODUCTION

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ABSTRACT

The aging laying hens are considered uneconomical due to low egg laying percentage, lower feed efficiency and decreased egg shell calcification. Dietary administration of organic acids can alleviate these problems by reducing the pH and increasing the length and depth of villi of the gastrointestinal tract enhancing thereby the digestibility of nutrients, absorption of minerals (Ca and P) and escalation of protein utilization through increased secretion and activity of the enzyme 'Pepsin', which is beneficial for both, the environment and the production economy. Paucity of information on this aspect inspired us to take up this study. Eighty H&N Brown Nick laying hens (79-88 weeks of age) were used to assess the impact of dietary supplementation of formic acid, propionic acid and their combination on the productive performance, egg quality, shell characteristics and some blood biochemical parameters during the late stage of production. Hens were randomly distributed into 4 groups. Each group contained 10 replicates with 2 hens/ replicate. The treatments were C (Basal diet), T1 (0.5 ml formic acid / kg diet), T2 (0.5 ml propionic acid / kg diet) and T3 (0.5 ml formic acid + 0.5 ml propionic acid / kg diet).The hens were given iso-nitrogenous (17.6% CP) and iso-caloric (2800 Kcal ME/Kg) basal diet. In comparison with the control, the treatments of acids and/or their mixture revealed a highly significant increase ($P<0.001$) in hen-day egg production and daily egg mass with reduction ($P<0.001$) in the feed intake and improvement ($P<0.001$) in the feed conversion ratio. The heaviest ($P<0.001$) egg weight was observed with formic acid (T1). Shell thickness, shell weight and shell surface area were significantly increased ($P<0.05$) with organic acids compared to the control. Propionic acid recorded the highest value ($P<0.05$) of plasma globulin and total antioxidant capacity (TAC) and the lowest ($P<0.05$) albumin/globulin ratio. The addition of formic acid alone (T1) or mixed with propionic acid (T3) caused significant reduction ($P<0.05$) in plasma cholesterol, while insignificant reduction in triglycerides was observed with formic acid treatment (T1). Plasma urea was significantly decreased ($P<0.05$) by the addition of acids compared to the control, while propionic acid (T2) and mixture of formic acid and propionic acid treatments (T3) recorded the lowest ($P<0.05$) plasma creatinine. The differences among treatments regarding alanine transaminase (ALT) and aspartic transaminase (AST) were non-significant ($P>0.05$). There are a tendency to increase ($P=0.07$) the gross energy content (kcal/ kg) of the internal egg components with propionic acid (T2) and acid mixture (T3) compared to the other treatments. It is concluded that organic acids supplementation in laying hens' diet during the late stage of production had positive effects on the productivity and the related economic traits, besides bolstering immunity, that conferred protection against infectious diseases, without any adverse effects on the liver and kidney functions..

KEY WORDS

Laying hens, Egg production, Egg composition, Organic acid, Shell characteristics

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INTRODUCTION

Organic acids are short chain fatty acids (C_1-C_7) widely exist in nature as normal components in plants or animal tissues. They are also produced during the microbial fermentation of carbohydrates in the cecum of chicken (Van Immerseel et al., 2006). There are many types of organic acids, such as formic acid, propionic acid, lactic acid, citric acid, and fumaric acid etc. Each one of these acids has different mode of action and different locations or sectors.

The aging laying hens are unable to produce appropriate egg number with a good shell quality than the younger ones. The fundamental reason of this case is related to decrease in the ability of older hens in absorbing calcium (Al-Batshan et al., 1994). Likewise, the length of the intestinal villi in older layers is also decreased (Schwarzer, 2006). This causes low egg laying percentage, lower feed efficiency and decreased egg shell calcification (Schwarzer, 2006).

The quality of feed additives is mostly determined by the efficiency of egg production. Organic acids can modify the pH of the gastrointestinal tract to improve the solubility, the digestion and the absorption of nutrients (Yesilbag and Çolpan, 2006). These are useful in increasing the percentage of egg production through maintaining the thickness of egg shells.

The improvement in the absorption of Ca and P by the use of dietary organic acids lowers the pH of the intestinal content and can beneficially affect the biomechanical indicators of the bones of high-productive laying hens (Swiatkiewicz et al., 2010).

Hen-day egg production at the beginning of egg lay (24-28 weeks) and during the peak period (34-38 weeks) was increased by using organic acids in laying hens diets (Yesilbag and Çolpan, 2006). Using lactic, citric, propionic and formic acids together have improved the productivity of hens and the egg quality characteristics such as shell strength, yolk color and shell quality (Park et al., 2009).

The main objective of this experiment is to study the effects of adding formic acid, propionic acid and their mixture in laying hens diet during the late stage of egg production (79-88 weeks of age) on the egg production performance, shell characteristics and some blood parameters.

MATERIALS AND METHODS

The current experiment was carried out in south Sinai research station (Ras-Suder City) belonging to the Desert Research Center of Egypt.

The experimental design and housing of birds: Eighty H&N Brown Nick hens at 79 weeks of age were randomly divided into 4 treatment groups with 10 replicates in each group and 2 hens / replicate.

The experimental groups constituted four groups, viz., control group (C: Basal diet), T1 (0.5ml formic acid/kg diet), T2 (0.5 ml propionic acid/kg diet) and T3 (Mixture: of T1&T2: 0.5 ml formic acid and 0.5 ml propionic acid/kg diet). The concentration of formic acid was 90% and pH was 3.47. One molar solution contains 74.096 gm of propionic acid with 3.96 pH. The hens were reared in wire cages of triple deck batteries. They were exposed to 16 hr of continuous light and were reared under the same environmental, management and hygienic conditions.

The ambient temperature (ºC) and relative humidity (%) were recorded using the electronic digital thermo-hygrometer. The average of indoor ambient temperature was 28.05±0.66ºC and relative humidity was 48.05 ±1.08% during the experimental period.

The birds were given iso-nitrogenous (17.7% CP) and iso-caloric (2806 Kcal ME/Kg) diet, and free access to feed and water. The dietary formula (NRC, 1994) is presented in Table 1.

Table 1: Composition and calculated nutrients of the experimental diet.

Diet composition						Calculated nutrients		
S.N.	Ingredient	%	S.N.	Ingredient	%	S.N.	Nutrient	Value
1	Yellow corn	58.13	7	Wheat bran	3.00	1	CP (%)	17.70
2	SBM 44%	19.90	8	DLM	0.07	2	ME (Kcal/kg)	2806
3	CGM 60%	5.70	9	Salt	0.30	3	Calcium (%)	3.99
4	SFO	1.70	10	VMP*	0.30	4	Average P (%)	0.36
5	Limestone	9.50	11	Total	100	5	DLM (%)	0.39
6	DCP	1.40	---	---	---	6	L-Lysine (%)	0.76

Abbreviations: SN 2. SBM 44% (Soybean meal 44%), SN 3. CGM 60% (Corn gluten meal 60%), SN 4. SFO (Sunflower Oil), SN 6. DCP (Di-Calcium Phosphate), SN 8. DLM (DL-Methionine), SN 10. VMP (Vitamins & Minerals Premix). *VMP: Vitamins and minerals premix, each 2.5 kg contain: Vit A 10000000 IU, Vit D3 2000000 IU, Vit E 10 g, Vit K 1000mg, Vit B12 10mg, Vit B1 1000mg, Vit B2 5000mg, Vit B6 1.5g, Pantothenic acid 10g, Niacin 30g, Folic acid 1g, Biotin 50mg, Iron 30g, Manganese 70g, Choline chloride 10g, Copper 4g, Zinc 50g, Selenium 100mg and Iodine 300mg.

The measurement of bird's weights: Individual live body weights were recorded at the beginning and the end of the experimental period (79 and 88 weeks of age, respectively). Feed intake was recorded at weekly intervals.

The internal egg components analysis: The chemical composition of the internal egg components (yolk and albumen) was determined (A.O.A.C., 2005) by using 3 eggs from each treatment. Gross energy content (kcal/kg) was determined by completely combusting in a bomb calorimeter.

The quality characteristics of egg and shell: Egg mass (g/hen/day) was calculated by using egg number and egg weight which were recorded daily. Feed conversion ratio (g feed intake/ g egg mass) was calculated as the amount of feed consumed divided by egg mass.

At 84 and 88 weeks of age, six eggs were taken from each treatment for measuring egg quality characteristics, such as egg weight, egg shape index, albumen (%), yolk (%), yolk index and haugh unit and shell quality traits which included shell weight, shell (%), thickness, density, shell surface area and shell weight per unit of surface area (SWUSA). The percentage of albumen, yolk and shell was calculated as their weights relative to egg weight (Carter, 1968). Shape index was calculated (Panda, 1996) as follow:

Egg shape index = (Egg width / Egg length) x 100

Haugh unit (Haugh, 1937) was calculated as:

Haugh unit= 100 x log $(H+7.57-1.7 \times W^{0.37})$

Where: H = Albumen height, W = Egg weight.

Shell thickness (ST) was measured without membrane by using micrometer. Shell surface area (SA) and shell weight per unit surface area (SWUSA) were calculated by the following equations (Nordstrom and Qusterhout, 1982):

SA (cm^2) = $3.9782 \times EW^{0.7056}$

SWUSA (mg/cm^2) = SW (mg) / SA (cm^2)

Where, 3.9782 = constant factor, EW = egg weight (g), SW= shell weight (g).

Shell density (SD) in g/cm^3 was estimated by the following equation (Curtis et al., 1985):

SD = SW (g)/SA (cm^2) $\times$ ST (cm)

Blood biochemical profiles: At the end of the experiment, three hens were taken at random from each treatment group and sacrificed by cervical dislocation. Blood samples were immediately taken, centrifuged at 3000 rpm for 20 minutes, and then plasma stored at -20ºC for later analysis. Blood parameters included plasma total protein, albumin, cholesterol, triglycerides, urea, creatinine, alanine transaminase (ALT), aspartic transaminase (AST) and total antioxidant capacity (TAC)**.** All samples were determined colorimetrically by using Biodiagnostic kits. Globulin was calculated by subtracting albumin from total protein. Albumin/globulin ratio was also estimated.

Statistical analysis: The data was analyzed by using General Linear Model (GLM) procedures by SAS program (SAS, 2002) using simple one-way analysis of variance according to this model :$Y_{ij} = \mu + T_i + e_{ij}$,

Where, Yij = Observation, µ = General mean, Ti = Random effect of treatment (i = 1, 2, 3 and 4), eij = Random error. Statistical differences between means were also estimated (Duncan, 1955).

RESULTS AND DISCUSSIONS

Productive performance: The data of hen-day egg production (%), egg weight (g) and daily egg mass (g) during the experimental period (79-88 weeks of age) are presented in Table 2.

Table 2: Effect of formic acid (T1), propionic acid (T2) and their mixture (T3) on productive performance of laying hens during the last stage of production (79-88 weeks of age).

Item	C	T1	T2	T3	SE	Sig
Hen-day egg production (%)	61.90[c]	79.22[a]	68.19b[c]	71.38[b]	2.54	***
Egg weight (g)	60.57[b]	62.40[a]	60.48[b]	60.00[c]	0.14	***
Daily egg mass (g)	37.63[c]	49.29[a]	41.27[bc]	42.80[b]	1.54	***
Feed intake (g/hen/day)	91.96[a]	85.92[b]	87.86[b]	86.51[b]	0.81	***
Feed conversion ratio (FI/E mass)	2.83[a]	1.81[c]	2.30[b]	2.17[b]	0.10	***
Initial body weight (g)	2067.50	2085.00	2020.00	2045.00	42.68	ns
Final body weight (g)	1845.00	1727.50	1758.33	1818.42	38.86	ns

a,b,c: Means within the same row showing different letters are significantly different. SE=Stander error, Sig=Significant, ns=not significant, * = (P<0.001).**

Egg production (%) was significantly (P<0.001) increased with the addition of formic acid (T1, by 17.3%) or its mixture with propionic acid (T3, by 9.5%), while a non-significant increase was observed with the addition of propionic acid (T2, by 6.3%) compared to the control treatment. Egg mass (g) followed the same trend as the egg production (%).

The highest significant value (P<0.001) of egg weight was observed with the formic acid treatment (T1), whereas mixture treatment (T3) recorded the lowest value compared to the control. This improvement may be due to the reduction in gastric pH that accelerates the conversion of pepsinogen to pepsin and, in turn, improves the absorption rate of proteins, amino acids and minerals (Park et al., 2009). Furthermore, formic and propionic acids have broader antimicrobial activities against bacteria, fungi and yeast (Doerr et al., 1995; Partanen and Morz, 1999). Formic and propionic acids administration to the hens reduces the acidity of crop and gizzard which avoid the colonization of pathogens on the intestinal wall and thus improves the total nutrient digestibility, feed efficiency and the egg laying rate (Wang et al., 2009).

The current findings are in agreement with the reports of several authors (Gama et al., 2000; Rahman et al., 2008; Soltan, 2008; Wang et al., 2009) who indicated that egg production was significantly increased in laying hens at older age which are fed on diets supplemented with organic acids. Likewise, the productivity of laying hens was improved by adding a blend of organic acids containing formic acid and propionic acid (Griggs et al., 2005) along with citric and lactic acids (Park et al., 2009) or a blend of encapsulated organic acids in the diet (Youssef et al., 2013).

Moreover, an improvement in egg weight was observed with the dietary supplementation of organic acids (Langhout and Sus, 2005; Shalaei et al., 2014). Therefore, the blend of organic acids or their salts, including formic acid or propionic acid, have been reported to increase the egg weight and egg production (Grashorn et al., 2013) and egg mass (Soltan, 2008; Attia et al., 2013; Dahiya et al., 2016) compared to the control diet.

On the contrary, other reports have indicated that the dietary supplementation of organic acids had no significant effect on egg production (Boling et al., 2000; Kaya et al., 2014; Shalaei et al., 2014), egg mass (Boling et al., 2000), and egg weight (Gama et al., 2000; Yalcin et al., 2000; Yesilbag and Çolpan, 2006; Swiatkiewicz et al., 2010b).

Feed intake and feed conversion ratio: The results in Table 2 indicated that the supplementation of organic acids (formic acid or propionic acid) or their mixture significantly decreased ($P<0.001$) the daily feed intake with a significant improvement ($P<0.001$) in feed conversion ratio compared to the control.

This improvement in feed conversion ratio (feed intake/ egg mass) may be due to the beneficial antibacterial and acidity effect of the organic acids on the intestine and histomorphology (Loddi et al., 2004) and the increase in the villus height (Adil et al., 2010) which support the enteric health and increase the nutrient utilization for improving the growth performance of poultry.

Indeed, several reports have proved that feed conversion ratio was significantly improved with the addition of organic acids (Langhout and Sus, 2005; Rahman et al., 2008; Soltan, 2008; Attia et al., 2013) or their blend (Grashorn et al., 2013; Youssef et al., 2013) in laying hens diet. On the contrary, it has been reported that feed conversion ratio was not significantly affected by the addition of organic acids mixture (Shalaei et al., 2014) and citric acid to laying hens diet (Boling et al., 2000).

On the other hand, it is reported (Cave, 1984; Pinchasov and Jensen, 1989) that propionic acid depressed feed intake but similar results were not reported in case of lactic acid (Cave, 1984). In contrast, feed intake was not significantly affected by organic acid treatment (Yesilbag and Çolpan, 2006, Rahman et al., 2008; Soltan, 2008; Swiatkiewicz et al., 2010).

Likewise, it is also reported that formic acid, calcium-formate and buffered propionic acid did not affect the feed utilization (Izat et al., 1990). The differences in these researches results could be due to the level and the type of organic acids which needed to increase the nutrient utilization by hens. In the current study, the reduction in feed intake and the improvement of feed conversion ratio are good indicators for the beneficial effect of formic and/or propionic acid in increasing the ability of hens to utilize the nutrients.

Initial and final body weights: The results of Table 2 revealed non-significant difference among treatments regarding the initial and final body weights. These findings agree with several reports (Boling et al., 2000; Yesilbag and Çolpan, 2006; Rahman et al., 2008; Kaya et al., 2014) and disagree with others (Soltan, 2008). The difference in results could be due to the age of hens, dosage of acids, the way of the acids administration, the duration of the experiment, and the difference in environmental conditions etc.

Egg and shell quality characteristics: The addition of organic acids or their mixture did not affect egg and shell quality traits at 84 weeks of age, except for the albumen (%) that was significantly (P<0.05) increased by formic acid addition (Table 3 and Table 4).

On a long run, the organic acids or their mixture were found to be more effective on the egg and shell quality traits, where egg weight (P<0.05), yolk index, haugh unit, shell weight, shell percent, shell thickness (P<0.05), surface area (P<0.05) and SWUSA were greater at 88 weeks of age compared to the control.

Table 3: Effect of formic acid (T1), propionic acid (T2) and their mixture (T3) on egg quality traits compared to the control (C) at 84 and 88 weeks of age.

Item/ Week	C	T1	T2	T3	SE	Sig
Egg weight (g)						
84 week	60.87	61.44	60.34	58.58	1.49	ns
88 week	59.72[b]	63.83[a]	57.38[b]	58.06[b]	1.29	*
Egg shape index						
84 week	74.84	77.98	75.78	77.05	0.96	ns
88 week	74.83	77.61	75.64	76.67	1.02	ns
Yolk (%)						
84 week	32.11	28.62	33.35	31.82	1.37	ns
88 week	30.20	33.85	33.55	32.56	2.04	ns
Yolk index						
84 week	39.65	42.73	40.83	41.60	1.53	ns
88 week	38.11[b]	40.83[ab]	42.72[a]	42.08[a]	1.21	t
Albumen (%)						
84 week	53.37[ab]	55.86[a]	50.50[b]	51.00[b]	1.38	*
88 week	58.40	51.39	51.97	53.65	2.80	ns
Haugh unit						
84 week	72.10	71.65	72.13	79.25	2.75	ns
88 week	75.89[b]	77.96[b]	88.63[a]	83.55[ab]	3.25	t

a,b,c: Means within the same row showing different letters are significantly different.
SE=Stander error, Sig=Significant, ns=not significant, *= (P<0.05), t = 0.05 < P < 0.10.

Table 4: Effect of formic acid (T1), propionic acid (T2) and their mixture (T3) on egg shell quality traits compared to the control (C) at 84 and 88 weeks of age.

Item/ Week	C	T1	T2	T3	SE	Sig
Shell weight (g)						
84 week	6.52	6.90	6.98	6.20	0.42	ns
88 week	5.47[b]	6.64[a]	6.44[ab]	6.44[ab]	0.33	*
Shell (%)						
84 week	10.72	11.26	11.56	10.53	0.62	ns
88 week	9.15[b]	10.41[ab]	11.28[a]	11.08[a]	0.56	*
Shell thickness (mm)						
84 week	0.452	0.526	0.516	0.439	0.06	ns
88 week	0.338[b]	0.420[a]	0.410[a]	0.448[a]	0.02	*
Shell surface area (cm2)						
84 week	72.22	72.66	71.78	70.29	1.26	ns
88 week	71.27[b]	74.68[a]	69.27[b]	69.86[b]	1.08	*
SWUSA (mg/cm2)						
84 week	90.30	95.04	97.19	87.79	5.28	ns
88 week	76.70[b]	88.89[ab]	93.27[a]	92.11[a]	4.56	*
Shell density (g/cm3)						
84 week	0.217	0.196	0.192	0.204	0.02	ns
88 week	0.230	0.212	0.245	0.208	0.02	ns

a,b,c: Means within the same row showing different letters are significantly different. SE=Stander error, Sig=Significant, ns=not significant, * Significant (P<0.05).

The improvement in shell thickness and weight may be related to the beneficial effect of organic acids addition on the intestinal histology and the absorption rate of Ca, P and protein (Soltan, 2008) which leads to increase in the Ca and protein deposits of the shell resulting in an increase in the shell weight and shell thickness. This is considered as a good indicator of shell quality. It is known that shell resistance to breakage decreases with age in hens but using the organic acids helps in restoring it. This useful effect of organic acids may be due to the reduction in pH in the upper intestinal tract which probably causes an increase in the availability of Ca and P and the stimulation of the length of the micro villi (Swiatkiewicz et al., 2010a).

These results are in agreement with the report that found a significant effect of organic acids mixture containing propionic acid at high level on egg shell thickness (Soltan, 2008). Likewise, the mixture containing lactic acid, citric acid, propionic acid and formic acid showed better values of shell quality (Park et al., 2009). Acetic acid supplementation in the drinking water of laying hens has also been found to have a significant effect in increasing the egg weight and shell thickness (Kadim et al. 2008). It is also reported that the diets containing 0.01% or 0.15% formic acid have significantly increased shell thickness in laying hens (Abbas et al., 2013).

It has also been reported that dietary organic acids and/or their mixture had no significant effect on egg shape index, yolk index, shell weight and haugh unit (Yesilbag and Çolpan, 2006; Rahman et al., 2008; Kaya et al., 2014; Sari, 2017) in laying hens. In contrast, feeding hens on diet containing lactic acid, acetic acid or phenyllactic acid have resulted in an improvement of haugh unit score (Yalcin et al., 2000; Kadim et al., 2008; Wang et al., 2009). On the other hand, another report has indicated a slight decrease in haugh unit in hens receiving 0.05% organic acid supplementation (Gama et al., 2000).

Blood biochemical profiles: The effects of adding organic acids to hens' diet on blood metabolites are shown in Table 5 and Table 6. There was a tendency to increase ($P<0.05$) the plasma total protein by propionic acid (T2) treatment, but no difference ($P>0.05$) was observed in plasma albumin among treatments. A/G ratio was significantly ($P<0.05$) reduced due to the significant ($P<0.05$) increase in plasma globulin (Table 5).

Table 5: Effect of formic acid (T1), propionic acid (T2) and their mixture (T3) on some blood biochemical parameters in laying hens.

Item	C	T1	T2	T3	SE	Sig
Total protein (g/dl)	4.64[b]	4.68[b]	6.07[a]	4.61[b]	0.42	t
Albumin (g/dl)	2.52	2.67	2.36	2.62	0.14	ns
Globulin (g/dl)	2.12[b]	2.01[b]	3.71[a]	1.98[b]	0.37	*
Albumin/Globulin ratio	1.23[a]	1.36[a]	0.68[b]	1.32[a]	0.13	*
TAC (mM/L)	0.102[b]	0.073[b]	0.314[a]	0.060[b]	0.06	*
Cholesterol (mg/dl)	154.37[a]	92.23[b]	149.00[a]	88.50[b]	15.72	*
Triglycerides (mg/dl)	435.9	369.8	435.6	457.1	105.03	ns

a,b: Means within the same row showing different letters are significantly different. SE=Stander error, Sig=Significant, ns=not significant, *= ($P<0.05$), t = $0.05 < P < 0.10$.

Abbreviation: TAC = Total Antioxidant Capacity

It is reported that a high globulin level and low A/G ratio signify better disease resistance and immune response (El-Kerdawy, 1996). Plasma globulin is a source of antibodies (gamma globulins) and indicates to a good immunity response.

This means that the addition of propionic acid in the laying hens' diet improved hen's immunity response by increasing the production of antibodies. Propionic acid has a wide antimicrobial activity and can affect the pathogenic bacteria and fungi (Doerr et al., 1995; Partanen and Mroz, 1999) by penetrating the bacterial cell wall. It inhibits the harmful effect of bacteria and fungi and decreases their numbers in the intestine (Suryanarayana et al., 2006). An earlier report has shown that broiler chicken fed on acidified diets had better immune response as indicated by a higher serum globulin level than the control (Kamal and Ragaa, 2014).

It has also been reported that organic acids supplementation have led to a higher percentage of gamma globulin in broilers than the control (Rahmani and Speer, 2005).The same trend was observed in respect of total antioxidant capacity (TAC), which increased ($P<0.05$) due to propionic acid supplementation in the diet (Table 5). In general, the measurement of various antioxidant components in serum of the birds is neither possible nor practical. Hence the total antioxidant capacity (TAC) is the best parameter to express the antioxidant status of the birds (Erel, 2004).

Indeed, the body usually keeps a balance between the production of free radicals and antioxidants. In the current study, propionic acid supplementation in the diet may support the body to produce more antioxidants which have an essential role in protecting the body cells from the hazards of oxidation. It has been reported that the addition of organic acid in duckling diet increased the total antioxidant capacity (Elnaggar and Abo-El Maaty, 2017)

The addition of formic acid alone (T1) or mixed with propionic acid (T3) in hens diets resulted in significant ($P<0.05$) reduction in plasma cholesterol (Table 5). This may be due to the reduction in the microbial pH as a result of addition of organic acids which inhibits the action of the important microbial enzymes that forces the bacterial cell to consume energy to release the acid protons, leading to the accumulation of intracellular acid anions (Young and Foegeding, 1993).

The results are in agreement with many earlier reports (Abdo and Zeinb, 2004; Kamal and Ragaa, 2014), but were in difference with some other reports (Yesilbag and Çolpan, 2006; Sari, 2017). On the other hand, our result is similar to the proposition of the author (Yesilbag and Çolpan, 2006), who did not observe significant effect of organic acid supplementation on plasma triglycerides (Table 5).

Plasma urea and creatinine are considered as indicators of the kidney function (Perrone et al., 1992), while ALT and AST are biochemical markers for liver function and health status (Nyblom et al., 2004; Che et al., 2011). In the current study, plasma urea was significantly ($P<0.05$) decreased in the treatment groups fed on organic acids compared to the control, while the lowest significant value ($P<0.05$) of plasma creatinine were noticed with propionic acid (T2) and the mixture (T3) treatments and there was no significant difference ($P>0.05$) between formic acid treatment (T1) and the control group (Table 6).

It is known that the reduction in blood urea and creatinine by organic acids supplementation is considered as indicators for better amino acids digestibility and protein utilization, where uric acid is the major end product of protein metabolism in poultry. Similar findings were also reported in quails (Hayat et al., 2014) and duckling (Elnaggar and Abo-El Maaty, 2017).

Table 6: Effect of formic acid (T1), propionic acid (T2) and their mixture (T3) on kidney and liver functions.

Item	C	T1	T2	T3	SE	Sig
Urea (mg/dl)	17.36[a]	10.81[b]	8.84[b]	9.06[b]	1.91	*
Creatinine (mg/dl)	0.409[ab]	0.533[a]	0.284[b]	0.267[b]	0.05	*
ALT (IU/L)	183.61	182.02	192.69	187.35	3.56	ns
AST (IU/L)	62.81	62.59	61.92	61.36	0.65	ns

a,b: Means within the same row showing different letters are significantly different. SE=Stander error, Sig=Significant, ns=not significant, *= (P<0.05).

Our study did not reveal significant differences among treatment groups in respect of ALT and AST (Table 6). It is in agreement with many reports in laying hens (Kaya et al.; 2014; Sari, 2017), broilers (Abdel-Fattah et al., 2008) and Japanese quail (Attia et al., 2013).

The internal egg components and chemical composition: The addition of organic acid did not affect the chemical composition content of the internal egg components (Table 7), except for the gross energy content that tended to be increased with the addition of propionic acid (T2) and the blend of propionic cid and formic acid (T3).

Table 7: Effect of formic acid (T1), propionic acid (T2) and their mixture (T3) on the chemical composition of the internal egg components (yolk and albumen) on dry matter basis (%)during the last stage of egg production (79-88 weeks of age).

Item	C	T1	T2	T3	SE	Sig
DM	26.10	25.57	25.73	25.17	0.62	ns
OM	95.97	96.50	97.20	97.83	0.42	ns
Protein	46.03	47.99	49.97	45.40	2.62	ns
Fat	36.30	34.36	34.35	35.50	0.96	ns
GE (kcal/kg)	6810[ab]	6997[b]	6992[a]	7015[a]	81.6	t

a,b: Means within the same row showing different letters are significantly different. SE=Stander error, Sig=Significant, ns=not significant, t = 0.05 < P < 0.10

DM= dry matter, OM= organic matter

This increase may be attributed to the short chain fatty acids supplementation (especially propionic acid) that is considered as fuels for the tissues of the host (Rémésy et al., 1992) and contribute to shaping the gut environment and can be used as energy sources by the host cells and the intestinal bacteria (Rios-covián et al., 2016).

Short chain fatty acids have high gross energy values (Freitag, 2007) and are used in different metabolic processes for energy generation including the production of ATP in the citric cycle (Diebold and Eidelshurger, 2006). Propionic acid contains almost 17.78 MJ/ kg, while formic acid contains 11.34 MJ/ kg as a net metabolizable energy (Papatsiros et al., 2012) which approximately equal 4248 kcal/ kg and 2709 kcal/ kg, respectively.

Protein (%) was improved by 1.96% (T1) and 3.94% (T2) than the other treatments. This may be due to improvement in the absorption rate of proteins and amino acids by dietary organic acids (Park et al., 2009). The decline in fat (%) was 1.94 (T1), 1.95 (T2) and 0.8% (T3) versus the control. This decline is considered as an indicator of low yolk cholesterol (Slaugh, 2002; Aghaii et al., 2010) and compatible with the reduction of plasma cholesterol in the current study. Almost, there are no any published reports about the effect of organic acids on the chemical composition of the internal egg components.

CONCLUSION

These results proved that adding formic acid and propionic acid individually or together in laying hens diets at the late stage of egg production was very useful for increasing egg production and improving feed conversion ratio. This improvement means more profit for the egg producers during the last stage of production. In addition, these feed additives do not have negative effects on the general health status of hens or the nutritive value of the produced eggs.

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UNDERTAKING

It is certified that the research paper '**THE EFFECT OF ORGANIC ACIDS ADDITION IN LAYING HENS DIETS ON EGG PRODUCTION, EGG QUALITY, SHELL CHARACTERISTICS AND SOME BLOOD CONSTITUENTS DURING THE LAST STAGE OF PRODUCTION'** is an original research work carried out by the author in Animal and Poultry Nutrition Department, Desert Research Center, El-Mataria, Cairo, Egypt. It has neither been published nor contemplated for publication elsewhere.

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