INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

EVALUATION OF DIFFERENT EGG QUALITY PARAMETERS FOR THE EFFECT OF DIETARY SUPPLEMENTATION OF SALTS OF ORGANIC ACIDS MIXTURE ON LAYING HENS

[a]Dahiya, R., [b]Berwal, R.S., [c]Lalit, [b]Sihag, S., [d]Dalal D.S. and [e*]Patil, C.S.
Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004
[1]M.V.Sc., [2]Professor, Department of Animal Nutrition, [3]P.hD. Scholar, [4]Professor, [5]Assistant Professor Department of Animal Genetics and Breeding, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana.
Corresponding author email: dr.cspatil03@gmail.com

ABSTRACT
The present study was carried out to determine the effect of dietary organic acids mixture supplementation on egg quality parameters in laying hens. A total 24 week old 140 white leghorn laying hens which were randomly distributed to seven dietary treatment groups, each containing 20 hens. The hens were fed (18% CP% and 2697 Kcal KgG[1] ME) i.e. Supplemented with T_1 (0 % control), T_2 (0.5% sodium-butyrate), T_3 (1.0% sodium-butyrate), T_4 (1.5% sodium-butyrate), T_5 (0.5% calcium-propionate), T_6 (1.0% calcium-propionate) and T_7 (1.5% calcium-propionate). The results of the study depicted that egg weight was significantly ($P<0.05$) increased at 0.5% level of salts of organic acids in the diet. Different levels of salts of organic acids had significant ($P<0.05$) effect on egg quality i.e. shell thickness, shape index and Haugh unit but did not significantly affect the albumen index and yolk index. The egg length and egg width were not significantly affected by supplementation of salts of organic acids in the diets of layers. Basal diet supplemented with salts of organic acids had significant ($P<0.05$) positive effect on shape index per cent and shell thickness (mm) over basal diet. The overall mean values of yolk index per cent ranged between 42.30 (T_3) to 43.16 (T_1). Cholesterol and low density lipoprotein (LDL) were significantly ($P<0.05$) reduced in supplemented group as compared to un-supplemented group and high density lipoprotein (HDL) was significantly ($P<0.05$) increased by dietary supplementation of salts of organic acids. The results of the present investigation concluded that egg quality was more profitable at 0.5% level of sodium-butyrate and 0.5% level of calcium-propionate which reduced the feed cost without affecting the egg quality.

KEYWORDS: Calcium-propionate, dietary organic acids, dietary treatments, egg quality, laying hens and sodium butyrate.

INTRODUCTION
India is emerging as the world's 2[nd] largest poultry market with an annual growth of more than 14%, producing 61 million tones or 3.6% of the global egg production and annual growth rate of egg production is 5-8% (4[th] international poultry and livestock expo, 2015). Antibiotics have been widely used in poultry production for decades to improve growth rate and feed conversion efficiency, however, their use as growth promoters in the poultry industry has been intensively controversial because of the development of bacterial resistance and potential consequences on the human health (Ratchliff, 2000). In response to this apparent threat, the European commission (EC) decided to phase out, and ultimately ban (January 1[st] 2006), the marketing and use of antibiotics as growth promoters in feed (EC Regulation No. 1831/2003). Organic acids and their salts are generally regarded as safe and have been approved by most member states of European union (EU) to be used as feed additives in the animal production (EFSA, 2011). The advantage of salts over acids is that they are generally odourless and easier to handle in the feed manufacturing process owing to their solid and less volatile form (Huyghebaert *et al.,* 2011). Organic acids can serve as a meaningful tool to controlling all enteric non-pathogenic and pathogenic especially acid-intolerant bacteria like *Escherichia coli, salmonella* and *campylobacter* species (Wolfenden *et al*., 2007). Non-antibiotic alternatives to antibiotic growth promoters have been proposed for use in animal diets due to concerns about the safety in both animals and humans. Eggshell quality is one of the most important issues in the poultry industry, influencing the economic profitability of egg production and hatchability. High breaking strength of eggshell and absence of shell defects are essential for protection against the penetration of pathogenic bacteria such as *salmonella sp.* into eggs (Swiatkiewicz *et al*., 2010). It affects the hatchability, regulates the gaseous exchange during incubation and affects the number of hatching eggs to be set in incubation. One of the main concerns is a decrease in eggshell quality as the hen ages, due to an increase in egg weight without an increase in the amount of calcium carbonate deposited on the eggshell. For this reason, the incidence of cracked eggs could even exceed 20% at the end of the laying period (Nys, 2001). Supplying the hen with an optimal Ca intake is the crucial in order to ensure the proper calcification of the eggshell, but increasing the Ca level in the diet to above 3.6-3.8% usually has no beneficial effect on eggshell quality. Organic acids salts are beneficial to increase uptake of minerals from gut to improve eggshell quality (Soltan, M.A., 2008 and Swiatkiewicz *et al.,* 2010). The aim of this study was to investigate the effects of organic acid

mixture at different levels of supplementation in the diet of laying hens on egg quality parameters of laying hens.

MATERIALS & METHODS

All the experimental procedures have been conducted in accordance with the guidelines laid down by the Institutional Ethics Committee. The investigation was conducted at poultry farm, Department of Animal Genetics and Breeding, College of Veterinary sciences, LUVAS, Hisar for the year 2016. For this study one hundred and forty single comb white leghorn laying hens at 24 weeks of age were randomly distributed to seven dietary treatment groups *i.e.* T_1 (control), T_2 (0.5% sodium-butyrate), T_3 (1.0% sodium-butyrate), T_4 (1.5% sodium-butyrate), T_5 (0.5% calcium-propionate), T_6 (1.0% calcium-propionate) and T_7 (1.5% calcium-propionate), consisting of five replications of four birds each in each treatment. Based upon the proximate composition and metabolizable energy of feed ingredients the layer's control ration having maize grain as energy source was formulated as per BIS (2007). All the diets were analysed for proximate principles (AOAC, 2007) and were randomly divided into 7 groups in Completely Randomized Design (CRD). The hens were housed individually in cages. All the diets were prepared to be isocaloric and nitrogenous. They were reared under identical conditions of environment and management of light, water, disease control etc. Feed and water were supplied ad lib. The different dietary treatments were, as given below:- T_1, Basal diet (Control) as per BIS, 2007 Standard; T_2, Basal diet + Sodium butyrate @ 0.5%; T_3, Basal diet + Sodium butyrate @ 1.0%; T_4, Basal diet + Sodium butyrate @ 1.5%; T_5, Basal diet + Calcium propionate @ 0.5%; T_6, Basal diet + Calcium propionate @ 1.0% and T_7, Basal diet + Calcium propionate @ 1.5%. Feed additives and supplements were premixed and then mixed with weighed quantity of feed ingredients to make a homogenous mixture of rations. The cost of different experimental diets T_1 (control), T_2, T_3, T_4, T_5, T_6 and T_7 were Rs. 22.23, 22.68, 23.13, 23.58, 22.65, 23.08 and 23.50/kg, respectively.

TABLE 1: The ingredients and chemical composition of control diet

Ingredient composition	
Ingredients	(Kg/100kg feed)
Maize	50
Soybean meal	13
Groundnut cake	7
DORP	12
Rice Polish	5
Fish Meal	6
Mineral Mixture	3
Salt	1
Shell Grit	3
Total	100

Feed additives included Spectromix-10g and Spectromix-BE-10g per 100kg feed

The study was undertaken from 24 to 40 weeks of age of layers in first phase of production cycle. The entire duration of study was divided into eight periods of 14 days each. After every two weeks, 35 eggs were collected randomly, one from each replication of each treatment to estimate egg quality parameters. The egg shell was broken at the middle portion with the help of blunt end of knife. The egg contents were poured on egg breaking glass stand. Following egg quality parameters were determined.

Egg quality parameters

Egg length and egg weight measured by the length of each egg was taken by using Vernier calliper. Shape index was calculated maximum width of egg divided by maximum length of egg. Shell thickness (mm) was measured by using Screw gauge. For this purpose, membrane removed pieces of shell were collected from three places, the average shell thickness was taken as the final reading. The albumen index was measured by average height of albumen divided by average width of albumen. Maximum length and the maximum width of thick albumen were measured with the help of Vernier calliper. The height of thick albumen was taken between the yolk and the outer border of thick albumen avoiding the chalaza. Albumen height was measured with the help of tripod spherometer with a least count of 0.001 mm after adjusting for the zero error on the plain glass plate. Yolk index is measured by the height of the yolk with the help of tripod spherometer and width by Vernier calliper and it was calculated by average height of yolk divided by average width of yolk and Haugh unit is the product of log of albumen height and egg weight and it was calculated by using the following formula (Haugh, 1937):

Haugh unit= $100 \log (H + 7.57 - 1.7W^{0.37})$. Where, H is Albumen height and W is Egg weight.

Cholesterol, HDL and LDL in egg yolk of layers

Cholesterol, HDL and LDL level in the egg yolk was estimated at the 38th weeks of age. Total lipids from sample were extracted according to the method of Angelo et al. (1987). Cholesterol value of extracted fat from egg yolk was estimated by using commercially available "ERBA Kit" in ERBA-EM-200 automatic analyzer (Erba Manheim, Germany).

Statistical analysis

The statistical analysis of data was performed using SPSS 21.0 version of Microsoft (SPSS, 2001). One way ANOVA was used for the differences between groups. When the p values were significant ($p<0.05$), a Duncan's multiple range test was performed (Duncan, D.B., 1995). All the data were expressed as mean ± standard errors.

RESULTS & DISCUSSION

Based upon the proximate composition and metabolizable energy of feed ingredients the layers' control ration was formulated as per BIS (2007) Standard. The ingredients and chemical composition of diet fed to layers in control group (T_1) is presented in table 1. The contents of crude protein, crude fiber, ether extract, nitrogen-free extract, and organic matter of basal diet (T1) were 18.04%, 4.34%, 3.61%, 66.21% and 92.20%, respectively. The calculated value of ME was 2697.17 kcal/kg feed.

The data in respect of the traits of egg quality *viz*., egg weight, shell thickness, egg length, egg width, shape index, albumen index, yolk index and Haugh unit at the end of each period (Biweekly) 26th, 28th, 30th, 32nd, 34th, 36th, 38th and 40th weeks of age of laying hens under different dietary treatments are given in tables 2, 3 and 4, respectively. Mean values of cholesterol, HDL and LDL in egg yolk of layers under different dietary treatments was depicted in table 5.

Egg weight

The cumulative values of egg weights (g) ranged from 51.64 (T_4) to 54.67 (T_5) (Table 3). The results of the study unveiled that egg weight (g) was significantly (P<0.05) higher in T_2 and T_5 groups as compared to T_1, T_3, T_4, T_6 and T_7 groups, indicating that egg weight was significantly (P<0.05) increased at 0.5% level of supplementation of salts of organic acids in the diet of layers. This improvement in egg weight at 0.5% level might be due to lower per cent hen day egg production at 0.5% level which, consequently increased the weight of eggs because these two traits are negatively correlated (Tomar, 2014). Comparable results were found by Kadim *et al.* (2008) who observed a significant (P<0.05) improvement in egg weight by supplementing the diets with various levels (200, 400 and 600 ppm) of acetic acid in laying hens between 30-40 weeks of age and it might be due to potentially ameliorating effect on some stressors, which allowed for an improvement in the weight of eggs. Moreover, Grashorn *et al.* (2012) and Youssef *et al.* (2013) who depicted that egg weight was significantly (P<0.05) improved by different dietary treatments as compared to control group (basal diet). In contrary to these findings, Rahman *et al.* (2008), Wang *et al.* (2009) and Bonos *et al.* (2011) reported that supplementation of salts of organic acids had no effect on average egg weight.

TABLE 2. Egg weight, Shell thickness and Egg lenght under different dietary treatments in laying hens

Parameters	Treatment						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Week	Egg weight (g)						
24-26	54.77^{b}±0.96	55.68^{c}±0.41	53.18^{ab}±0.48	52.50^{a}±0.52	56.18^{c}±0.87	54.38^{b}±0.53	53.56^{ab}±0.64
26-28	53.81^{bc}±0.61	54.43^{c}±0.44	52.25^{ab}±0.33	51.69^{a}±0.93	54.91^{c}±0.44	53.48^{bc}±0.65	53.05^{b}±0.65
28-30	52.93^{bc}±0.59	53.61^{c}±0.68	51.48^{a}±0.56	50.97^{a}±0.38	53.78^{c}±0.59	52.77^{bc}±0.65	52.21^{b}±0.64
30-32	52.24^{bc}±0.83	52.91^{c}±0.75	50.68^{a}±0.43	50.10^{a}±0.70	53.35^{c}±0.93	52.04^{b}±0.90	51.51^{ab}±0.48
32-34	51.77^{ab}±0.50	52.45^{b}±0.67	50.28^{a}±1.13	51.67^{ab}±0.74	53.11^{c}±0.96	51.72^{ab}±0.60	51.27^{ab}±0.82
34-36	50.83^{ab}±0.53	51.71^{bc}±0.72	49.65^{a}±1.17	49.13^{a}±0.73	52.21^{c}±0.86	51.02^{b}±0.63	50.53^{ab}±0.99
36-38	54.34^{ab}±0.63	56.51^{c}±0.70	53.78^{a}±1.15	53.30^{a}±0.62	56.87^{c}±0.87	54.68^{b}±0.64	54.16^{ab}±0.82
38-40	55.57^{b}±0.63	57.35^{c}±1.01	54.28^{a}±1.27	53.80^{a}±0.76	57.71^{c}±1.07	55.38^{b}±0.71	54.96^{ab}±0.62
MEAN	53.28^{b}±0.66	54.33^{c}±0.68	51.94^{a}±0.81	51.64^{a}±0.67	54.76^{c}±0.82	53.19^{b}±0.66	52.65^{ab}±0.70
	Shell thickness (mm)						
24-26	0.282±0.007	0.288±0.007	0.293±0.006	0.290±0.008	0.287±0.006	0.291±0.007	0.289±0.008
26-28	0.288±0.008	0.292±0.007	0.304±0.006	0.301±0.006	0.291±0.007	0.300±0.005	0.299±0.008
28-30	0.290^{a}±0.009	0.308^{b}±0.005	0.329^{c}±0.006	0.322^{c}±0.008	0.306^{b}±0.007	0.324^{c}±0.008	0.319^{c}±0.007
30-32	0.304^{a}±0.006	0.314^{ab}±0.008	0.338^{b}±0.006	0.332^{b}±0.005	0.313^{ab}±0.008	0.333^{b}±0.007	0.328^{b}±0.003
32-34	0.312^{a}±0.006	0.322^{a}±0.006	0.345^{b}±0.010	0.340^{b}±0.007	0.315^{a}±0.007	0.340^{b}±0.009	0.336^{b}±0.006
34-36	0.317^{a}±0.003	0.328^{a}±0.008	0.352^{b}±0.005	0.346^{b}±0.005	0.321^{a}±0.008	0.349^{b}±0.008	0.340^{b}±0.008
36-38	0.319^{a}±0.003	0.328^{a}±0.002	0.358^{b}±0.004	0.354^{b}±0.005	0.322^{a}±0.006	0.352^{b}±0.004	0.348^{b}±0.004
38-40	0.322^{a}±0.005	0.332^{a}±0.005	0.365^{b}±0.007	0.360^{b}±0.008	0.331^{a}±0.004	0.359^{b}±0.003	0.356^{b}±0.002
MEAN	0.304^{a}±0.005	0.314^{a}±0.004	0.335^{b}±0.006	0.330^{b}±0.004	0.311^{a}±0.005	0.331^{b}±0.006	0.326^{b}±0.004
	Egg length (cm)						
24-26	5.82^{b}±0.10	5.70^{b}±0.05	5.34^{a}±0.06	5.58^{b}±0.04	5.34^{a}±0.08	5.58^{b}±0.05	5.72^{b}±0.11
26-28	5.84±0.04	5.54±0.06	5.62±0.04	5.58±0.07	5.64±0.07	5.60±0.10	5.70±0.13
28-30	5.62±0.08	5.58±0.05	5.60±0.08	5.60±0.05	5.54±0.08	5.54±0.08	5.68±0.05
30-32	5.58 ±0.05	5.60±0.05	5.60±0.09	5.64±0.06	5.68±0.05	5.56±0.05	5.62±0.03
32-34	5.58^{a}±0.04	5.78^{b}±0.10	5.90^{b}±0.16	5.54^{a}±0.07	5.42^{a}±0.08	5.56^{a}±0.02	5.48^{a}±0.07
34-36	5.80±0.10	5.66±0.06	5.86±0.09	5.62±0.03	5.60±0.03	5.62±0.10	5.72±0.08
36-38	5.82^{ab}±0.06	5.76^{a}±0.04	5.66^{a}±0.05	5.72^{a}±0.03	5.76^{a}±0.10	5.66^{a}±0.08	5.94^{b}±0.07
38-40	5.74^{b}±0.12	5.78^{b}±0.07	5.50^{a}±0.06	5.68^{b}±0.05	5.68^{b}±0.05	5.66^{b}±0.08	5.64^{b}±0.05
MEAN	5.72±0.07	5.67±0.05	5.63±0.04	5.62±0.05	5.58±0.06	5.59±0.07	5.68±0.08

The mean values within same column with different superscripts differ significantly (P< 0.05).

Shell thickness

The cumulative values of shell thickness (mm) ranged from 0.304 (T_1, control) to 0.335 (T_3, 1.0% sodium-butyrate). The results of the present investigation depicted that shell thickness was significantly (P<0.05) improved by supplementing the diets with salts of organic acids in the layers and it might be due to increased mineral and protein absorption which reflected on the increased calcium and protein deposition of the shell (Soltan, 2008). In the present study, it was observed that there was increased metabolizability and retention of nutrients which might be the reason of increased shell thickness. Similar results were also reported by Wang *et al.* (2009), Youssef *et al.* (2013) and Kaya *et al.* (2013) who found a significant (P<0.05) increase in shell thickness by supplementing the diets with prebiotics and organic acids in the layers. By contrast, Swiatkiewicz *et al.* (2010) observed no significant effect on shell thickness among treatment groups. Moreover, Yesilbag and Colpan (2006) observed that shell thickness was not significantly improved by supplementing the basal diet with organic acid mixture in laying hens and it might be attributed to organic acid mixture used in the study.

Egg length and Egg width

The results of the study also depicted that egg length and egg width were not significantly affected by the treatments. These results of present study are in resemblance with results of Kaya *et al.* (2013) and Attia *et al.* (2013) who reported that supplementation of salts of organic acids had no effect on egg length and egg width. By contrast to these findings, Kadim *et al.* (2008) observed that egg length and egg width of supplemented treatment groups were higher than control grouped.

TABLE 3. Egg width, Shape index and Albumin index under different dietary treatments in laying hens

Paramet	Treatment						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Week	Egg width (cm)						
24-26	4.18±0.04	4.24±0.07	4.08±0.03	4.10±0.03	4.12±0.03	4.12±0.04	4.12±0.05
26-28	4.14±0.04	4.10±0.04	4.14±0.05	4.12±0.03	4.14±0.02	4.06±0.04	4.14±0.06
28-30	4.14±0.05	4.16±0.02	4.18±0.04	4.16±0.03	4.12±0.03	4.10±0.04	4.06±0.04
30-32	4.12 ±0.05	4.14±0.06	4.18±0.03	4.24±0.05	4.18±0.08	4.22±0.03	4.26±0.05
32-34	4.12^a±0.05	4.16^a±0.04	4.32^b±0.05	4.20^a±0.04	4.06^a±0.02	4.16^a±0.05	4.08^a±0.05
34-36	4.12^a±0.05	4.20^a±0.06	4.34^b±0.06	4.24^a±0.06	4.18^a±0.03	4.20^a±0.04	4.26^a±0.05
36-38	4.22±0.08	4.14±0.02	4.26±0.05	4.26±0.02	4.24±0.02	4.26±0.05	4.34±0.05
38-40	4.22±0.06	4.14±0.07	4.16±0.05	4.20±0.07	4.18±0.08	4.24±0.05	4.28±0.08
MEAN	4.15±0.05	4.16±0.06	4.21±0.07	4.19±0.06	4.15±0.04	4.17±0.03	4.19±0.04
	Shape index (%)						
24-26	71.82^a±1.05	73.38^b±1.74	76.40^{bc}±0.22	73.47^b±0.83	77.15^c±0.77	73.83^b±1.2	72.02^a±1.82
26-28	70.89^a±0.81	74.00^c±0.75	73.66^c±0.93	73.83^c±1.27	73.40^c±1.19	72.50^b±1.2	72.63^b±1.82
28-30	73.66^b±0.79	74.55^c±0.75	74.64^c±0.52	74.28^c±1.46	74.36^c±1.34	74.00^{bc}±1.7	71.47^a±0.75
30-32	73.83^a±0.72	73.92^a±0.76	74.64^b±0.76	75.17^b±1.61	73.59^a±1.19	75.89^c±1.6	75.80^c±1.67
32-34	73.83^b±0.50	71.97^a±1.40	73.22^b±2.63	75.81^c±0.97	74.90^{bc}±0.88	74.82^{bc}±0.8	74.45^{bc}±0.44
34-36	71.03^a±0.89	74.20^b±0.87	74.06^b±1.55	74.44^b±1.14	75.00^c±0.65	74.73^{bc}±1.4	74.47^b±0.60
36-38	72.50^{ab}±1.19	71.87^a±0.84	75.26^c±0.40	74.47^c±0.56	73.61^b±1.45	75.26^c±0.9	73.06^b±0.84
38-40	73.51^b±1.04	71.62^a±1.40	75.63^d±1.68	73.94^b±0.93	73.59^b±1.43	74.91^c±1.1	72.88^{ab}±0.72
MEAN	72.63^a±0.87	73.18^b±0.79	74.68^c±0.72	74.42^c±0.84	74.45^c±0.74	74.49^c±0.5	73.34^b±0.89
	Albumen index (%)						
24-26	6.95^b±0.06	6.72^a±0.05	6.95^b±0.07	6.93^b±0.06	6.81^{ab}±0.05	6.91^b±0.03	6.68^a±0.05
26-28	6.92±0.12	6.97±0.14	7.04±0.11	7.00±0.16	6.88±0.13	6.98±0.14	6.92±0.13
28-30	7.02±0.07	7.06±0.07	7.16±0.11	7.11±0.09	6.95±0.08	7.08±0.09	7.03±0.09
30-32	7.04±0.16	7.11±0.18	7.25±0.16	7.18±0.18	6.98±0.16	7.13±0.15	7.05±0.16
32-34	7.12±0.16	7.23±0.13	7.34±0.13	7.29±0.16	7.04±0.14	7.22±0.16	7.16±0.11
34-36	7.08^a±0.17	6.90^a±0.11	7.42^b±0.14	7.25^b±0.15	7.00^a±0.15	7.15^{ab}±0.14	7.02^a±0.11
36-38	7.11^a±0.08	7.23^{ab}±0.05	7.65^b±0.08	7.39^b±0.06	7.06^a±0.07	7.29^{ab}±0.07	7.15^a±0.06
38-40	7.02^a±0.10	7.18^{ab}±0.08	7.52^b±0.11	7.32^b±0.09	6.92^a±0.11	7.28^b±0.10	7.11^{ab}±0.09
MEAN	7.03±0.11	7.05±0.10	7.29±0.11	7.18±0.12	6.95±0.11	7.13±0.11	7.01±0.10

The mean values within same column with different superscripts differ significantly (P< 0.05).

Shape index

The results of the study also depicted that shape index per cent was significantly (P<0.05) improved among different dietary treatments as compared to control group. This finding is in contrary with Soltan (2008) and Attia *et al.* (2013) who revealed that supplementation of salts of organic acids had no effect on shape index. Moreover, Bonos *et al.* (2011) depicted that shape index was significantly (P<0.05) decreased in supplemented group as compared to control group.

Albumen and yolk index

The albumen index per cent and yolk index per cent were not significantly affected by supplementation of salts of organic acids in the diets of layers. These results are in resemblance with Kaya *et al.* (2013) and Youssef *et al.* (2013) who found a non-significant effect on albumen index and yolk index among different dietary treatments as compared to control group. By contrast to these findings, Rahman *et al*. (2008) and Soltan (2008) showed a slight deterioration of albumen index in layers with different inclusion levels of organic acids mixture in the basal diet which might be attributed due to increased albumen per cent on organic acids mixture supplementation and older age of laying hen might be responsible for decreasing albumen index per cent.

Haugh unit

The cumulative values of Haugh unit ranged between 79.92 (T_2, 0.5% Sodium-butyrate) to 82.78 (T_4, 1.5% Sodium-butyrate). Haugh unit was significantly (P<0.05) improved by supplementing the basal diet with 1.0% and 1.5% levels of salts of organic acids in the layers and this improvement might be due to reduction in the weight of eggs at the above said levels of salts of organic acids in the diets of laying hens. The results are in consistent with Attia *et al.* (2013) who found an improvement in Haugh unit of groups fed diets supplemented with different concentrations (1.5, 3.0 and 6 %) of acetic acid which might be due to decrease bacterial contamination of eggs resulting in improved keeping quality of eggs. Moreover, Kadim *et al.* (2008), Wang *et al.* (2009), Bonos *et al.* (2011) who reported that Haugh unit was significantly (P<0.05) improved by different dietary treatments as compared to control group. By contrast, Rahman *et al.* (2008) and Youssef *et al.* (2013) depicted that Haugh unit was not significantly affected by different dietary treatments as compared to control group. In nutshell, the egg quality was improved by supplementation of salts of organic acids in the ration of laying hens.

TABLE 4. Yolk index and Haugh unit under different dietary treatments in laying hens

Parameters	Treatment						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Week	Yolk index (%)						
24-26	42.58±0.14	42.32±0.16	42.11±0.15	42.18±0.16	42.40±0.16	42.28±0.15	42.40±0.15
26-28	42.75^{ab}±0.17	42.98^{b}±0.18	42.25^{a}±0.16	42.54^{ab}±0.17	43.54^{c}±0.12	42.68^{ab}±0.15	42.62^{a}±0.17
28-30	42.95±0.17	42.76±0.19	42.45±0.16	42.68±0.19	42.84±0.17	42.76±0.17	42.82±0.18
30-32	43.68±0.04	43.06±0.05	42.64±0.07	42.88±0.05	43.28±0.04	43.08±0.04	43.32±0.05
32-34	44.32±0.12	43.72±0.11	42.88±0.09	43.48±0.10	43.94±0.13	43.70±0.13	43.92±0.10
34-36	45.18^{b}±0.09	44.98^{b}±0.07	43.40^{a}±0.13	43.96^{ab}±0.34	45.68^{c}±0.07	44.54^{ab}±0.07	44.75^{ab}±0.11
36-38	42.95±0.08	42.28±0.21	41.85±0.13	42.04±0.25	42.68±0.11	42.48±0.08	42.54±0.10
38-40	41.72^{ab}±0.14	41.63^{ab}±0.15	40.66^{a}±0.18	41.11^{ab}±0.15	42.28^{b}±0.13	41.40^{ab}±0.14	41.36^{ab}±0.16
MEAN	43.16±0.12	42.76±0.14	42.30±0.13	42.57±0.17	42.98±0.12	42.82±0.11	42.86±0.12
	Haugh unit						
24-26	76.41^{a}±1.08	76.28^{a}±0.38	76.68^{ab}±0.39	76.95^{b}±0.34	76.13^{a}±0.67	76.54^{a}±0.66	76.78^{ab}±0.90
26-28	77.64^{a}±0.59	77.48^{a}±0.88	78.48^{b}±0.34	78.95^{b}±0.71	77.42^{a}±0.41	78.28^{b}±0.44	78.68^{b}±0.62
28-30	78.34^{a}±0.41	78.24^{a}±0.10	79.54^{b}±0.68	80.02^{b}±0.11	78.18^{a}±0.89	79.32^{b}±0.59	79.68^{b}±0.97
30-32	79.48^{a}±0.41	79.38^{a}±0.29	80.68^{b}±0.90	81.28^{b}±0.42	79.26^{a}±0.43	80.38^{b}±0.45	80.54^{b}±0.33
32-34	81.42^{a}±0.75	81.24^{a}±1.36	83.48^{b}±0.64	83.95^{b}±0.79	81.02^{a}±0.10	83.08^{b}±0.64	83.54^{b}±0.35
34-36	84.68^{a}±0.71	84.28^{a}±0.61	85.64^{b}±0.37	86.52^{c}±0.90	84.04^{a}±0.37	85.10^{b}±0.90	86.17^{c}±0.67
36-38	82.75±0.34	82.68±0.28	82.95±0.69	83.24±0.84	82.58±0.65	82.85±0.63	82.98±0.34
38-40	81.14±0.65	80.98±0.89	81.28±0.62	81.64±0.88	80.84±0.35	81.04±0.65	81.17±0.34
MEAN	80.13^{a}±0.62	79.92^{a}±0.54	81.68^{bc}±0.48	82.78^{c}±0.39	79.74^{a}±0.59	81.02^{b}±0.64	82.26^{c}±0.55

The mean values within same column with different superscripts differ significantly ($P< 0.05$).

Cholesterol, HDL and LDL in egg yolk of layers under different dietary treatments

The mean values of total cholesterol; HDL and LDL in egg yolk ranged between 12.38 (T_3) to 13.42 (T_1), 5.62 (T_1) to 6.84 (T_6) and 5.48 (T_6) to 7.24 (T_1) mg/g egg yolk, respectively. The results of the study depicted that egg yolk concentration of total cholesterol and LDL were significantly ($P<0.05$) reduced by supplementing the diets with 1.0% and 1.5% levels of salts of organic acids in their different dietary combinations and HDL value was significantly ($P<0.05$) higher in laying hens fed diets supplemented with 1.0% and 1.5% than hens fed control diet. This reduction in total cholesterol and LDL might be due to organic acid acidification (Kamal *et al.*, 2014). In the present study, the reduction in cholesterol level might be due to reduction in concentration of cholesterol in serum. In contrary to these observations, Singh (2012) found a non-significant effect on concentration of total cholesterol; LDL and HDL in egg yolk. Millet *et al.* (2006) observed that average value of total cholesterol is 14.11 mg/g egg yolk in laying hens fed standard diets.

TABLE 5: Mean values of cholesterol, HDL and LDL in egg yolk of layers under different dietary treatments

Treatments	Cholesterol (mg/g egg yolk)	HDL (mg/g egg yolk)	LDL (mg/g egg yolk)
T_1	13.42^{b} ±0.049	5.62^{a}±0.020	7.24^{b}±0.030
T_2	13.04^{b}±0.043	5.85^{a}±0.020	6.98^{b}±0.020
T_3	12.38^{a}±0.030	6.52^{b}±0.010	5.65^{a}±0.020
T_4	12.52^{a}±0.026	6.38^{b}±0.010	5.82^{a}±0.010
T_5	13.15^{b}±0.041	5.96^{a}±0.020	6.84^{b}±0.020
T_6	12.48^{a}±0.018	6.84^{b}±0.010	5.48^{a}±0.010
T_7	12.68^{a}±0.034	6.62^{b}±0.010	5.73^{a}±0.020

The mean values within same column with different superscripts differ significantly ($P< 0.05$).

CONCLUSIONS

From the obtained results it can be concluded that supplementation of salts of organic acid increases egg weight at 0.5% level, improve shape index per cent and shell thickness (mm) over basal diet and haugh unit at the level of 1.0 and 1.5% in the ration of layers during the period of 24 to 40 weeks of age. Improvement in shell quality parameters may be attributed to the beneficial effect of organic acids on Ca and P absorption. Cholesterol and LDL were reduced and HDL was increased by dietary supplementation of salts of organic acids which may be due to the acidification effect of organic acids. The differential effect of inclusion of salts of organic acids in laying hen diets may be confounded by variations in gut flora and environmental condition. As a conclusion of these findings, it is thought that organic acids may be beneficial when used in laying hen diets.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Vice Chancellor and Head of the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary And Animal Sciences, Hisar, Haryana for providing research facilities for the successful completion of this study.

REFERENCES

Angelo, A.J. St., Vercellotti, J.R., Legendre, M.G., Vinnett, C.H., Kuan, J.W. and James, C.Jr. (1987) Chemical instrumental analysis of warmed over flavor in beef. *J. Food Sci.*, **52**(5): 1163-1168.

AOAC International (2013) Official Methods of analysis of AOAC International (OMA) Gaithersburg, Maryland.

Attia, Y.A., El-Hamid, A.E., Ellakany, F., Bovera, F., Al-Harthi, M.A. and Ghazaly, S.A. (2013) Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of acetic acid. *Ital. J. Anim. Sci.*, vol. **12**: 2.

BIS (2007) Requirement for chicken feeds. IS: 1374-2007, Manak Bhawan, 9 Bahadurshah Zafar Marg, New Delhi-110001.

Bonos, E., Christaki, E. and Florou-Paneri, P. (2011) Effects of dietary Mannan-Oligosaccharides and Calcium formate on performance and egg quality of Japanese quail. *J. F. Sci., Eng.:*289-296.

Duncan, D.B. (1995) Multiple range and multiple F-test. *Biometrics*, 11: 1-42.

EFSA (2011) Scientific opinion on the safety and efficacy of propionic acid, sodium propionate, calcium propionate and ammonium propionate for all animal species. EFSA Journal, **9**(12): 2446.

Grashorn, M.A., Gruzauskas, R., Dauksiene, A., Jarule, V., Alencikiene, G. and Slausgalvis, V. (2012) Influence of organic acids on quality and sensory attributes of chicken eggs. Arch. Geflügelk. **77:** 29-34.

Haugh, R.R. (1937) The haugh unit for measuring egg quality. U.S. Egg Poult. Mag., **43**: 552-555.

Huyghebaert, G., Ducatelle, R., Van Immerseel, F. (2011) An update on alternatives to antimicrobial growth promoters for broilers. The veterinary journal, **187**: 182-188.

International Poultry and Livestock Expo (2015) Banglore international exhibition centre (BIEC), Banglore, India. http://www.iplexpo.com.

Kadim, I.T., Al-Marzooqi, W., Mahgoub, O., Al-Jabri, A. and Al-Waheebi, S.K. (2008) Effect of acetic acid supplementation on egg quality characteristics of commercial laying hen during hot season. *Intl. J. Poult. Sci*., **7**: 1015–1021.

Kamal, A.M and Ragaa, N.M. (2014) Effect of dietary supplementation of organic acids on performance and serum biochemistry of broiler chicken. Nature and science, **12**(2).

Kaya, H., Kaya, A., Gul, M. and Celebi, S. (2013) Effect of zeolite and organic acid mixture Supplementation in the layer diet on performance, egg quality traits and some blood parameters.*J. Anim. vet. Adv*., **12**(6):782-787.

Millet, S., De Ceulaer, K., Van Paemel, M. and Raes, K. (2006) Lipid profile of Araucana hens compared with Lohmann selected leghorn and ISA Brown hens given diets with different fat sources. *British Poult. Sci.*, **47**(3): 294-300.

Nys, Y. (2001) Recent developments in layer nutrition for optimizing shell quality. *13th Euro. Sym. Poult. Nutr*, Blankenberge Belgium, pp. 45–52.

Rahman, M.S., Howlider, M. A. R., Mahiuddin, M. and Rahman, M.M. (2008) Effect of supplementation of organic acid on laying performance, body fat and egg quality of hens. *Bang. J. Anim. Sci*., **37**(2): 74-81.

Ratcliff, J. (2000) Antibiotic ban-a European perspective. Proceeding of the 47th Maryland Nutrition Conferences for Food Manufacturers, Pp. 135-152.

Singh, S.D. (2012) Effect of feeding pearl millet on performance of layers. *M.V.Sc. Thesis, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.*

Soltan, M.A. (2008) Effect of organic acid supplementation on egg production, egg quality and some blood serum parameters in laying hens. *Intl. J. Poult. Sci.*, **7**: 613-621.

SPSS. 2001. Statistical Package for Social Sciences. SPSS Inc., 444 Michigan Avenue, Chicago, IL, USA.

Swiatkiewicz, S., Koreleski, J. and Arczewska, A. (2010) Laying performance and egg shell quality in laying hens fed diets supplemented with prebiotics and organic acids. *Czech J. Anim. Sci.*, **55**(7): 294-306.

Tomar, A.K. (2014) Genetic improvement in productivity of egg type chicken through selection. *M.V.Sc. Thesis, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.*

Wang, J.P., Yoo, J.S., Lee, J.H., Zhou, T.X., Jang, H.D., Kim, H.J and Kim, I.H (2009) Effects of phenyllactic acid on production performance, egg quality parameters and blood characteristics in laying hens. *J. Appl. Poult. Res.* **18**: 203-209.

Wolfenden, A.D., Vicente, J.L., Higgins, J.P., Andreatti, R.L., Filho, S.E., Higgins, B. and Hargis, M (2007) Effect of Organic Acids and Probiotics on Salmonella enteritidis Infection in Broiler Chickens. *Int. J. Poult. Sci*., **6**: 403-405.

Yesilbag, D. and Colpan, I. (2006) Effects of organic acid supplemented diets on growth performance, egg production, quality and on serum parameters in laying hens. Revue Med. Vet. **157**: 280-284.

Youssef, A.W., Hassan, H.M.A., Ali, H.M. and Mohamed, M.A. (2013) Effect of supplementation of probiotics, probiotics and organic acid on layer performance and egg quality. *Asian J. Poult. Sci.*, **7**(2): 65-74.