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Supplementation of lemon juice through drinking water on performance, egg quality, serum biochemical indices and combat heat stress in laying pullets

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ABSTRACT

Numerous feed additives, such as probiotics, prebiotics, organic acids, and enzymes, have the potential to optimize weight gain, feed efficiency and maintain the acid-base balance which eventually contributes to improve production performance. The purpose of the current study was to investigate the possible advantages of lemon juice (LJ) on laying performance, egg quality, serum biochemical properties, and layers' ability to recover from heat stress. Ninety-six Hy-Line Brown layers all 29 weeks were randomly assigned to four treatments, each with six replications, and were given a six-hour daily drinking experiment. The birds were provided with 0% (control), 0.5%, 1% and 1.5% LJ respectively for 6 weeks. The temperature, humidity, hen day egg production (HDEP) and egg weight (EW) were recorded. Egg quality indices were measured bi-weekly. An analysis of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), packed cell volume (PCV), heterophil (H), lymphocyte (L) count, and heterophil-lymphocyte (H/L) ratio was performed on the blood sample collected at the end of the experiment. The studied parameters among the various treated groups showed that the egg weight, feed conversion ratio (FCR), egg quality parameters, HDL, TG, AST, heterophil count, lymphocyte count and H/L ratio did not differ significantly ($P > 0.05$). HDEP, egg mass output (EMO) and FI differed significantly ($P < 0.01$) compared to the control group. The significant reduction in TC, LDL, glucose, ALT, increase in feed intake, HDEP and PCV showed that LJ had a positive effect in reducing the adverse effects of heat stress in laying pullets. Taken together, it may be concluded that LJ can be used to improve the laying performance and reduce the heat stress in layers.

1. Introduction

Egg is known as nutritious food. It has all the nutrients required for a person in order to fulfill the desired nutritional requirement. It is rich in protein, vitamin, mineral, fat etc. Egg of 58 gm weight contains energy 78 kcal, protein 6.5 gm, carbohydrate trace, fat 5.8 gm, cholesterol 225 mg, retinol equivalent 98 µg, vitamin D 0.9 µg, riboflavin 0.24 mg, folate 26 µg, vitamin B12 1.3 µg, choline 83.2 mg, biotin 10 µg, phosphorus 104 mg, iron 0.99 mg, zinc 0.68 mg, Iodine 28 µg, selenium 5.7 µg (Ruxton et al. 2010). Simon (2009) asserts that poultry, specifically chicken, is considered the least expensive source of animal protein in the form of eggs and meat in the world. In Bangladesh, poultry makes up between 22 and 27 percent of all animal protein. (Hamid et al. 2017). Despite having the majority of the poultry producers from the temperate region of the world, a large portion of the producers are from the hot and humid climatic countries too (Ahmed et al. 2008). The temperature of the later countries often remains over 35 °C with a high humidity (more than 85% relative humidity). The deep body temperature of the layer chickens is 41 °C. It becomes too difficult for the layers to combat the excessive heat as their whole body is covered with feathers. Therefore, the production of good quality eggs with maintaining health of laying hens is one of the most critical goals of the industry (Gültepe et al. 2019). The impact of high temperature on young chickens and laying hens has been investigated so far (Ashraf et al. 2013; Montanhini Neto et al. 2013), broilers (Hasheimi et al. 2013; Sakomura et al. 2013; Sohail et al. 2013). The main concern is to find the way to combat this stress caused by the high ambient temperature and humidity. Heat stress has been shown to

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have a detrimental impact on the welfare and output of broilers and laying hens (Lara et al. 2013) by reducing feed intake, feed efficiency that results in poor egg quality (Bozkurt et al. 2012). Laying hens require specific nutritional requirements in high temperatures to increase egg output and egg shell quality (Irandoust et al. 2012). A number of antibiotic alternatives, such as organic acids, probiotics, prebiotics, and enzymes, have been reported to be beneficial for layers and broilers, since they increased weight gain (Haque et al. 2010), feed conversion efficiency (Koivunen et al. 2016, immune status (Al-Khalaifa et al. 2019, carcass weight, and carcass quality (Islam et al. 2012).

Lemon (*Citrus limon L.*) is an evergreen native plant of Asia and belongs to family Rutaceae. It contains potassium (145 mg/100 g fruit), calcium (61 mg), vitamin C (40 to 50 mg/100 g), vitamins A, B1, B2, and B3, and 27 Kcal/100 g. It also includes volatile oil (which makes up 2.5% of the peel), limonene, alpha-terpinene, alpha-pinene, citral, coumarins, mucilage, and bioflavonoids. Additionally, some published reports (Fisher and Phillips, 2006; Manners, 2007;), suggest that the majority of the bioflavonoids in lemon come from the pith and peel. The oil exhibits some bacteriostatic and antiviral activity that is believed to be caused by the presence of citral and linalool (Fisher and Phillips, 2006; Manners 2007). Lemons also include some active antioxidant components, such as flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins (Ibrahim et al. 2011). Ascorbic acid is added as an additional nutrient to the diet to improve poultry performance by improving body weight and reducing mortality (Shewita et al. 2019). It has been demonstrated that supplementing laying hens with ascorbic acid increases egg production, egg weight, and egg shell thickness (Daghir, 2008). Wan et al. (2021) concluded that supplementing laying hens with fresh lemons improved their late-laying performance and antioxidant capacity. It also outperformed citric acid and flavomycin in terms of enhancing albumen quality, immunological status, and lipid metabolism. Omer et al. (2019) found that adding lemon-onion-garlic juice (LOG) in laying hens' diets significantly improved feed conversion throughout the second stage of egg collection. Gültepe et al. (2019) discovered that egg yolk color was lighter in higher usage and Haugh unit increased. As per the report of Tavakkoli et al. (2014) adding lemon juice to drinking water greatly decreased the number of eggs with cracked and fragile shells during heat stress. Gültepe et al. (2019) discovered in their research that the serum values of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), low-density lipoprotein (LDL), total cholesterol (TC), glucose was unaffected by the LJ juice. Wan et al. (2021) claimed a finding that fresh lemon (FM) improved serum antioxidant enzyme activities and reduced serum high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG) and total cholesterol (TC) compared to other groups, indicating favorable effects on antioxidative status and lipid metabolism. Gültepe et al. (2019) found that egg production increased in 1% and 2.5% LJ groups. Shihab et al. (2019) have focused effects of using ionized water on performance of Japanese quails from 6 weeks to 18 weeks of age and concluded that acidic drinking water has led to greater hen day egg production (HDEP) than water with neutral pH. Gültepe et al. (2019) discovered that red blood cell counts increased in the 1% LJ group while blood lymphocytes decreased in the 2.5% LJ group compared to the control. Behboudi et al. (2016) found that the individual addition of lemon juice or thyme had no significant impact on FI, BWG, FCR, H/L ratio, TC concentration; however, the combined effect of the two additives improved performance and decreased H/L ratio and serum TG concentration. Lymphocytes, eosinophils, total red blood cell count, hemoglobin, and hematocrit were found to be decreased in response to heat stress, whereas mean corpuscular volume, mean corpuscular hemoglobin, heterophil count, and H/L ratio were found to be increased in the study of Gogoi et al. (2021). Therefore, the present experiment was conducted to evaluate the effects of supplementation of lemon juice in drinking water of the laying pullets on the improvement of laying performance, egg quality, serum biochemical indices, and heat stress alleviation on layers.

2. Materials and Methods

2.1. Experimental birds

A total of 96 Hy-line Brown laying birds aged 29 weeks were randomly collected and randomly allocated for this experiment. They were placed in two three-tire shaped cages and provided with equal care and managements. Duration of the experiment was 6 weeks. The work was conducted in compliance with the Bangladesh Agricultural University, Mymensingh Guidelines for Animal Welfare and Experimentation Ethics Committee (Ethical Approval Number: AWEEC/BAU/2023 (39).

2.2. Drinking experiment procedures

The drinking water contained the lemon juice supplied for 6 hours (from 10 am to 4 pm). Lemon juice was added in the drinking water either at 0% (control), 0.5%, 1.0% or 1.5%. The doses of LJ have been decided based on the previous research findings (Gültepe et al. 2019). Each treatment contained 6 replications containing 4-layers in each replication. Fresh lemons were purchased from local market and squeezed to extract juice. Then after proper mixing, the lemon juice was added in the respective treatments at the described levels. The birds were supplied drinking water according to the treatments every day at 10 am, and the remaining water was measured at 4 pm at the end of the experiment. The temperature, humidity, egg production and egg weight of the individual replication were measured daily. The feed consumption was measured weekly. After every two weeks, one egg per replication was collected randomly from individual replication to measure the external and internal egg quality. Blood from the wing web was gathered at the end of the feeding trial from each replication to collect the data of serum biochemical and hematological parameters of different drinking water treated birds.

2.3. Laying performance

Data of HDEP, EW, FI, and temperature (°C), humidity (%) were recorded. The egg mass output (EMO) was calculated with the data of per cent of HDEP and average EW in grams. FCR was calculated dividing FI in grams by EMO in grams. The eggs were collected and analyzed for the quality parameters. The shape index of an egg was calculated from the width and length of the egg by using appropriate formula. The shell thickness (mm) was measured by egg shell thickness meter (Ogawa Seiki Co. Ltd, Tokyo, Japan). The albumen index and Haugh unit of egg white was calculated from the egg. For determining the yolk index, the width and height of the egg yolk were measured with the help of the slide calipers and micrometer respectively without the yolk being removed from the intact albumen. Yolk color score was observed by comparing with the roche yolk color fan (RYC, F. Hofman-la Roche and Ltd., Switzerland).

2.4. Serum biochemical parameters

In terms of serum biochemical analysis, blood was first collected from wing web and taken into a falcon tube and placed at 45° (slanting position) for the complete separation of the serum, then it was centrifuged at 3000 rpm at 10 °C for 15 minutes. The serum was transferred into Eppendorf tube and preserved between -12^{0}C and -20 °C until further analysis. The TC, TG, HDL, LDL, glucose, ALT, and AST were determined from serum by using clinical lab test methods of using spectrophotometer (Spectronic, Genesis5, USA). TC and HDL cholesterol were determined using the methods established previously. At the end of the drinking trial, little amount of blood was also taken into another 12 falcon tubes with anticoagulant for the tests of packed cell volume (PCV) and 1 drop of blood from each 12 samples into 12 different slides to make a smear for the tests of H/L ratio. PCV was measured by using Wintrobe hematocrit tube.

2.5. Statistical analyses

Following the guidelines of completely randomized design (CRD), all collected and computed data were statistically analyzed using the One-Way ANOVA approach utilizing Statistical Computer Package Program 20.00 (SPSS Inc. Chicago, USA 2013). The value is presented as mean ± standard error. Duncan's Multiple Range Test (DMRT) was used to compare the significance of various treatments. P-values of 0.05 or lower were regarded as significant.

3. Results

3.1. Production performance

The overall laying performance on different treatments has shown in the Table 1. The supplementation of different levels of LJ through the drinking water of layers showed significant increase in FI (P = 0.001), HDEP (P = 0.001) and EMO (P = 0.047). However, it had no significant effect (P > 0.05) on egg weight and FCR. For hen day egg production, the higher values were demonstrated in 1.5% LJ supplemented group.

3.2. Egg quality traits

The effect of supplementation of different levels of LJ on the external and internal egg quality parameters is represented in Table 2. The supplementation of different levels of LJ did not showed any significant effect on both external and internal egg quality parameters except yolk index in 30 weeks. The significant increase (P<0.05) in yolk index was found in 1.5% LJ supplemented groups.

3.3. Serum biochemistry

In case of serum biochemical and hematological properties, the supplementation of different levels of LJ showed significant differences in some properties that demonstrated in Table 3. The TC, LDL, glucose, ALT significantly decreased (P<0.01) and PCV significantly increased (P<0.05) with the supplementation of different levels of LJ. However, TG, HDL, AST, heterophil-lymphocyte count and their ratio revealed no statistically significant variations (P>0.05) across the treatments.

Table 1. Effects of LJ levels on Hy-Line Brown layers production performance (29–34 weeks)

Parameters	Treatments				P-value	LS
	Control	0.5% LJ	1% LJ	1.5% LJ		
EW (g)	62.983±0.33	63.794±0.81	63.040±0.72	62.022±0.75	0.357	NS
FI (g/b/d)	117.469^b±0.28	119.472^a±0.27	119.553^a±0.07	119.461^a±0.15	0.001	**
HDEP (%)	93.353^c±0.28	96.627^b±0.40	96.925^b±0.36	98.611^a±0.20	0.001	**
EMO (g/b/d)	58.798^b±0.28	61.653^a±1.03	61.104^a±0.72	61.160^a±0.64	0.047	*
FCR	1.991±0.02	1.931±0.03	1.948±0.02	1.941±0.02	0.317	NS

Where, LS = level of significance; EW = average egg weight (g/b); FI = feed Intake (g/b/d); HDEP = hen day egg production (%); EMO = egg mass output (g/b/d); FCR = feed conversion ratio; NS = non-significant; ** = P<0.01; * = P<0.05; ^{a,b,c,d,e} = superscript, means bearing dissimilar superscript in a row, differs significantly at the stated level of probability; Mean ± Standard Error.

Table 2. Effects of varying LJ levels on the Hy-Line Brown layers' internal and exterior egg quality characteristics (29–34 weeks)

Age of layers (weeks)	Treatments				P-value	LS
	Control	0.5% LJ	1% LJ	1.5% LJ		
Shape Index						
30	0.78±0.58	0.79±0.68	0.79±0.97	0.77±0.80	0.32	NS
32	0.80±0.26	0.78±0.71	0.78±0.85	0.77±1.09	0.18	NS
34	0.77±0.88	0.78±0.57	0.77±0.80	0.77±0.94	0.72	NS
Shell thickness (mm)						
30	0.38±0.01	0.39±0.01	0.38±0.01	0.39±0.01	0.58	NS
32	0.37±0.01	0.37±0.01	0.35±0.01	0.36±0.01	0.25	NS
34	0.37±0.01	0.38±0.01	0.37±0.01	0.38±0.01	0.86	NS
Percent of shell (%)						
30	10.01±0.09	9.91±0.30	9.72±0.24	10.37±0.21	0.25	NS
32	9.65±0.11	10.10±0.20	9.49±0.17	9.81±0.17	0.10	NS
34	10.07±0.12	10.05±0.21	9.96±0.21	9.86±0.24	0.87	NS
Albumen Index						
30	0.13±0.01	0.12±0.01	0.13±0.01	0.13±0.005	0.68	NS
32	0.18±0.003	0.17±0.01	0.18±0.01	0.18±0.004	0.56	NS
34	0.15±0.004	0.15±0.005	0.14±0.01	0.14±0.005	0.11	NS
Haugh Unit						
30	98.41±1.69	94.12±4.35	98.98±1.88	101.97±1.34	0.23	NS
32	109.05±0.60	109.37±1.48	109.97±1.30	111±0.28	0.57	NS
34	104.37^{a}±0.67	104.67^{a}±0.70	101.27^{b}±1.06	102.77^{b}±1.11	0.05	*
Yolk Index						
30	0.44^{b}±0.01	0.45^{b}±0.01	0.46^{ab}±0.01	0.48^{a}±0.01	0.04	*
32	0.48±0.01	0.48±0.004	0.497±0.004	0.498±0.01	0.19	NS
34	0.45±0.01	0.46±0.004	0.45±0.01	0.46±0.01	0.50	NS
YCS						
30	6.67±0.21	6.17±0.17	6.33±0.33	6.83±0.31	0.29	NS
32	7±0.26	7±0.26	6.83±0.31	7±0.26	0.96	NS
34	6.33±0.33	7±0.26	6.50±0.43	6.50±0.22	0.50	NS

Where LS = level of significance; NS = non-significant; YCS = Yolk color score; * = $p<0.05$; ^{a,b,c,d,e} = superscript, means bearing dissimilar superscript in a row, differs significantly at the stated level of probability; data shown in Table is mean ± SE; SE = Standard Error. LS = Level of Significance.

Table 3. Effect of different levels of LJ on serum biochemical properties of the Hy-Line Brown layers (at 34 weeks)

Parameters	Treatments				P-value	LS
	Control	0.5% LJ	1% LJ	1.5% LJ		
TC (mg/dL)	190.42^{a}±13.25	132.88^{b}±15.48	106.14^{b}±5.07	109.30^{b}±2.26	0.0016	**
TG (mg/dL)	163.85±2.70	148.34±3.32	145.71±8.82	132.23±11.36	0.0960	NS
HDL (mg/dL)	27.07±0.99	27.39±0.57	29.29±1.78	30.24±1.24	0.2795	NS
LDL (mg/dL)	137.34^{a}±14.37	72.49^{b}±17.46	46.11^{b}±3.96	49.94^{b}±2.07	0.0017	**
Glucose (mg/dL)	118.48^{a}±13.34	104.65^{ab}±5.06	80.44^{b}±3.83	48.05^{c}±2.95	0.0008	***
AST (U/L)	177.67±12.19	116.97±27.49	103.44±34.03	114.00±25.37	0.2498	NS
ALT (U/L)	8.37^{a}±1.94	5.03^{ab}±1.52	4.50^{ab}±0.47	1.53^{b}±0.18	0.0312	*
PCV (%)	26.667^{b}±0.33	29.667^{a}±0.67	30^{a}±0.58	30.333^{a}±0.88	0.013	*
H/L ratio	0.44±0.02	0.41±0.03	0.41±0.025	0.40±0.03	0.860	NS
Lymp. count (%)	69.33±0.88	69.67±2.96	70±1.53	70.67±1.76	0.966	NS
Heter. count (%)	30.33±1.67	28.67±1.33	28.33±2.52	28.33±1.76	0.774	NS

Where, TG = triglycerides; HDL = high-density lipoprotein; LDL = low-density lipoprotein; AST = aspartate aminotransferase; ALT = alanine aminotransferase; TC = total Cholesterol (mg/dl); PCV = packed cell volume; Lymp. count = lymphocyte count; Heter. count = heterophile count; H/L ratio = heterophile-lymphocyte ratio; * = $p<0.05$; ^{a,b,c,d,e} = superscript, means bearing dissimilar superscript in a row, differs significantly at the stated level of probability; data shown in Table is mean ± standard Error. LS = Level of Significance.

4. Discussion

4.1. Production performance

In this study, it is found that supplementation of LJ has no affect on drinking water (Table 4). Among the laying performance, the feed intake, HDEP and EMO were improved significantly (P,0.05) compared to the control group. Vitamin C is rich in Lemon Juice (Pisoschi et al. 2011) and the presence of vitamin C might be attributed to ameliorate the adverse effects of heat stress by maintaining normal homeostasis in laying hens. Thus, the supplemented groups exhibited better performance as compared to the control. Likewise, dietary supplementation of vitamin C had been found to increase body weight, feed intake and feed efficiency in broilers in heat stressed condition. Wan et al. (2021) found that supplementation of fresh lemon improved the feed intake in layer groups of 79-96 weeks age. This study remains in agreement with these statements that ascorbic acid content of Lemon juice improves the feed intake and feed utilization in layers while disagreement with Gültepe et al. (2019) concluded that supplementation of LJ had no effect on feed intake in older laying hen (57 weeks). The ascorbic acid content of LJ, known as anti-stress vitamin, can promote digestion, resist oxidation and are conductive to growth. They can improve the conversion of digested feed into eggs. Therefore, the supplementation of LJ could improve the feed intake in layers. From the results of this experiment, it was stated that HDEP of the Hy-Line Brown layers of the 29-34 weeks showed significant increase on different levels of LJ supplementation. LJ contain numerous phytochemicals, including polyphenols, terpenes, and tannins. It contains slightly more citric acid than lime juice (about 47 g/L), nearly twice the citric acid of grapefruit juice, and about five times the amount of citric acid found in orange juice. It is also a good source of vitamin C and antioxidants, which can help support immune function. Thus, higher HDEP in treatment groups in our study may be the combined effect of all those substances present in LJ. Overall highest value observed in 1.5% LJ supplementation. This statement remained in agreement with Gültepe et al. (2019), Daghir (2008), Wan et al. (2021), Omer et al. (2019) who found that egg production increased in LJ supplementation on laying hens of different ages. LJ contains acidic pH (2.39±0.05) and the citric acid of LJ is the key reason for the acidic pH. The acidic drinking water (pH 5) had resulted in higher HDEP (%) than the water with neutral pH (Shihab et al. 2019). Additionally, according to Wan et al. (2021), fresh lemon may improve the conversion of digested meal into eggs. Consequently, LJ supplementation may be advantageous in HDEP in layers.

Drinking water supplementation of LJ showed significant impact on EMO. Addition of ascorbic acid in layers improved HDEP, FCR, egg weight and EMO (Ahmed et al. 2008; Skrivan et al. 2013). This experiment agreed with Omer et al. (2019), Ahmed et al. (2008), Skrivan et al. (2013) whereas differed with Gültepe et al. (2019). Further, alike findings of Gültepe et al. (2019) and Omer et al. (2019) stated no effect of LJ supplementation on egg weight in older laying hens (57 weeks) and of laying hens of 30-week-old in lemon-onion-garlic juice supplementation.

4.2. Egg quality traits

This study demonstrated that supplementation of LJ had no significant impact on both external and internal egg quality parameters. The average temperature and humidity during the experimental period have shown at Table 5. External egg quality specially egg shell quality deteriorates as the temperature increases. Because, high temperature negatively affects the feed intake, therefore, the egg production and egg quality (Daghir 2008; Bozkurt et al. 2012). The deleterious effects of heat stress on production performance and egg shell quality can be determined by the status of Ca^{2+} and the ability of gastrointestinal cells to transport calcium. Besides, hyperventilation, the respiratory alkalosis, and alteration of acid-base balance become the reason of the loss of carbon dioxide from the blood and bicarbonate from the blood and body fluids. Therefore, lower amount of bicarbonate concentration in the lumen of the shell gland decreases the egg shell quality. Ascorbic acid content of the LJ helps to restrict the increase of body temperature during heat stress up to 35 °C. This study agreed with the findings of Gültepe et al. (2019), Ezzat et al. (2017), Omer et al. (2019) who found no significant effect of LJ on external egg quality parameters on laying hens of different ages; however, in disagreement with Tavakkoli et al. (2014) and Daghir (2008) who found less amount of broken and fragile shell eggs in older laying hens (50 weeks) during heat stress.

Table 4. Weekly water intake (ml) of Hy-Line Brown layers (29–34 weeks)

Age (week)	Treatments				P-value	LS
	Control	0.5% LJ	1% LJ	1.5% LJ		
29	625.6 ± 27.09	642.4 ± 17.04	592.1 ± 22.32	586.5 ± 9.93	0.085	NS
30	652.2 ± 38.44	614.2 ± 30.21	562.7 ± 39.89	563.7 ± 33.77	0.119	NS
31	670.2 ± 30.51	656.0 ± 27.35	606.7 ± 31.50	622.6 ± 20.61	0.153	NS
32	648.1 ± 13.56	640.5 ± 21.63	585.5 ± 28.32	607.9 ± 16.20	0.063	NS
33	711.2 ± 17.78	697.6 ± 19.59	658.0 ± 28.47	650.1 ± 22.73	0.092	NS
34	721.2 ± 19.28	721.7 ± 12.56	656.7 ± 42.35	675.2 ± 35.39	0.173	NS

Where, LS = level of significance; NS = non-significant; Mean ± Standard Error.

Table 5. Weekly temperature and humidity of different tiers in the layer cage

Age (Week)	Temperature (℃) (AM)	Temperature (℃) (PM)	Humidity (%) (AM)	Humidity (%) (PM)
29	27.5	28.0	92.5	90.5
30	28.5	28.5	94.0	89.5
31	29.0	29.5	90.0	83.0
32	29.0	29.5	89.5	85.5
33	29.5	31.0	87.5	78.0
34	31.0	32.5	81.0	71.0

Citric acid, malic acid and other flavor substances of LJ can improve the nutrient absorption, therefore assist protein synthesis and increase the feed palatability (Yakhkeshi et al., 2014). As a result, it facilitates the protein synthesis in the magnum of the oviduct of laying hens which results in better albumen quality. The haugh unit (HU) value significantly ($p<0.05$) decreases with the supplementation of LJ at 34 weeks but the HU values were not statistically different in other ages of laying pullets tested here (Table 2). Ezzat et al. (2017) stated that acidic water ($_p$H 5) did not affect the egg quality traits (yolk diameter, yolk height, yolk index, albumen height) of Japanese quail. Goliomytis et al. (2014) stated that dietary hesperidin supplementation facilitated egg yolk oxidative stability. This statement agreed with the findings of Gültepe et al. (2019) who found that LJ had no effect on albumen index and yolk index of the eggs of older laying hens (57 weeks). In this experiment, we observed the yolk index values increased significantly ($p<0.05$) with the addition of LJ in 30 weeks of age but not continued at later ages (32 and 34 weeks). This experiment also differed with the findings of Wan et al. (2021) and Omer et al. (2019) who found an increase in Haugh unit and albumen index in older laying hen (79-96 weeks) as well as in yolk color and yolk percentage in the layers (30 weeks). The hesperidin content of LJ resulted in the reduction of yolk traits in older laying hens (Gültepe et al. 2019; Xu et al. 2008).

4.3. Serum biochemistry

The data of serum biochemical properties have been presented in the Table 3. The results revealed that TC, LDL, glucose, and ALT significantly ($P<0.05$) decreased and PCV significantly increased with the supplementation of different levels of LJ on the layers. These results of serum biochemical indices agreed with the findings of few researchers who found reduction in TC, TG, LDL, HDL, ALT and AST on LJ supplementation in rats (Choi et al. 2004), in rabbits (Omer et al. 2015) and in laying hens of different ages (Omer et al. 2019; Wan et al. 2021). Data presented in this research had a disagreement with Gültepe et al. (2019) and Goliomytis et al. (2014) who found no significant effect of LJ on TC, LDL, glucose and ALT on LJ supplementation in older laying hen (57 weeks) and also with Behboudi et al. (2016) who stated that supplementation with LJ significantly reduced TG content in broilers. The blood serum biochemistry indicates the condition of an organism. The hesperidin content of LJ acts as a cholesterol lowering agent through decreasing activity of hepatic HMG-CoA reductase in rats (Choi et al. 2004). The TC, LDL, HDL, TG content of serum directly relates to lipid metabolism. Reduction in those parameters indicates the favorable effect of LJ on lipid metabolism. Lower serum cholesterol inhibits the growth of bacteria and reduces the oxidative stress. Therefore, the supplementation of LJ in laying hens might have a beneficial impact on lowering cholesterol content indicating higher lipid metabolism and reduction in oxidative stress.

The heterophil count, H/L ratio increased numerically and lymphocyte count reduced numerically with the supplementation of LJ. This statement agreed with the supplementation of LJ had no effect on heterophil count, lymphocyte count, H/L ratio in heat stressed broilers (Behboudi et al. 2016) and also agreed with the statement on having no significant effect in heterophil count in older laying hens (Gültepe et al. 2019). Therefore, supplementation of LJ might not have an effect on the heterophil count, lymphocyte count and H/L ratio in the laying hen of 29-34 weeks-age. The findings of Duangjinda et al. (2017) stated that increased amount of heterophil count and H/L ratio and also reduced amount of lymphocyte count can be determined as an exposure of birds to heat stress. PCV is one of the most important factors to identify the heat stress. The heat is released from cells by losing water to intestinal fluid and blood circulation. So, the birds exposed to extreme heat stress had lower PCV levels. As a result, an increase of water in plasma leads to the lower PCV (Abdel-Azeem et al. 2007). This statement agreed with the findings of Duangjinda et al. (2017), who found lower PCV content in the hematological properties of heat stressed birds.

5. Conclusion

Supplementation of LJ might be used in layers and could play significant role in the reduction of heat stress. Moreover, supplementation of LJ had no indicative effect on external and internal egg quality parameters. Furthermore, this research showed that TC, LDL, glucose and ALT significantly reduced and PVC significantly increased that eventually help to reduce heat stress. Therefore, it could be concluded that supplementation of 1.5% LJ with drinking water may reduce heat stress due to exposure to hot climate and lead to improved production performances of commercial layers.

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Authors' contributions

Jannatul Fatima Keya and Lamia Khatun Tanim conducted the experiment and prepared the manuscript. Musabbir Ahammed critically review the manuscript and Bipul Chandra Ray helped in data analysis and manuscript preparation. Bapon Dey supervise the entire the research activities and improved the manuscript quality.

Conflict of interest

The authors have declared that no competing interests exist.

References

Ahmed W, Ahmad S, Kamran Z 2008. Response of Laying Hens to Vitamin C Supplementation through Drinking Water under Sub-Tropical Conditions. Avian Biology Research, 1(2): 59-63. https://doi.org/10.3184/175815508X360461

Al-Khalaifa H, Al-Nasser A, Al-Surayee T, Al-Kandari S, Al-Enzi N, Al-Sharrah T, Ragheb G, Al-Qalaf S, Mohammed A, 2019. Effect of dietary probiotics and prebiotics on the performance of broiler chickens. Poultry Science, 98(10): 4465-4479. https://doi.org/10.3382/ps/pez282

Ashraf S, Zaneb H, Yousaf MS, Ijaz A, Sohail MU, Muti S, Usman MM, Ijaz S, Rehman H, 2013. Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. Journal of animal physiology and animal nutrition, 97: 68-73. https://doi.org/10.1111/jpn.12041

Behboudi H, Esmaeilipour O, Mirmahmoudi R, Mazhari M, 2016. The influence of drinking water containing lemon juice and thyme supplemented diet on performance and some blood parameters of broilers under heat stress. Iranian Journal of Applied Animal Science, 6(1): 169-174.

Bozkurt M, Küçükyilmaz K, Catli AU, Çınar M, Bintaş E, Çöven F, 2012. Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. Poultry Science, 91(6): 1379-1386. https://doi.org/10.3382/ps.2011-02023

Choi GS, Lee S, Jeong TS, Lee MK, Lee JS, Jung UJ, Kim HJ, Park YB, Bok SH, Choi MS, 2004. Evaluation of hesperetin 7-O-lauryl ether as lipid-lowering agent in high-cholesterol-fed rats. Bioorganic & Medicinal Chemistry, 12(13): 3599-3605. https://doi.org/10.1016/j.bmc.2004.04.020

Chowdhury VS, Tomonaga S, Nishimura S, Tabata S, Cockrem JF, Tsutsui K, Furuse M,2012. Hypothalamic gonadotropin-inhibitory hormone precursor mRNA is increased during depressed food intake in heat-exposed chicks. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 162(3): 227-233. https://doi.org/10.1016/j.cbpa.2012.03.009

Chowdhury VS, Tomonaga S, Nishimura S, Tabata S, Furuse M, 2012. Physiological and behavioral responses of young chicks to high ambient temperature. The Journal of Poultry Science, 49: 212-218. https://doi.org/10.2141/jpsa.011071

Daghir NJ, 2008. Poultry industry in hot climates. Cromwell Press, Trowbridge, London.

Duangjinda M, Tunim S, Duangdaen C, Boonkum W, 2017. Hsp70 genotypes and heat tolerance of commercial and native chickens reared in hot and humid conditions. Brazilian Journal of Poultry Science, 19(01): 07-18. https://doi.org/10.1590/1806-9061-2016-0245

Ezzat HN, Shihab IM, Hussein MA, 2017. Effects of ionized water on certain egg quality traits and the levels of proteins and enzymes in the blood of the Japanese quail Coturnix japonica. International Journal of Poultry Science, 16: 69-80. https://doi.org/10.3923/ijps.2017.69.80

Fisher K, Phillips CA, 2006. The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* in vitro and in food systems. Journal of Applied Microbiology, 101(6): 1232-1240. https://doi.org/10.1111/j.1365-2672.2006.03035.x

Gogoi S, Kolluri G, Tyagi JS, Marappan G, Manickam K, Narayan R, 2021. Impact of heat stress on broilers with varying body weights: Elucidating their interactive role through physiological signatures. Journal of Thermal Biology, 97: 102840. https://doi.org/10.1016/j.jtherbio.2021.102840

Goliomytis M, Orfanou H, Petrou E, Charismiadou MA, Simitzis PE, Deligeorgis SG, 2014. Effect of hesperidin dietary supplementation on hen performance, egg quality and yolk oxidative stability. British Poultry Science, 55: 98-104. https://doi.org/10.1080/00071668.2013.870328

Gultepe EE, Iqbal A, Cetingul IS, Uyarlar C, Ozcinar U, Bayram I, 2019. Effects of lemon juice on performance, egg quality trait, and some blood parameters of laying hens in the late phase of production. Acta Veterinaria Eurasia, 45(2): 56-63. 10.26650/actavet.2019.19005

Hamid MA, Rahman, MA, Ahmed S, Hossain KM, 2017. Status of poultry industry in Bangladesh and the role of private sector for its development. Asian Journal of Poultry Science, 11(1), 1-13. https://doi.org/10.3923/ajpsaj.2017.1.13

Haque MN, Islam KM, Akbar MA, Chowdhury R, Khatun M, Karim MR, Kemppainen BW, 2010. Effect of dietary citric acid, flavomycin and their combination on the performance, tibia ash and immune status of broiler. Canadian Journal of Animal Science, 90(1): 57-63. https://doi.org/10.4141/CJAS09048

Hasheimi SR, Zulkifli I, Somchit MN, Zunita Z, Loh TC, Soleimani AF, Tang SC, 2013. Dietary supplementation of *Zingiber officinale* and *Zingiber zerumbet* to heat-stressed broiler chickens and its effect on heat shock protein 70 expression, blood parameters and body temperature. Journal of Animal Physiology and Animal Nutrition, 97(4): 632-638. https://doi.org/10.1111/j.1439-0396.2012.01302.x

Ibrahim MR, El-Banna HM, Omara II, Suliman MA, 2011. Evaluation of nutritive value of some citrus pulp as feedstuffs in rabbit diets. Pakistan Journal of Nutrition, 10(7): 667-674. https://scialert.net/abstract/?doi=pjn.2011.667.674

Irandoust H, Samie AH, Rahmani HR, Edriss MA, Mateos GG, 2012. Influence of source of fat and supplementation of the diet with vitamin E and C on performance and egg quality of laying hens from forty-four to fifty-six weeks of age. Animal Feed Science and Technology, 177(1-2): 75-85. https://doi.org/10.1016/j.anifeedsci.2012.06.004

Islam KMS, Schaeublin H, Wenk C, Wanner M, Liesegang A, 2012. Effect of dietary citric acid on the performance and mineral metabolism of broiler. Journal of Animal Physiology and Animal Nutrition, 96(5): 808-817. https://doi.org/10.1111/j.1439-0396.2011.01225.x

Koivunen E, Talvio E, Valkonen E, Tupasela T, Tuunainen P, Valaja J, 2016. Use of semi-leafless peas (*Pisum sativum L*) with enzyme addition in diets for broilers. Agricultural and Food Science. https://doi.org/10.23986/afsci.56122

Lara LJ, Rostagno MH, 2013. Impact of heat stress on poultry production. Animals, 3(2): 356-369. https://doi.org/10.3390/ani3020356

Manners GD, 2007. Citrus limonoids: analysis, bioactivity, and biomedical prospects. Journal of Agricultural and Food Chemistry, 55(21): 8285-8294. https://doi.org/10.1021/jf071797h

Montanhini Neto R, Ceccantini ML, Fernandes JIM, 2013. Effects of methionine source, arginine: lysine ratio and sodium chloride level in the diets of grower broilers reared under high-temperature conditions. Brazilian Journal of Poultry Science, 15: 151-160. https://doi.org/10.1590/S1516-635X2013000200012

Omer HA, El-Mallah GM, Abdel-Magid SS, Bassuony NI, Ahmed SM, El-Ghamry AKA, 2019. Impact of adding natural bioactive mixture composed of lemon, onion, and garlic juice at different levels on productive performance, egg quality, and some blood parameters of commercial laying hens. Bulletin of the National Research Centre, 43(1): 1-10. https://doi.org/10.1186/s42269-019-0160-4

Pisoschi AM, Pop A, Negulescu GP, Pisoschi A, 2011. Determination of ascorbic acid content of some fruit juices and wine by voltammetry performed at Pt and carbon paste electrodes. Molecules, 16(2): 1349-1365. https://doi.org/10.3390/molecules16021349

Ruxton CHS, Derbyshire, E, Gibson S, 2010. The nutritional properties and health benefits of eggs. Nutrition and Food Science, 40(3), 263-279. https://doi.org/10.1108/00346651011043961

Sakomura NK, Barbosa NAA, Longo FA, Da Silva EP, Bonato MA, Fernandes JBK, 2013. Effect of dietary betaine supplementation on the performance, carcass yield, and intestinal morphometrics of broilers submitted to heat stress. Brazilian Journal of Poultry Science, 15: 105-112. https://doi.org/10.1590/S1516-635X2013000200005

Shewita, R.S., El-Naggar, K. and El-Naby, W.S.A. (2019). Influence of dietary vitamin C on growth performance, blood biochemical parameters and transcript levels of heat shock proteins in high stocking density reared broiler chickens. Slov. Vet. Res., 56(22): 129-138.

Shihab IM, Ezzat HN, Hussein MA, 2019. Effect of using ionized water on some productive and physiological performance of Japanese quails. Poultry Science, 98(10): 5146-5151. https://doi.org/10.3382/ps/pey590

Simon PC, 2009. Commercial egg and poultry meat production and consumption and poultry trade worldwide. In Proceedings of the 6th International Poultry Show and Seminar, Dhaka, Bangladesh. March.

Skrivan M, Marounek M, Englmaierová M, and Skrivan ova V, 2013. Influence of dietary vitamin C and selenium, alone and in combination, on the performance of laying hens and quality of eggs. Czech Journal of Animal Science, 58: 91-97. https://doi.org/10.17221/6619-CJAS

Sohail MU, Hume ME, Byrd JA, Nisbet DJ, Ijaz A, Sohail A, Shabbir MZ, Rehman H, 2012. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poultry science, 91(9): 2235-2240. https://doi.org/10.3382/ps.2012-02182

Tavakkoli H, Masallanejad SS, and Salandari S, 2014. Effect of lemon juice on the egg shell quality of layers subjected to heat stress. International Journal of Advance Biological Biomedical

Wan Y, Ma R, Qi R, Li Y, Liu W, Li J, Liu L, Zheng J, Yue J, Zhan K, 2021. Dietary fresh lemon improves the albumen quality, immune status and lipid metabolism of Jingfen laying hens during the late laying period. Italian Journal of Animal Science, 20(1): 834-841. https://doi.org/10.1080/1828051X.2020.1870174

Xu G, Liu D, Chen J, Ye X, Ma Y, Shi J, 2008. Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chemistry, 106(2): 545-551. https://doi.org/10.1016/j.foodchem.2007.06.046

Yakhkeshi S, Shawrang P, Rahimi S, 2014. Effect of some acidifiers on gastrointestinal tract characteristics and performance of broiler chickens, 143-149.