

## EFFECT OF ONION JUICE (*ALLIUM CEPA*) ON PERFORMANCE, EGG QUALITY TRAITS, BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN LAYING HENS

A. Iqbal<sup>1\*</sup> and I. Bayram<sup>1</sup>

<sup>1</sup> Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey.

\*Corresponding author e-mail: aamir\_vet@yahoo.com

### ABSTRACT

The present study aimed to evaluate the effect of onion juice through drinking water, on the production, egg quality, hematological and immune parameters in laying hens. A total of 240 Babcock hens (40 weeks old) were divided randomly into 5 groups (48 birds in each) and each group was further subdivided into 8 replicates, containing 6 birds in each subgroup. Onion juice at the rate of 0%, 0.25%, 0.5%, 1%, and 2% was added to the drinking water offered to groups A, B, C, D, and E, respectively for 12 weeks. Egg weight, and mean water consumption was increased significantly ( $P \leq 0.05$ ) in group C. Moreover, egg mass also increased in group E supplemented with 2% onion juice. Regarding immunity, the 2% Onion juice group produced higher IgG at 6 weeks and 12 weeks of study as compared to control and other supplemented groups in the immunological response of laying hens vaccinated against the Newcastle Disease virus. For serological parameters, at the 6th week, cholesterol, LDL, and calcium significantly ( $P \leq 0.05$ ) increased in group E however, in the 10th-week only ALT level increased in group B over control. For hematological parameters, only the hemoglobin level was found to be higher in the 1% onion juice group. It is concluded that the addition of 0.5mg/L onion juice in the laying hens via water had meaningfully increased egg weight and water intake while supplementation of 2mg/L onion juice had ominously increased egg mass and immunity concerning an increase in IgG level in laying hens. Though, other production and performance parameters, egg quality traits, hematological parameters, and serological parameters persisted non-significant.

**Keywords:** Onion juice, Laying hen, Egg yield, Immunity, Blood parameters.

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### INTRODUCTION

For many decades, antibiotics have been used as feed additives in poultry ration (Harms, 1986). Due to the potential for residues of antibiotics in animal products and bacterial resistance in the human body, nowadays, scientists are working to replace these additives with herbs. In developed countries, antibiotic-related feed additives are banned to use. (Nasir and Grashorn, 2006). Mostly spices and plant extract can be used to increase the health condition and performance of the animals (Goodarzi and Nanekarani, 2014). Now probiotics are being used in feed additives instead of antibiotics, in poultry rations. (Bill Revington, 2002).

There are several studies conducted in which scientist found beneficial effects of plant or vegetable oils and their extracts on different growth parameters (Soltan *et al.*, 2008), antioxidants properties (Bakhiet *et al.*, 1995), and also on eggs production (Rahimi *et al.*, 2011).

In a study, garlic juice was provided to laying hens at the rate of 0%, 0.25%, 0.5%, and 1% of their body weight. It was concluded that garlic juice significantly improved the egg quality of laying hens (Ghazaghi *et al.*, 2014). Use of essential oils in the chicken diet can decrease cholesterol level because

essential oil contains active substances which affect 3-hydroxyl- 3- menthylglutaryl coenzyme A reductase in the liver. After all, this coenzyme is mainly responsible for cholesterol synthesis in the liver (Brown *et al.*, 1990).

Onion (*Allium cepa L.*) is cultivated almost in every country but mostly in China, the USA, and India (Ebesunun *et al.*, 2007). The Onion belongs to the Allium genus. Scientist believes that Onion is originated near to Central and East Asia (Ebesunun *et al.*, 2007). Onions have anti-oxidant, anti- hyper lipid-emic, hypoglycemic, anti-hypertensive, anti-thrombin properties, and also used against inflammatory reaction (Lampe, 1999). Cholesterol in the serum is greatly reduced when dehydrated onions were used in experimental rats having high cholesterol (Vidyavati *et al.*, 2010). According to Aji *et al.* (2011), the beneficial effects of onion bulbs were observed on broilers chickens' growth yield. Broilers' diets containing onions bulbs can significantly decrease triglycerides and cholesterol levels in blood serum (Goodarzi and Landy, 2013).

In monogastric models, abundant research has been done on the effects of dietary oregano plant materials. Several positive effects of dietary oregano plant materials have been reported on performance, gut morphology, health, immunity, and product quality in quails (Cetingul *et al.*

2010, Rahman *et al.* 2018). Gültepe *et al.* (2019) observed some positive effects of lemon juice as a water supplement on egg production during the late phase production cycle of laying hens. Furthermore, Çetingül *et al.* (2019) reported that supplementation of Pomegranate Molasses with drinking water to laying hens may affect some quality parameters in eggs after 30 days of storage. *Pistacia terebinthus* seeds at 20 and 40 g kg<sup>-1</sup> supplementation levels could be used to extend the shelf life of eggs without any adverse effect on egg quality (Gultepe 2018). (Rahman *et al.* 2017) the study indicated that supplementation of *Mentha Piperita* oil and its juice in the laying hen's diet had no significant effect on egg quality traits during storage for 15 and 30 days at 4°C.

Administering the extracts of onions in rabbits greatly reduced liver, aorta, and serum triglycerides and also reduced liver and serum protein. Onion extracts greatly increase the growth performance of broilers chickens (Goodarzi and Landy, 2013). Onion extract exhibited nontoxic and nonpathogenic effects and was considered as a safe feed additive (An *et al.*, 2015). Onion has a mode of action similar to antibiotics. Antibody productions were higher in chicken blood serum in which onion juice was a supplement in their feed (Yamamoto *et al.*, 1998). Sebastian *et al.* (1979) study observed that if we inject onion extract in rabbits, it significantly decrease protein and triglycerides in the serum.

Keeping in view the above points, the present study aimed to investigate the effects of different levels of Onion juice via drinking water on performance, egg quality, and serological parameters in laying hens. Multiple plants extracts including *Onion Juice* have been used for since long as nutraceutical but proper administration needs characterization and quantification of active ingredients or bioactive compounds in Onion Juice, so the current study was planned to investigate the potential of *Onion Juice* to be used in water and its influence on feed efficiency, immunity, and other biological factors in laying hens.

## MATERIALS AND METHODS

**Experimental design and management:** A total of 240 Babcock white laying hens (40 weeks old) were divided into 5 groups (48 birds in each) and each group was further subdivided into 8 replicates, containing 6 birds in each subgroup. Total 5 groups A, B, C, D, and E were added onion juice in drinking water at the rate of 0%, 0.25%, 0.5%, 1%, and 2% respectively for 12 weeks. The lighting schedule of 16 hour light and 8-hour darkness was followed. Water and feed were offered *ad libitum*. Birds were vaccinated against the New Castle virus at the start of the trial for antibody determination in blood. All Onion juice treatment groups including the control group were fed a basal diet prepared to meet the needs of laying

hens (Table.1) as recommended by the National Research Council (1994).

**Data Collection and Analyses:** The body weights were recorded at the start, at the 6th week, and end of the experiment (12 weeks). Feed intake was measured weekly. Feed conversion was calculated as the ratio of g of feed consumed per g of egg weight produced. Eggs produced were recorded daily and weekly average egg production was calculated. Eggs were weighed once a week. Three eggs from each replicate were collected randomly at the start, mid, and end of the trial and analyzed for egg quality parameters like egg weight, eggshell thickness, color index of the yolk, Albumin index, and Haugh unit. At the end of the trial, three birds from each replicate were selected and blood samples were collected from the heart. Blood samples were drawn and placed in anti-coagulant containing tubes and without anticoagulant for serum separation. Complete blood cell count, blood glucose, cholesterol, calcium, High-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein (TP), Liver enzymes like Aspartate transaminase (AST), Alanine transaminase (ALT), total antioxidant status (TAS) and total oxidant status (TOS) were analyzed by commercially available rapid plasma regain mono test kit. Further serum anti-ND antibody titer was also determined.

**Data Collection and Analyses:** Egg production and feed consumption were recorded weekly. Eggs were weighed once per week. Egg mass was calculated as follows:  
Egg mass = Percent egg production × average egg weight in grams  
Feed conversion ratio (FCR) values were calculated as follows:

$$\text{FCR} = \text{feed consumption (g)/egg mass (g)}$$

**Statistical analysis:** The model assumptions of normality and homogeneity of variance were examined by Shapiro-Wilk and Levene tests, respectively. The statistical analysis was performed with MedCalc software (MedCalc Software bvba, Oostend, Belgium, version 17.5). One-way ANOVA was used for group comparison followed by Tukey-Kramer for post-hoc. All data were expressed as mean ± SEM. The significance level was considered as  $p \leq 0.05$ .

## RESULTS

The results of the present study revealed that egg weight was significantly ( $P \leq 0.01$ ) increased by 0.5% of the onion juice group (group C) as compared to other treatment and control groups. Similarly, water consumption was also significantly ( $P \leq 0.05$ ) increased by 0.5% onion juice supplemented group. Moreover, egg mass significantly ( $P \leq 0.05$ ) increased in group E (2% onion juice). However, body weight, feed consumption,

egg production, and feed conversion ratio similar in all groups ( $P>0.05$ ) (Table 2).

Regarding egg quality parameters, mean egg weight increased significantly in group C. However, egg mass significantly increased in group E supplemented with 2% onion juice, while eggshell thickness, egg yolk color, Haugh unit, albumen index, and yolk index did not differ ( $P>0.05$ ) (Table 3).

For serological parameters, after seven weeks of analyses, cholesterol, LDL, and calcium levels significantly increased in group E while other parameters such as glucose, HDL, AST, ALT, Total protein, TAS,

and TOS did not differ however, after 12 weeks only ALT level significantly increased in group B over control. For immunity, the group supplemented with 2% onion juice (group E) produced higher IgG after eight weeks and twelve weeks of study which shows an increase in the immunological response of laying hens vaccinated against the Newcastle (Table 4 and 5).

For hematological parameters, only hemoglobin (Hb) concentration increased in group D with 1% onion juice. However, RBC, MCV, MCH, MCHC, platelet, RDWC, and hematocrit level were similar in all groups (Table 6).

**Table 1. Composition of basal diets & Onion juice ingredients.**

<b>Ingredients</b>	<b>%, as-fed basis</b>
<b>Feed Ingredients</b>	<b>Inclusion %</b>
Corn gran	57.50
Sunflower meal, 32 %hp	15.42
Full fat soya	10.00
Soybean meal, 44%	5.90
Limestone	8.54
Dicalcium phosphate	2.06
Salt	0.25
Vitamin-mineral mix	0.25
L-lysine hydrochloride	0.05
DL-methionine	0.03
<b>Calculated values</b>	
DM	90.5
CP	16.0
ME.kcal/kg	2750
Ca	3.83
Av.P	0.43
Na	0.14
Met+Sis	0.62
Lysine	0.74
Treonin	0.57
Tryptophane	0.19
Linoleic acid	2.23

<sup>1</sup>. Provided per kg of diet: Vitamin A:12.000.000 IU, Vitamin D3:3.000.000IU, Vitamin E:35.000, Vitamin K3:3.500, Vitamin B1:2.750IU, Vitamin B2:5.500IU, Nicotinamid: 30.000IU, Ca-D-Panhotenate:10.000IU, Vitamin B6: 4.000IU, Vitamin B12-15IU, Folic acid:1.000IU, D-Biotin: 50IU, Cholin clorid:150.000IU, Manganese: 80.000mg, Iron: 60.000 mg, Zinc:60.000 mg, Copper:5.000 mg, Iodine:2.000 mg, Cobalt: 500 mg, Selenium: 150 mg, Antioxidant:15.000 mg

<b>Ingredients of Onion Juice</b>	
<b>Onion juice Ingredients</b>	<b>mg/L</b>
Gallic Acid	2.659
Protocatechuic acid	0.240
Syringic acid	1.208
Vanilic	4.439
Caffeic	40.131
P-coumaric	0.239
Cinnamic	0.388
Gentisic acid	11.699
Epicatechin	24.962
Ellagic	10.599

Table 2. Performance Parameters (1-12 weeks)

Group	Daily Feed Cons (g)	Mean Daily egg production (%)	FCR (Kg feed/Kg Egg)	Egg weight (g)	Egg Mass (g/hen/day)	Water Intake (L/hen/day)
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Control	100.6±2.2	61.4±4.3	3.5±0.40	50.9±2.5 <sup>ab</sup>	32.9±3.4 <sup>ab</sup>	0.35±0.05 <sup>bc</sup>
0.25%Onion	101.3±3.5	59.1±4.6	4.2±0.53	48.1±0.7 <sup>a</sup>	29.6±2.7 <sup>a</sup>	0.32±0.02 <sup>ab</sup>
0.5 % Onion	105.5±1.8	67.8±1.1	2.7±0.07	58.6±0.6 <sup>c</sup>	40.7±1.1 <sup>bc</sup>	0.36±0.02 <sup>c</sup>
1% Onion	102.3±4.0	61.9±5.3	4.6±1.01	49.1±2.7 <sup>a</sup>	32.3±3.6 <sup>ab</sup>	0.31±0.01 <sup>a</sup>
2% Onion	105.1±2.1	72.2± 2.7	2.7± 0.16	56.2± 1.4 <sup>bc</sup>	42.6± 2.5 <sup>c</sup>	0.33±0.02 <sup>ab</sup>
<b>P</b>	0.678	0.141	0.072	<b>0.001</b>	<b>0.010</b>	<b>0.001</b>

a, shows the lowest increase, ab shows moderate, bc shows a bit and c shows highest increase

Table 3. Egg quality parameters

Group	Egg weight(g)	Shell Thick. (mm)	Egg Mass	Haugh Unit	Yolk color (Roche fan)	Albumen index (%)	Yolk index (%)
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Control	50.9±2.5 <sup>ab</sup>	0.34±0.10	32.9±3.4 <sup>ab</sup>	88.19±0.52	11.09±0.07	9.69±0.14	41.08±0.42
0.25%Onion	48.1±0.7 <sup>a</sup>	0.35±0.06	29.6±2.7 <sup>a</sup>	89.70±0.57	11.33±0.06	9.99±0.14	42.65±0.44
0.5 %Onion	58.6±0.6 <sup>c</sup>	0.34±0.08	40.7±1.1 <sup>bc</sup>	88.14±1.68	11.26±0.06	9.63±0.95	46.17±0.39
1% Onion	49.1±2.7 <sup>a</sup>	0.35±0.01	32.3±3.6 <sup>ab</sup>	90.46±0.58	11.32±0.06	10.51±0.13	49.92±0.79
2% Onion	56.2± 1.4 <sup>bc</sup>	0.36±0.012	42.6± 2.5 <sup>c</sup>	87.89±0.52	11.55±0.06	9.49±0.13	42.03±0.79
<b>P</b>	<b>0.001</b>	0.643	<b>0.010</b>	0.634	<b>0.411</b>	0.476	0.409

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Table 4. Mid at 6th week serological Parameters.

Groups	Glucose (mg/dl)	CHO (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	AST (U/L)	ALT (U/L)	TP (g/dl)	IgG	TAS	Ca (mg/dl)	TOS
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Control	203.25±2.18	104.12±0.7	14.75±0.7	46.37±3.7 <sup>ab</sup>	212.62±14.5	0.82±0.02	6.23±0.3	157.3±22.4 <sup>b</sup>	1.6±0.08	23.3±1.24 <sup>ab</sup>	71.4±14.3
0.25% Onion	197.87±6.75	132.87±6.9 <sup>ab</sup>	18.62±2.4	61.62±9.0 <sup>bc</sup>	228.12±19.5	1.05±0.3	6.15±0.2	54.8±4.1 <sup>a</sup>	1.5±0.22	22.1±2.07 <sup>a</sup>	89.5±20.5
0.5 % Onion	200.75±3.80	108.75±12.1 <sup>a</sup>	14.87±0.6	48.50±6.7 <sup>ab</sup>	205.12±8.0	0.90±0.01	5.61±0.2	116.5±28.0 <sup>b</sup>	1.5±0.08	22.8±0.94 <sup>a</sup>	100.1±18.2
1% Onion	200.75±3.50	97.00±7.9 <sup>a</sup>	16.87±2.6	41.62±4.4 <sup>a</sup>	239.37±28.2	0.86±0.19	5.88±0.3	144.1±20.2 <sup>b</sup>	1.5±0.16	20.3±1.88 <sup>a</sup>	56.8±9.6
2% Onion	196.42±7.74	152.28±8.7 <sup>b</sup>	13.71±0.5	71.28±4.2 <sup>c</sup>	190.28±6.5	1.58±0.73	6.08±0.1	215.4±9.3 <sup>c</sup>	1.1±0.10	27.6±0.6 <sup>b</sup>	57.1±2.5
<b>P</b>	0.894	<b>0.019</b>	0.297	<b>0.011</b>	0.354	0.527	0.554	<b>0.000</b>	0.274	<b>0.031</b>	0.178

a, shows the lowest increase, ab shows moderate, bc shows a bit and c shows highest increase

Table 5. Final serological Parameters.

Groups	Glucose (mg/dl)	CHO (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	AST (U/L)	ALT (U/L)	TP (g/dl)	IgG	TAS	Ca (mg/dl)	TOS
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Control	171.8±7.7	110.5±4.5	21.3±1.0	44.6±4.43	308.5±25.8	1.9±0.34 <sup>a</sup>	8.7±0.41	25.6±4.7 <sup>a</sup>	2.01±0.14	20.1±1.2	257.9±23.3
0.25%Onion	172.0±3.7	155.6±17.6	19.3±0.9	71.5±8.44	303.3±25.5	5.1±1.22 <sup>b</sup>	9.4±0.61	35.8±12.4 <sup>ab</sup>	1.85±0.31	22.2±2.4	273.1±31.5
0.5 %Onion	174.1±8.0	120.6±9.3	19.7±0.9	54.1±8.62	295.1±18.0	1.8±0.45 <sup>a</sup>	7.6±0.42	100.3±41.4 <sup>b</sup>	1.88±0.12	22.2±1.8	178.9±36.6

<b>1% Onion</b>	155.8± 11.3	178.5± 53.6	23.0± 3.6	52.7± 7.59	333.2± 47.6	2.8± 0.82 <sup>ab</sup>	8.9± 0.66	232.6± 19.4 <sup>c</sup>	2.00± 0.19	22.4± 1.7	212.6± 24.0
<b>2% Onion</b>	172.7± 6.6	138.5± 13.3	18.5± 0.7	60.2± 7.59	281.6± 10.3	4.3± 0.96 <sup>ab</sup>	8.6± 0.44	263.6± 18.0 <sup>c</sup>	1.93± 0.08	25.5± 0.8	225.8± 11.8
<b>P</b>	0.465	0.387	0.456	0.155	0.770	<b>0.027</b>	0.214	<b>0.000</b>	0.965	0.314	0.127

a, shows the lowest increase, ab shows moderate, bc shows a bit and c shows highest increase

**Table. 6 Hematological parameters.**

Group	HCT (10 <sup>9</sup> /l)	RDWC (10 <sup>9</sup> /l)	RBC (10 <sup>12</sup> /l)	He (g/l)	MCV (fl)	MCH (pg)	MCHC (g/l)	PLT (10 <sup>9</sup> /l)
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
<b>Control</b>	30.6±1.5	10.66±0.5	2.61±0.10	11.58±0.46 <sup>a</sup>	116.90±2.24	44.38±0.96	38.00±1.90	23.00±5.5
<b>0.25%Onion</b>	36.6±4.7	13.51±1.5	3.08±0.34	13.85±1.68 <sup>ab</sup>	117.84±3.1	44.67±0.72	38.05±2.41	38.85±6.0
<b>0.5 %Onion</b>	27.05±2.2	11.21±0.6	2.34±0.19	10.74±1.11 <sup>a</sup>	115.76±2.1	45.66±1.79	39.41±4.1	27.77±5.3
<b>1% Onion</b>	31.62±6.1	12.37±0.5	2.63±0.51	15.34±1.35 <sup>b</sup>	120.81±1.3	40.77±7.07	33.94±15.6	27.57±5.5
<b>2% Onion</b>	31.21±0.9	11.15±0.4	2.70±0.09	12.35±0.40 <sup>ab</sup>	115.42±1.1	45.76±1.35	39.61± 2.6	32.75±6.7
<b>P</b>	0.444	0.167	0.470	<b>0.049</b>	0.394	0.813	0.583	0.448

a, shows the lowest increase, ab shows moderate, bc shows a bit and c shows highest increase

RBC: Red blood cell count. He: Hemoglobin. MCV: Mean corpuscular volume. MCH: Mean corpuscular hemoglobin. MCHC: Mean corpuscular hemoglobin concentration. PLT: Platelet. MPV: Mean platelets volume, Red cell distribution Width Counts (RDWC), Hematocrit (HCT).

## DISCUSSION

According to Abdel-Wareth *et al.* (2014), supplementing the rations of laying hens with herbal products and their extracts had a positive effect on feed consumption. Ghasemi *et al.* (2010) demonstrated that using mixtures of garlic and thyme in the poultry diet did not affect feed intake. In contrast, some other studies observed that feed intake was reduced when herbal products like leaf oil (*Laurus nobilis* L.), sage leaf oil (*Salvia triloba* L.), myrtle leaf oil (*Myrtus communis*), and fennel seed were provided in the diet (Çabuk *et al.*, 2006). Similarly, In our present study with supplementation of onion juice, overall feed intake also did not show any significant difference between the groups as compared with control, although there was numerical higher feed intake in groups C and E it's statistically non-significant.

Abdel-Wareth *et al.* (2014) observed a positive effect of peppermint on feed conversion ratio (FCR) due to the supplementation of thyme in the diet of laying hens. However other studies showed that feed conversion ratio increased with the addition of herbal extract in the diet of both laying and broilers hens (Mamoun *et al.*, 2014; Aji *et al.*, 2011). In contrast, some other researchers did not observe a positive effect of plant originated product on feed conversion ratio (FCR) in poultry birds (Lee *et al.*, 2003; Christaki *et al.*, 2012). In our study, the overall feed conversion ratio during the 0-12 weeks showed no statistically significant between the groups however, the onion juice groups' values were better than the control group numerically.

Recently, Liu *et al.* 2018 observed that the taste sense of birds may be more sophisticated and developed than previously thought. Unlike the dietary supplementation, the highest dose of onion juice in the present study did not change the water and feed flavor due to different concentrations of onion juice content.

Researcher's observed that egg weight and egg mass were improved with the supplementation of oregano and thyme in the diet of laying hens during 68-72 weeks of age (Khan *et al.*, 2012). Similarly, Mansoub *et al.* (2011) also observed improved egg production and egg weight with the supplementation of herbal extract in the diet of laying hens. Similarly, in our overall 1-12 weeks study, the highest egg weight was observed in group C (58.6g) and lowest in groups B (48.1g) onion juice. Statistically, there was a significant difference in group C as compared with other treatment and control groups. Similarly, for egg mass, in our overall 1-12 weeks study, addition of 0.5mg/L onion juice in the laying hens via water had increased egg weight

Jang *et al.* (2004) studied that with the addition of herbal products, body weight increased. However, some others showed that the bodyweight of broiler hens remained unchanged with the addition of herbal products (Çabuk *et al.*, 2006). Moreover, Ragab and Hassan. (2007) observed no positive effect on the live weight of laying hens with the supplementation of carrot meal in their diet. In contrast, Glatz *et al.* (2017), found positive results on the live weight with carrot meal addition in laying hens diet. In the present study, body weight remained non-significant throughout the experimental period. Supplementation of different doses of Onion juice showed no effect on the body weight between the groups

as compared to the control group after the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup>-month application of different doses of onion juices.

Previous studies observed that the supplementation of garlic juice did not produce any positive effect on egg production in laying hens (Mahmoud *et al.*, 2010). Similarly, Rahimi *et al.* (2011) also observed no effect of the herbal extract on egg production in laying hens. In Denmark, carrot meal is commonly used as an organic forage in organic egg production (Rizal *et al.*, 2010). Overall 1 to 12 weeks trial period, egg production data showed non-significant differences in egg production percentage however 2% onion group showed higher value for egg production than the other group as numerically though there was no statistically significant.

Recently, Liu *et al.* (2018) provided an update about poultry taste buds indicating that the poultry taste buds are better developed than previously thought. Therefore, in our study 0.5% onion juice supplemented group C dose changed the water flavor. In our study, the increase of water consumption in the moderate dose of onion juice supplemented group might be explained by this hypothesis. Researchers observed that increase in egg weight had a direct correlation with water consumption. An increase in egg weight will increase water consumption (Medway *et al.*, 1957). Our result was consistent with the result of Medway *et al.* (1957) we also observed a significant increase in both egg weight and water consumption.

For egg quality parameters, supplementation of 70 g/day purple carrots increased egg yolk parameters. Moreover, purple carrots were found beneficial for the yolk and egg mass (Hammershøj *et al.*, 2010). Moreover, some other studies observed that with the addition of 5% carrots in the diet of laying hen increased egg yolk color, however, eggshell thickness, Haugh unit, and egg shape index remained unaffected (Ishikawa *et al.*, 1999). The no-effect in yolk color value might be linked to low in feed intake in all groups that why YC remained non-significant during initial, mid, and final egg analyses.

Regarding, Haugh unit Bölükbaşı and Erhan. (2007) observed that different egg quality parameters such as Haugh unit, yolk color, and eggshell thickness increased with the supplementation of 0.1%-0.5% thyme in the diet of laying hens. In contrast, feed supplementation with herbs and its extract has no positive effect on egg quality parameters (Navid *et al.*, 2014). Haugh unit (HU) results remained non-significant in all treatment groups during the initial, mid, and end of trial egg analysis as compared with the control group. It showed that Onion juice supplementation did not exert any effect on the Haugh unit of eggs.

The decrease of different egg quality parameters such as egg-breaking strength and eggshell thickness has a direct correlation with loss of moisture through eggshell pores and also with the loss of CO<sub>2</sub> from the albumin

(Williams, 1992). Herbal plant and their extract could be used to improve different performance parameters and egg storage time. Some researchers observed a positive effect of herbal extracts on egg quality and production (Aji *et al.*, 2011; Khan *et al.*, 2012). Contrary to the previous study, during our study, eggshell thickness means values did not show any significant difference between the groups during the analysis of eggs at 30, 60, and 90 days.

Some researchers have reported that the supplementation of plants extracts did not generate any positive effects on alkaline phosphate, glucose, calcium, and phosphorus levels in laying hens (Abdel-Wareth *et al.*, 2014), while other studies have reported the positive effects of herbal products on plasma triglycerides and meat cholesterol levels (Paraskeuas *et al.*, 2017). In our study, some serological parameters such as glucose, HDL, AST, total protein, TAS, and TOS level did not affect the supplementation of different levels of onion juice during mid and final serological analyses. Other studies have reported the positive effects of herbal products on plasma triglycerides and meat cholesterol levels (Paraskeuas *et al.*, 2017). Similarly, in our study serological parameters exhibited a significant difference in cholesterol, LDL, IgG, and Calcium during mid-serological analyses while in final serological analyses ALT and IgG showed significant variation.

Previous studies showed that immunoglobulin G (IgG) significantly increased with the addition of 3 and 6 g/kg of thyme in the diet of laying hens (Abd El-Hack *et al.*, 2015). Similarly, carvacrol and thyme at the dose rate 60, 100, and 200 mg/kg significantly increased immunoglobulin G (IgG) in broilers hens (Hashemipour *et al.*, 2013). In our study, immunity was improved with higher IgG values in laying hens at 2% onion juice supplemented group E as compared to other treatment and control groups during mid and final serological analyses which indicate significant results in the immunological response of laying hens vaccinated against the Newcastle virus.

Lee *et al.* 2018 founded that myricetin inhibited both Cyclooxygenase-2 (COX-2) and nuclear factor kappa B (NF-kappaB) trans-activation in phorbol ester-treated JB6 P+ cells. The COX-2 is an inducible isoform member of the cyclooxygenase enzyme family, which is regulated by growth factors and different cytokines such as IL1 $\beta$ , IL6, or TNF $\alpha$ , therefore overexpressed during inflammation. The NF-kappaB is a family of inducible transcription factors that play a key role in the immune system. Transcription of genes which are responsibly regulating the inflammation, proliferation, and differentiation of immune cells are induced by primary activation of the NF- $\kappa$ B pathway. Hence, myricetin could have anti-inflammatory as well as antioxidant effects through COX-2 inhibition pathway like nonsteroidal anti-inflammatory drugs. In the present study, the significant

effect of onion juice on serum IgG levels might be explained by these complex mechanisms. Further studies are needed to evaluate both the antioxidant and anti-inflammatory potential of onion juice.

Different research has shown different results regarding the effects of plant products on blood parameters in laying hens (Oleforuh-Okoleh *et al.*, 2015). According to Samour *et al.* (2006), the plant extract did not show any positive effect on blood parameters in laying hens. Different studies showed the different results of plant extract on blood parameters in laying hens (Oleforuh-Okoleh *et al.*, 2015). However other studies did not find any positive results of plant extract on blood parameters (Samour *et al.*, 2006). In our study, we also observed that different hematological parameters such as the red blood cells count (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets count, red cell distribution width counts (RDWC) and hematocrit percentage remained non-significant between all the treatments group as compared with the control group during the final analysis of blood sample. In our complete 12 weeks trail, total Hb concentration showed some variation in some groups. All the treatment groups exhibited no difference compared with the control group but the supplemented group D showed significant difference as compared with other treatment and control groups.

**Conclusion:** The results of the current study indicated that supplementation of 0.5% onion juice in the laying hens via water significantly improved egg weight and water consumption while supplementation of 2% onion juice had significantly increased egg mass and immunity regarding an increase in IgG level in laying hens. However, other production and performance parameters including egg quality traits, hematological aspects, and serological parameters remained non-significant. It is recommended to conduct more extensive research studies to explore the effect of this herbal product with a wide range of dose levels on various parameters.

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