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EFFECT OF DRIED TOMATO POMACE AS ALTERNATIVE TO VITAMIN C SUPPLEMENTED DIETS IN HEMATOLOGICAL INDICES AND OXIDATIVE STABILITY OF EGG YOLK OF LAYING HENS IN HIGH-AMBIENT TEMPERATURE

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ABSTRACT : The present experiment was aimed to estimate the influence of dried tomato pomace (DTP) as alternative to vitamin C supplemented diet on the oxidative stability and hematological indices in the egg yolk of laying chickens treated with high heat stress. Two hundred laying chickens from Lohman brown strain at the age of 34 weeks, were distributed into five treatments, 4 replicates 10 chickens (40/treatment) maintained at high ambient temperature. Chickens were fed laying diet without addition, laying diet with addition of vitamin C (300mg/kg), and 1, 2, 3% of DTP respectively. Although blood indices of chickens was similar (p>0.05) and DTP decreased triglyceride concentration, glucose, and cholesterol. The oxidative status for cholesterol egg yolk was improved through the use of diets supplemented with vitamin C and DTP as well as decreased MDA values. The result of the study indicate that DTP, and vitamin C supplementing modulates the oxidation-antioxidation system of hematological indices and egg yolk peroxidation in laying chickens treated with high ambient temperature.

Key words : Heat stress, dried tomato pomace, vitamin C, antioxidant, hematology indice.

INTRODUCTION

The effect of higher heat stress was considered as a main causes of growth performance decreased, egg production, nutrient, availability, immunity, and quality in laying chickens as well as broiler chickens. Furthermore the environmental temperature above 32°C, large production losses are readily evident (Kirunda *et al*, 2001; Mahmoud *et al*, 2003; Sahin *et al*, 2008; Mahmoud *et al*, 2004; Rozenboim *et al*, 2007; Lara *et al*, 2013). Unsaturated fatty acids were in high levels in the egg yolk which leads to higher susceptibility to lipid oxidation. Several studies were conducted to estimate the antioxidants effect in chicken diets to improve quality of eggs as well, increase the shelf life due to the effect of oxidation that cause damage in eggs during the storage period (da Cruz *et al*, 2016).

The production of active oxygen materials (ROS), could be increased due to high temperature degree which may result in deterioration to critical biomolecules including fatty acids, DNA and proteins, which leads to reduce welfare performance. Vitamin E and C inclusion were detected to be beneficial in reducing heat stress associated with physiological responses as well as improving heat resistance by their antioxidant effects (Ipek *et al*, 2007; Sahin *et al*, 2003; Kucuk *et al*, 2003; Ahmed *et al*, 2008; Chung *et al*, 2005; Sahin *et al*, 2006).

Dried Tomato Pomace (DTP) consist of 44% seed, 56% pulp and skin, seed was the main part of pomace that consist of 22.2-33.9% protein, 35.1% total dietary fiber, 20.5- 29.5% fat and 3.9-9.6% ash (Rahmatnejad *et al*,(2009). Additionally, per gram of dried pomace powder (naturally dried) included CP (11%), fat (4.5%), β-carotene (0.13 mg), lycopene (0.8 mg), vitamin C (1.73 mg), and α-tocopherol (0.07 mg) (Sahin *et al*, 2008).

Tomato items contain phytochemicals that may have wellbeing points of interest as well, considered as a source of important ingredients like lycopene, vitamin A, phenols, folate, vitamin C and flavonoids which were potential bioactive compound included in tomato (Agarwal and Rao, 2000; Beecher, 1988). As well as lycopene and pigment were the carotenoid with high amplitude of oxygen captivate because the existence of two non-conjugated double bands resulted in a higher activity (Moreira and Shami, 2004).

Different antioxidants can form together an integrated antioxidant system and they were important for protecting against damage causing by toxic products of cell metabolism as well, free radicals thus the addition of substances with potential antioxidant effect to chicken diets can increase their antioxidant activity (Ajakaiye *et al*, 2011; Sahin *et al*, 2006; Surai, 2006). Previous researches have detected the feasibility of feeding tomato by- product in chicken diet and revealed that addition of DTP to laying chicken diets performed comparable performance with chickens fed diet supplemented with corn meal and soybean meal (Dotas *et al*, 1999), or diet supplemented with different levels reach to 100 g/Kg in laying chickens (Jafari *et al*, 2006). However, an addition of DTP (150 g/kg) to the laying chicken diets reduced some of the egg parameters and raised feed conversion ratio (Jafari *et al*, 2006). Others revealed that feeding laying hens on DTP increased feed intake and egg traits, whereas egg yolk cholesterol content and serum were insignificantly affected (Nobakht and Safamehr, 2007). Furthermore, some researchers pointed that diet supplemented with DTP did not affect production traits, however, increased the color of egg yolk in laying chicken (Mansoori *et al*, 2008). Cholesterol of blood plasma, glucose, triglyceride contents, albumin, serum, globulin, and low-density lipoprotein did not be affected by the addition of DTP to laying chicken diets (Safamehr and Nobakht, 2007). Sahin *et al* (2008) reported that a beneficial compounds in the tomato such as vitamin C, lycopene, vitamin A, flavonoids, folate and phenols. Therefore, the current experiment was aimed to investigate the influence of adding DTP as an alternative to vitamin C to laying chicken diets, exposed to heat stress on hematological indices and stability of eggs stored at 4°C.

MATERIALS AND METHODS

The current experiment was carried out at the Poultry Research, Abu-Ghrab (longitude 44.8", latitude 33.7"), Ministry of Agriculture, during (June, July and August, 2014). A total of 200 laying hens (Lohman brown layer), 34 week old, with mean body weight 1750 ± 150 g were assigned to 5 groups with 4 replicate of 10 hens in completely random design. The hens were kept in floor pens (2 × 3m) on litter, under lighting schedule 16 hours/d lighting and 8 hours/d darkness with a temperature degree of 34± 2^{0}C and the humidity was 55% through the whole experiment period in addition, the pens were provided with nipples system drinker and hanging fodders. The dietary treatments were provided *ad libitum* with free access for drinking water as well as the basal diet considered as control according to NRC (1994) recommendation for laying chickens. The rations (Table 1) prepared for different groups, as fallow, group 1: control diet, without inclusion of DTP; group 2: basal diet containing 300 mg/Kg vitamin C. group 3, 4 and 5 basal diet containing 1, 2 and 3% DTP. Preparation DTP: wet tomato pomace was obtained after processing tomato juice (Iran Origen) and was air dried to final moisture content in order to obtain DTP (9% moisture) and ground through a 2 mm mesh. The nutrient analysis of DTP was assumed as reported by Mansoori *et al* (2008). Meal form was used in the diets formation and based on soybean and corn meal. The diets were formulated to be is nitrogenous (16.5%) and is caloric (2736 Kcal/Kg). According to Lohman layer guide, and the hens were offered 120 g at 8 o'clock in the morning. All diet have the same level of amino acids.

Blood parameters

Three hens of per replicate (12 hens per treatment) were randomly chosen at the end of the experimental period for blood collecting whereas amount of 5 ml of blood sample were pulled of the brachial vein (Campbell, 1995). The collected blood samples (1ml) were transferred to Ethylen Diamin Tetra Acid (EDTA) tube in order to determine white and red blood cells, lymphocytes, hemoglobin and cell volume (Siegel and Gross, 1983). The rest amount (4 ml) of blood samples were centrifuged at 3000 ×g for 15 minutes to isolate the blood serum to determinate the blood biochemical traits involving triglyceride, cholesterol, glucose, total protein, and albumin and were spectrophotometrically determined by using a commercial kit (Stanbio Laboratory Boerne TX). The difference between total protein and albumen represents the calculated globulin.

Yolk cholesterol and blood plasma low density lipoprotein (LDL) were calculated during the last week of trail. According to Folch *et al* (1956) method that adjusted by Nix and Washburm (1974), extract cholesterol from two eggs per replicate was done. Yolk Molondialdehyde (MDA) was determined during 14, 28 and 42 day of storage eggs at 4^{0}C, using method of Witte *et al* (1970).

One-way analysis of variance (ANOVA) test general linear model (SAS, 2012) was used for statistical analysis. Duncan Multiple Test (1955) at a significance level of 0.05 was used to compare the significant difference between values.

RESULTS

Table 2 indicated the influence of dietary treatments on blood cells. Showed all traits was affected by treatments with exception, hemoglobin concentration. In this experiment, PCV% was higher of the hens fed 3% DTP

Table 1 : Chemical analysis and Dietary content of the diet.

Ingredients	Diets treatments			
	Control	DTP1	DTP2	DTP3
Yellow corn	49	49	49	49
Wheat grain	5	5	5	5
Barley	12.29	11.39	10.49	9.69
Soybean meal	19	18.9	18.8	18.6
DTP	0	1	2	3
Proteins concentration (40%)*	5	5	5	5
Hydrogenated plant fat	1	1	1	1
Calcium diphosphate	1.8	1.8	1.8	1.8
Limestone	6.82	6.82	6.82	6.82
Salt NaCl	0.09	0.09	0.09	0.09
Total	100%	100%	100%	100%
Chemical analysis**				
Metabolism energy (kcal/kg)	2742.8	2739.6	2736.3	2733.4
Crude protein (%)	16.452	16.459	16.466	16.44
Lysine (%)	0.89	0.89	0.89	0.89
Cysteine (%)	0.28	0.28	0.28	0.28
Methionine (%)	0.38	0.38	0.38	0.38
Methionine + cysteine (%)	0.66	0.66	0.66	0.66
Arginine (%)	0.93	0.93	0.93	0.93
Phosphorus (%)	0.55	0.55	0.55	0.55
Linoleic acid (%)	1.85	1.85	1.85	1.85
Calcium (%)	3.46	3.46	3.46	3.46
Sodium	0.164	0.164	0.164	0.164
Chlorine	0.164	0.164	0.164	0.164
Vitamin E (mg/kg)	25	25	25	25
Vitamin C	300	300	300	300

* Proteins level for the feeding of poultry (Breedcom-5 special) produced by the Dutch company WAFI, Metabolism energy (kilo= 2100, raw fat 5%, raw fiber, 2%, raw protein, 40%, phosphorus 2%, calcium 8%, lysine 3.75%, methionine 2.85%, methionine + cystine 3.20%, sodium 2.20%, 500 mg vitamin E per kg protein), **According to the values of the chemical composition of the feed materials found in the composition of the diet according to the American Research Council (NRC, 1994).

Table 2 : The effects of feeding vitamin C and DTP on blood cells in laying chickens exposed to high ambient temperature.

Treatments	Blood Parameters			
	PCV %[1]	HP g/100 ml[2]	WBC× 10^3/mm^3[3]	H/L ratio[4]
Control	24 b	7.33	32.98 a	0.58 a
Vitamin C	29 ab	9.00	24.83 b	0.40 b
DTP 1%	29 ab	9.00	25.58 b	0.37 b
DTP 2%	29 ab	9.00	22.03 d	0.43 b
DTP 3%	31 a	10.33	18.40 c	0.37 b
SEM	2	2	0.00045	0.00062
P- value	0.05	N.S	0.05	0.05

DTP: dried tomato pomace, Value are means=12n, 1. Packet cell volume, 2. Hemoglobin, 3. White blood cells, 4. Heterophil/ Lmphocyte. At the same column, the means with at least one common letter, were insignificant (p>0.05), SEM: standard error means,

Table 3 : The influence of adding DTP and vitamin C to the diet on the plasma Biochemistry parameters in laying chickens under heat stress.

Treatments	Plasma Biochemistry parameters			
	Glucose (mg/100 ml)	Total protein (g/100 ml)	Albumin (mg/100 ml)	Globulin (g/100 ml)
Control	220 a	3.055 b	1.625 b	1.415 b
Vitamin C	130 b	5.130 a	2.760 a	2.400 a
DTP 1%	128 b	4.995 a	2.625 a	2.355 a
DTP 2%	130 b	5.130 a	2.830 a	2.330 a
DTP 3%	132 b	5.130 a	1.88 a	2.500 a
SEM	2	0.00045	0.00045	0.00045
P- value	0.05	0.05	0.05	0.05

Value are means= 12n, at the same column, the values with at least one letter, were insignificant (p>0.05), SEM: standard error means, DTP: dried tomato pomace.

and at least on control group (p< 0.05). Also WBC values was decreased as supplemented DTP diets improved (p<0.05), with regard to H/L ratio (p<0.01) and control treatment showed the highest values compared to the other treated groups.

Data from Table 3 showed that the glucose concentration was decreased in treated groups and at the highest in control group whereas the highest total protein was in treatments groups and the lowest in control group (p<0.05). Significantly, the differences between supplemented treatments and control treatment were revealed, with highest value in treated groups and the lowest in control group.

The influence of adding DTP and vitamin C to the chicken diets on blood serum means was showed in Table 4. The addition of vitamin C and DTP decreased serum triglyceride, cholesterol and non-HDL concentration. Blood serum concentration of HDL increased supplementation vitamin C and DTP.

Table 5 represents the influence of experimental treatments on MDA concentration in egg yolk storage at 4°C for 14.28 and 42 days. Results showed that inclusion of vitamin C and DTP at level 1, 2 and 3% in the laying hens, significantly decrease MDA concentration in comparative to control group. However highest value of MDA concentration was observed at control groups and the lowest value was in DTP3% group of lowed by DTP 2 group, vitamin C group and DTP1% group (p<0.05) also MDA concentration increased as the eggs stoking period progressed. The result for egg yolk peroxidation of laying chickens treated with high heat stress are presented in Fig. 1. Inclusions vitamin C or different levels

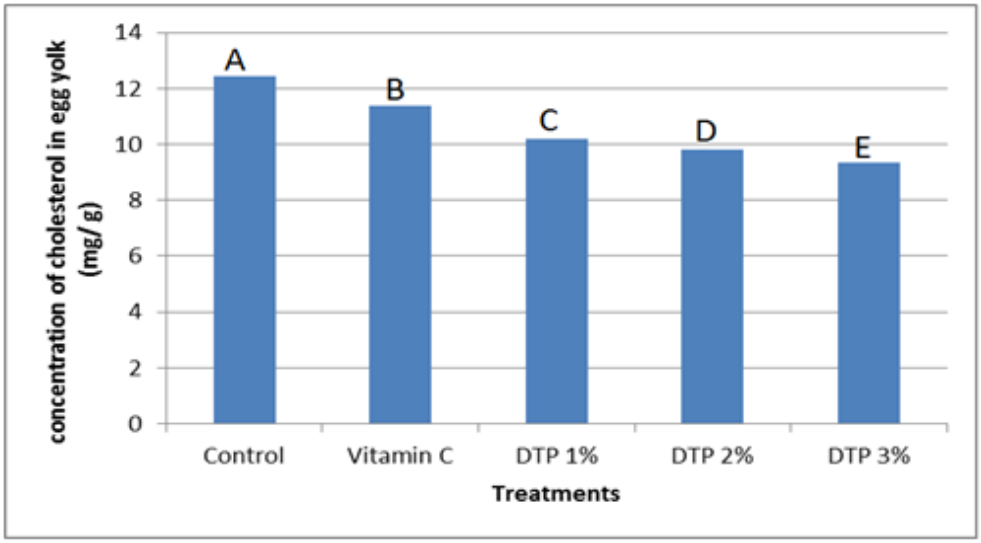

Fig. 1 : Egg yolk cholesterol concentration.

Table 4 : Effect of adding DTP and vitamin C on blood serum profile of laying chickens exposed to high ambient temperature.

Treatments	Parameters (mg/100 ml)			
	Triglycerides	Cholesterol	HDL-C	non-HDL
Control	1233 a	164 a	44 c	119 a
Vitamin C	884 b	99 a	61 b	37 a
DTP 1%	819 b	113 a	65 ab	47 a
DTP 2%	754 d	121 a	72 a	48 a
DTP 3%	468 c	100 a	71 a	29 a
SEM	2	2	2	2
P- value	0.05	0.05	0.05	0.05

Value are means = 12n, at the same column, the means with at least one common letter, were insignificant (p>0.05), SEM: standard error means, DTP: dried tomato pomace.

Table 5 : The influence of adding DTP and vitamin C on the egg yolk peroxidation (MDA), from egg stored at 4Cæ%, to 14, 28 and 42 days period.

Treatments	MDA (Malondialdehyde) Conant ration				
	Fresh	14 day	28 day	42 day	Mean
Control	0.335	0.355	0.965	1.070	0.681 a
Vitamin C	0.230	0.230	0.250	0.310	0.255 b
DTP 1%	0.251	0.245	0.275	0.325	0.274 b
DTP 2%	0.220	0. 230	0.240	0.250	0.235 cb
DTP 3%	0.180	0.190	0.200	0.230	0.200 c
SEM	0.0045	0.0045	0.0045	0.0045	0.0045
P. value	0.05	0.05	0.05	0.05	0.05

At the same column, the values with at least one common letter, were insignificant (p>0.05), DTP: dried tomato pomace, Value are means= 12n, SEM: standard error means

of DTP in laying hens diet, egg yolk peroxidation value reduced with vitamin C and with DTP increased.

DISCUSSION

The addition of DTP and vitamin C enhanced the performance in terms of blood cells, lipid profile, blood biochemistry, decreased egg yolk cholesterol and increase egg yolk stability in laying hens exposed to high ambient temperature (34°C± 1.5). Regarding the supplementation of dietary vitamin C, findings of this research were consistent with results of other works (Sahin *et al*, 2003; Kucuk *et al*, 2003; Chung *et al*, 2005), which found that vitamin C supplementation was a valuable in lessening some of heat stress associated with physical responses and enhancing heat resistance by their antioxidant influences. Several studies have suggested that vitamin C has a role as synergistically and entered in a few biochemical reactions and its role was due to it is reversible oxidation and reduction traits inside the cells such as mixed function including integration of oxygen in the substrate (McDowell, 1989; Gey, 1998; Frei *et al*, 1990). Additionally, vitamin C itself assumes a critical part not just in exchanging radical counterparts from lipid stages to fluid compartments but also in reacting with all aggressive oxygen types under development of individual idle radical (Gey, 1998). Serum MDA concentration diminished when vitamin C and E were added, demonstrating antioxidant impacts of them (Kucuk *et al*, 2003). Heat stress leads to an expanded generation of MDA in blood serum and liver, hence bringing about creation of free radicals (Bollengier-Lee *et al*, 1999; Halliwell and Gutteridge, 1989). In attempted to alternate the negative effects of heat stress, a few nutritional methodologies such as diet supplementation with antioxidant, vitamins, minerals and phytochemical have been estimated (Sahin *et al*, 2008; Selim *et al*, 2013; Habibian *et al*, 2014). Tomato pomace, contain antioxidant compounds including flavonoids, á-tocopherol, lycopene, folate, vitamin, â-carotene, and

phenolic (Sahin *et al*, 2008; Hosseini-Vashan *et al*, 2015; Selim *et al*, 2013). Among dietary carotenoids, lycopene is a strong antioxidant that prevents ROS production and associated with undesirable effects (Palozza *et al*, 2011) as well as exhibits cholesterol and lowering some cancers effect in human beings (Gerster, 1997; Ševcíková *et al*, 2008). Additionally, addition of tomato puree at the level 1% resulted in an increase in the antioxidant amplitude and decreased MDA value under heat stress conditions of broiler chickens (Selim *et al*, 2013).

However, levels of DTP had a significant influence on blood traits in layer chickens. In country to the control treatment, these findings were consistent with another research results of Safamehr *et al* (2011), Jalalinasab *et al* (2014). Several researches have demonstrated the feasibility of feeding tomato residues in poultry. Moreover, several studies have detected that addition of DTP to laying chicken diets increased egg production, feed intake, yolk color (Nobakht and Safamehr, 2007; Jafari *et al*, 2006; Jalalinasab *et al*, 2014; Mansoori *et al*, 2008). However, insignificant effect of feeding DTP on low-density lipoproteins (LDL) and cholesterol of blood plasma in laying hens was revealed (Nobakht and Safamehr, 2007) as well as on the triglyceride contents albumin, glucose and globulin (Rahmatnejad *et al*, 2009).

Moreover, tomato's pectin increase the lecithin cholesterol acyl transferase activity in blood plasma and reduced ($p<0.05$) the cholesterol of blood serum in rats. Blum *et al* (2006) pointed the increased level of HDL-cholesterol in diets rich with tomato (Cohen, 2002; Giovannucci *et al*, 2002). The effects of lycopene on the enlistment of foam cell arrangement by adjusted LDL was explored (Napolitano *et al*, 2007). Their outcomes revealed that lycopene may decrease the macrophage foam cell formation resulted by adjustment of LDL by lessening lipid combination as well as down managing the activity and expression of scavenger receptor activity. Lycopene has a compelling free radical scavenging movement, and this activity could be valuable to chickens due to harmful free radicals were shaped under heat stress condition, fast growth, high reproduction rates and escalated metabolism states of poultry industry. Ševcíková *et al* (2008) found a critical part in the antioxidant defense system can be played by lycopene.

CONCLUSION

Vitamin C Supplementation (300 mg/kg) or levels 1, 2 and 3% of DTP improved some blood parameters, decreased triglycerides, MDA and increased HDL cholesterol concentration of heat stressed laying hens. Although DTP, 1, 2 and 3% in the diet showed to be a substitute for vitamin C in layer ration producing comparable or even superior to vitamin C.

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