treated mice developed massive liver necrosis (Chow and Hong, 2002). Oxidative damage which is a consequence of excessive oxidative stress and/or insufficient antioxidant potential (Chow, 1979; 1991; Sies, 1986; Halliwell, 1987), is the other consequence of nitrate or nitrite treating in animals. There are very limited reports regarding the possible detrimental effects of nitrate or nitrite on poultry performance. Water nitrate has been reported to decrease the growth rate of animals especially broiler chickens (Barton et al., 1986). In slow growing native breed (Balady), dietary sodium nitrate (4.2 gr/kg) have retarded growth and caused methaemoglobinaemia (Atef et al., 1991). No information is available about the negative effects of nitrate consumption on lying performance, blood indices and enzymes activities in broiler breeder. Hence, recent experiment was conducted to investigate the effects of dietary sodium nitrite consumption on egg quality and quantity parameters and some blood indices of native breeder hens of West Azerbaijan hens. Pyruvate and lactate are the end products in the process of glycolysis (Mannen et al., 1997). Lactate dehydrogenase (LDH) is an oxidoreductase enzyme which catalyzes the interconversion of lactate and pyruvate. When disease or injury affects tissues containing LDH, the cells release LDH into the bloodstream and caused the higher levels of LDH than normal levels. Therefore, LDH is the most often indices to evaluate the of tissues damages hence LDH activity was investiageted as the liver damage or toxicity indicator (Daneshyar et al., 2010). Moreover both the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes are released to the blood stream during the liver damages (Shim et al., 2004; Daneshyar et al., 2009) and hence were assessed in recent experiment. Liver injury such as massive liver necrosis (Chow and Hong, 2002) has been reported in nitrite toxicity.

MATERIALS AND METHODS

One hundred twenty-weeks-old hens (West Azerbaijan native hens) were used in this experiment. West Azerbaijan native hens are held in West Azerbaijan native hen support and breeding center which have been selected for egg production and body weight gain during 14 generations. These birds were divided between 10 replicate pens with 10 birds each. Five pens were allocated to each experimental group. One group was fed the control diet and the other was fed the same control diet supplemented with 4.2 g/kg sodium nitrate (Atef et al., 1991), and defined as the CD and ND groups respectively. Environmental temperature was set at 21°C. Sixteen h light and 8 h dark were used during the experimental period. Each bird was given 110 g of the experimental diets per day for seven weeks (Table 1).

Table 1. Composition of experimental diets

Ingredients (%)	Control diet	Sodium nitrate diet
Corn (8% protein)	59.88	59.88
Soybean meal (44% protein)	21.50	21.50
Soybean oil	3.75	3.75
Dicalcium phosphate	1.50	1.50
Oyster shell	11.64	11.64
Dl-methionine	0.11	0.11
Vitamin and mineral premix[1]	0.50	0.50
Salt	0.37	0.37
Sand	0.75	0.33
Sodium nitrate	0.00	0.42
Total	100	100
Calculated analysis		
ME (kcal/kg)	2,800	2,800
CP (%)	14.00	14.00
Calcium (%)	3.40	3.40
Available phosphorus (%)	0.34	0.34
Sodium (%)	0.18	0.18
Arginine (%)	0.74	0.74
Methionine+cystine (%)	0.59	0.59
Lysine (%)	0.68	0.68
Tryptophan (%)	0.14	0.14

[1] Supplied per kilogram of diet: retinol, 9,000 IU; cholecaciferol, 2,000 IU; alpha tocopherol, 181 IU; cyanocobalamin, 15 μg; riboflavin, 4.4 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 μg; thiamine, 4 mg; calcium, 99 mg; zinc, 84 mg; manganese, 60 mg; iodine, 0.922 mg; selenium, 0.2 mg; copper, 10 mg.

Before the experiment, the birds were adopted to the experimental diets for two weeks. The experimental diets were formulated to meet or exceed the broiler breeder requirements (Lesson and Summers, 2005). Hens were given free access to water. Then eggs were collected daily for five weeks, and their related egg mass and egg production were calculated and reported weekly. Two eggs from each pen collected for assess the egg quality parameters (shell stiffness, shell thickness, yolk color and haugh units) at first 3 consecutive days of each week (Saturday, Sunday and Monday). Egg shell thickness was measured using a micrometer (OSK-13496, Ogawa Seiki CO., LTD. Japan). Albumin height was determined using a special instrument (OSK-13471, Ogawa Seiki CO., LTD. Japan) and used for calculation of Haugh unit according the formula of Eisen et al. (1962) that presented below. Shell stiffness and yolk color were measured by egg shell intensity meter (Model 1618, Tokyo, Japan) and Roche color fan system respectively. At the end of the experiment (wk 32 of age), one hen per pen selected randomly and used for taking the blood samples. Blood samples were taken from the wing vein by venoject and transferred to anticoagulant tubes (EDTA). Then the blood samples were

centrifugated at 5,000 rpm for 5 min. The plasma samples were stored at -20°C until the later analysis. Plasma samples were thawed in lab and their total antioxidant capacity were determined using Randox total antioxidant status test kit (Randox Laboratories Ltd, UK). Moreover, plasma total protein, total cholesterol and triglyceride contents and LDH, AST, and ALT activities were determined enzymatically by Pars Azmon kits (Pars Azmon Co., Iran) using a spectrophotometer (Alcyon 300, USA). The experimental data were analyzed based a completely randomized design with 2 treatments and 5 replicate each. The blood indices data were subjected to GLM procedure of SAS (SAS Institute, 2002) whereas egg quantity and quality parameters were analyzed according the MIXED procedure of SAS by repeated measures. Duncan's multiple range test was used to separate the means when treatment means were significant (p≤0.05).

$$HU = 100 \log[H - \frac{\sqrt{G}(30W^{.0.37} - 100)}{100} + 1.9]$$

HU = Haugh unit
W = Egg weight (g)
H = Albumin height
G = 32.2

RESULTS AND DISCUSSION

The effect of dietary sodium nitrate consumption on egg weight, egg production and egg mass are shown in Table 2. No effect of sodium nitrate was observed on egg production and egg mass during wks 28 and 29 of age (p>0.05), but sodium nitrate decreased the both egg production and egg mass afterwards (during wks 30, 31 and 32 of age) (p<0.05). Moreover both the egg production and egg mass were increased over time (p<0.05). Dietary sodium nitrite declined the egg weight (p<0.001) at the last two weeks of experimental period (p<0.01). No significant treatment× time interaction was observed for egg weight, egg production and egg mass (p>0.05). The time increasing effect on egg production is related to the normal increase trend of egg production before peak (wk 28). This increasing trend in egg production has caused the same trend for egg mass. We started the experiment on wk 26 and egg collection on wk 28 of age.

Dietary sodium nitrate didn't affect the shell stiffness, shell thickness and Haugh unit (Table 3) (p>0.05). Furthermore, no effect of time or treatment×time were observed for shell stiffness (p>0.05). Over time, shell thickness was decreased while Haugh unit increased (p<0.05). Although a treatment effect was observed for yolk colour (p<0.05), no significant differences were observed

Table 2. Weekly egg production, egg mass and egg weight of birds fed the control diet (CD) or diets supplemented with 4.2 g/kg (ND)

	Treatment	Egg weight (gr)	Egg mass (gr)	Egg production (%)
Wk 28	CD	51.77	23.53	43.02[a]
	ND	47.48	20.54	40.14[a]
	Pooled SEM	1.46	1.81	3.30
	p value	0.15	0.44	0.68
Wk 29	CD	52.16	28.55	53.14[a]
	ND	49.74	22.55	43.71[a]
	Pooled SEM	1.36	2.05	3.56
	p value	0.40	0.15	0.20
Wk 30	CD	52.35	29.70[a]	56.55[a]
	ND	50.62	23.01[b]	44.0[b]
	Pooled SEM	0.88	1.57	2.98
	p value	0.36	0.021	0.025
Wk 31	CD	53.90[a]	33.53[a]	62.0[a]
	ND	51.77b	27.62b	47.0[b]
	Pooled SEM	0.56	1.40	3.22
	p value	0.003	0.023	0.008
Wk 32	CD	55.84[a]	34.69[a]	64.0[a]
	ND	50.39[b]	25.58b	46.0[b]
	Pooled SEM	1.21	2.20	4.47
	p value	0.01	0.03	0.02
Source of variation	Treatment	0.0007	0.02	0.05
	Time	0.10	0.0001	0.001
	Treatment×time	0.43	0.54	0.24

[a-b] Means in each column and time with no common superscript differ significantly (p<0.05).

Table 3. Weekly shell stiffness, shell thickness, haugh unit and yolk colour of eggs[1] from birds fed the control diet (CD) or diet supplemented with 4.2 g/kg sodium nitrate (ND)

	Treatment	Shell stiffness (kg/cm$^{2)}$)	Shell thickness (μm)	Haugh unit	Yolk color
Wk 28	CD	2.24	31.60	83.80	5.06
	ND	2.26	30.53	80.56	5.63
	SEM	0.19	0.49	1.72	0.20
	p value	0.95	0.28	0.35	0.15
Wk 29	CD	2.10	29.20	81.66	6.37
	ND	2.87	29.30	87.33	6.84
	SEM	0.34	0.52	1.590	0.12
	p value	0.26	0.93	0.070	0.08
Wk 30	CD	1.93	26.74	85.51	6.33
	ND	2.19	29.57	83.04	6.80
	SEM	0.14	0.97	1.05	0.15
	p value	0.37	0.14	0.24	0.10
Wk 31	CD	2.14	25.14	87.03	6.53
	ND	2.42	25.57	86.49	6.93
	SEM	0.15	0.48	1.35	0.13
	p value	0.36	0.66	0.84	0.13
Week 32	CD	2.18	25.80	87.24	6.55
	ND	2.40	25.53	86.72	6.94
	SEM	0.15	0.46	1.44	0.13
	p value	0.45	0.77	0.87	0.12
Source of variation	Treatment	0.34	0.38	0.72	0.02
	Time	0.23	0.0001	0.008	0.0001
	Treatment×time	0.92	0.41	0.03	0.89

[1] Thirty eggs per treatment (six eggs per replicate) were used for egg quality indices.

between the treatment during different weeks (p>0.05). Decreasing trend of shell thickness over time in recent experiment is possibly related to lower availability of calcium for more produced eggs. Because both the egg production and egg mass increased by time. The increased yolk color content of both groups during time is because of higher content of dietary pigments. Before the experiment, a diet with lower corn (wheat-corn-soybean meal diets) was used which was changed to experimental diets with higher corn content (corn-soybean diets). Higher color score of yolk is well recognized by dietary supplementation of pigments (Esfahani-Mashhour et al., 2009). In addition, higher color index of eggs from sodium nitrate fed birds could be connected to the higher access to the pigments in their smaller eggs (lower egg weight). No report is available regarding the plasma TP or TG changes by nitrate or nitrite consumption in birds. We expected some changes in the metabolism of TP and TG or the enzyme activities, because nitrate or nitrite results in formation of methemoglobin and low ability of blood cells to carry oxygen (Ger et al., 1996) and hypoxemia consequently. But as shown in Table 4, none of the plasma TP and TG or the activity of ALT, AST and LDH enzymes (wk 32 of age) were affected by dietary consumption of sodium nitrate at the end of experiment (p>0.05).

Sodium nitrite decreased both the TAC and TC at wk 32 of age (p<0.001). Lower plasma TAC content of ND birds is due to the higher peroxide production. Peroxide is a reactive oxygen specious (ROS) that cause oxidative damage in body. Excessive levels of ROS or proxynitrite

Table 4. Plasma total antioxidant capacity (TAC), total cholesterol (TC), total protein (TP) and total triglyceride (TG) and plasma activities of lactate dehydrogenase (LDH), aspartate aminoteransferase (AST) and alanine aminotransferase (ALT) of birds[1] fed the control diet (CD) or diets supplemented with 4.2 g/kg (ND) at the end of experiment (wk 32)

Parameters	CD	ND	Pooled SEM	p value
TAC (mmol/L)	1.06^a	0.66^b	0.08	0.0001
TC (mg/dl)	151.50^a	94.00^b	10.36	0.0001
ALT (U/L)	8.750	6.400	0.85	0.18
AST (U/L)	184.00	187.20	8.44	0.86
TP (gr/dl)	5.500	4.94	0.19	0.10
TG (mg/dl)	699.00	569.00	95.07	0.53
LDH (U/L)	501.30	718.80	76.95	0.17

$^{a-b}$ Means in each row with no common superscript differ significantly (p<0.05).

[1] Five birds per treatment (one per replicate) were used for determination of blood indices.

results in oxidative stress and causing severe damage to cellular components and even lipid peroxidation (Seven et al., 2010).

Rather than peroxide, nitrites and nitrates are the key oxidation products as well as ready sources of NO, which in turn react rapidly with superoxide to form reactive $ONOO^-$ (proxynitrite) (Chow et al., 2001). Proxynitrite causes the formation of methemoglobine, oxidative damages and consequently body stress and hypoxia. This process decreases the ATP production and as a result diminishes the egg mass and egg production that observed in our study. Formation of methemoglobin interferes with the ability of blood cells to carry oxygen. Fatal toxic methemoglobinemia has been indicated due to high levels of nitrite in drinking water or occupational exposure to nitrite (Ger et al., 1996). Declined plasma TC of nitrate fed birds in recent experiment may be because of cholesterol oxidation by produced free radicals from nitric oxide (Drexler et al., 1991). Liao et al. (1995) have shown that oxidized LDL decreases the expression of endothelial nitric oxide synthase. Moreover their reports indicated that nitric oxide generation is decreased in hypercholesterolemia.

The results of this study indicate that consumption of sodium nitrate in native hens diminishes the body antioxidant capacity and then induces hypoxia which is consequently decreases the egg weight, egg production and egg mass. Furthermore, declined plasma TC of nitrate fed birds possibly is due to cholesterol oxidation effect of produced free radicals from nitric oxide. Higher yolk color index of sodium nitrate fed birds related to the higher availability of pigments for their smaller eggs.

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