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Effect of Strain, Type of Natural Antioxidant and Sulphate Ion on Productive, Physiological and Hatching Performance of Native Laying Hens

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Abstract: A total number of 480 hens and 48 cocks from Inshas and Dokki4 strains of 32 weeks old were divided into 12 groups with 2 replicates each (20 hens+2 cocks). The experiment had a 2×3×2 factorial arrangement of treatments with two strains (Inshas and Dokki4), three types of natural antioxidants in the diet (control, 0.25% thyme and 0.25% anise) and two levels of sodium sulphate (0 and 0.5%). The experimental groups were fed on the experimental diets from 32 to 44 weeks of age. After 4 weeks of the experiment, the eggs were collected from each treatment were incubated weekly in the incubator. Fertility % and hatchability % of the total eggs and fertile eggs were calculated and the main results obtained can be summarized as follows: Addition of thyme or anise to laying hens diet numerically increased egg number and improved feed conversion. Addition of thyme or anise increased antioxidant capacity in plasma, while decreased LDL, HDL, total cholesterol, triglyceride and total lipids in blood plasma, liver and yolk extract. Addition of thyme tended to improve fertility and hatchability while anise tended to decrease these parameters. The response to sulphate depended on the strain and type of natural antioxidant. The combination of thyme and sulphate is the most successful additive for improving fertility and hatchability under the condition of this study.

Key words: Hen strain, natural antioxidant, sulphate ion, physiological and hatching performance

Introduction

The broiler breeder egg hatchability ranged from 79 to 82% whereas turkey egg hatchability ranged from 76 to 80% and this mean 20% loss of incubated broiler and turkey eggs had a large economic impact on the industry (Schaal and Cherian, 2007). Chick embryos may be subjected to stress caused by excessive production of heat during the latter part of egg incubation (Tullett, 1990). Chick embryo development is associated with an accumulation of polyunsaturated fatty acids in tissue lipids (Speake *et al.*, 1998) making them susceptible to lipid peroxidation (Surai, 1999a). Therefore, the integrated antioxidant systems in the chicken tissues are responsible for protection of polyunsaturated fatty acids, protein and DNA from damaging effect of free radicals and toxic products of their metabolism (Surai *et al.*, 2003). The antioxidant system of developing embryo and newly hatched chick includes fat-soluble antioxidants such as vitamin E (Surai, 1999b) carotenoids (Surai *et al.*, 2001 a and b) and water-soluble antioxidants including ascorbic acid and reduced glutathione (GSH), as well as antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase and catalase (Surai, 1999b and 2002). The enhancement of the antioxidant system of the developing chick as a result of maternal diet manipulation presents great opportunities for poultry producers. Recently, Liaurado *et al.* (1997) reported a 5.

6% hatchability improvement by adding 6 mg canthaxanthin/kg commercial broiler breeder's diet. Carotenoids used by (Surai *et al.*, 2001 a and b) and Canthaxanthin used by Surai *et al.* (2003) have higher molecular weight but other antioxidants like thymol or carvacrol (the main phenolic components in thyme) have a lower molecular weight. It is tempting to assume that supplementation with exogenous natural antioxidants or pharmacologic use of synthetic low molecular weight antioxidants can offer relatively simple and effective ways to control oxidative stress (Modriansky *et al.*, 2002). The thyme species have higher antioxidant capacity (Miguel *et al.*, 2004). Gulcin *et al.* (2003) found that extract anise (*Pimpinella anisum* L.) seed possessed antioxidant and antimicrobial activity. Botsoglou *et al.* (1997) found that hens fed thyme have a lower concentration of malonaldehyde in yolk compared with hen fed control diets and indicated that possible transfer of the antioxidant constituents of thyme into the hen through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into the yolk. Cao *et al.* (1998) found with human that the increased serum antioxidant capacity after the treatments of strawberries and spinach and indicated the possible absorption of phenolic compounds in these diets. Miller (1974) suggested that the sulphate may be a partial substitute for methionine when the latter was present at

Table 1: Composition and calculated analysis of the control basal diet

Ingredients	%
Yellow corn	63.50
Soybean meal (44%)	24.57
Wheat bran	2.00
Lime stone	7.77
Premix*	0.30
Salt	0.30
Di-calcium phosphate	1.50
DL- methionine	0.06
Total	100.00
Calculated analysis**	
CP%	16.000
Kcal ME /kg	2703.340
Crude fiber%	3.470
Crude fat %	2.860
Calcium %	3.320
Available phosphorus %	0.406
Lysine %	0.889
Methionine %	0.350
Methionine+Cystine %	0.620
Sodium%	0.135

*Premix contain per 3kg vit A 12 000 000, vit D3 3000 000 IU, vit E 50000mg, vit K3 3000mg, vit B1 2000mg, vit B2 7500mg, vit B6 3500 mg, vit B12 15mg, Pantothenic acid 12000mg, Niacin 30000mg, Biotin 150mg, Folic acid 1500mg, Choline 300gm, Selenium 300mg, Copper 10000mg, Iron 40000mg, Manganese 80000mg, Zinc 80000mg, Iodine 2000mg, Cobalt 250 mg and CaCO₃ to 3000g, **According to Egyptian Feed Composition Tables for Animal and Poultry Feedstuffs (2001)

inadequate dietary levels. Sulphate also, decreased the negative effects of toxicity due to aflatoxicosis (Qota *et al.*, 2005; Ali *et al.*, 2006). Yeh and Yen (2006) showed that the supplementation of natural phenolic acids through a balanced diet containing enough fruits and vegetables could be the most effective in inducing of phase II sulphate conjugation enzyme. Qi *et al.* (2005) found that high sulphate content ulvans had more effective scavenging activity on hydroxyl radical than natural ulvan. Yuan *et al.* (2005) found that the oversulphated and acetylated derived of carrageenan oligosaccharides exhibited higher antioxidant activity than the polysaccharides and oligosaccharides in certain antioxidant systems and indicated that the sulphation and acetylation can increase the scavenging activity. Azuma *et al.* (2000) found that absorbed flavonoids are present in the common blood circulation in the form of glucuronide, sulphate and methylate conjugates are excreted via urine or bile while chlorogenic acid (important phenolic antioxidants) remained intact in the small intestine. Ninfali *et al.* (2005) suggest that not all of the ingested phenolics can reach the plasma, but those escape in blood stream lead to a significant increase in total plasmatic antioxidant capacity, both when they are in the free a glyconic form and when they are glucuronide or sulphate conjugated form. Bartelt and Kirinya (1976) found that liver slices from geese, ducks and chickens contained high sulphate conjugation enzyme activities.

The present study was designed to assess the effect of strain, type of natural antioxidant and sulphate ion on productive, physiological and hatching performance of two strains of native laying hens.

Materials and Methods

This study was conducted at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. A total number of 480 hens plus 48 cocks from Inshas and Dokki4 strains of 32 weeks old were equally divided into 12 groups of 2 replicates (20 hens+2 cocks each) and housed in floor pens (280 cm long×220 cm wide).

Six experimental diets were prepared from a hen basal diet (Table 1). The experiment had a 2×3×2 factorial arrangement of treatments with two strains (Inshas and Dokki4), three types of natural antioxidants (control, 0.25% thyme and 0.25% anise) and two levels of sodium sulphate (0 and 0.5%). The experimental diets were offered for the two strains (240 hens from each strain) from 32-44 weeks of age as follows:

- 1) The basal diet without any supplement serves as a control diet (C-diet)
- 2) C-diet+0.25% thyme (T-diet)
- 3) C-diet+0.25% anise (A-diet)
- 4) C-diet+0.5% sodium sulphate
- 5) T-diet+0.5% sodium sulphate
- 6) A-diet+0.5% sodium sulphate

Characteristics investigated included Weight Gain (WG), Egg Number (EN), Egg Weight (EW), Egg Mass (EM), Feed Intake (FI), Feed Conversion (FC) and some reproductive performance. EM was calculated as gram egg per hen per experiment period. FI was calculated as gram feed per hen per day. FC was calculated as gram feed consumed per gram egg produced. At 6th and 12th weeks of the experiment, a total number of 240 eggs (20 eggs from each treatment) was taken to determine egg quality. At the end of the experimental period (44 weeks of age), 3 hens from each treatment were randomly selected, weighed, slaughtered and sacrificed to obtain relative organs weight and tissues analysis.

Blood samples were taken (3 from each treatment) after slaughter into dry clean centrifuge tubes containing drops of heparin, centrifuged at 4000 rpm for 15 minutes and the clear plasma was separated and stored in deep freezer at -20°C. Blood constituents including Ca, P, Low Density Lipoprotein (LDL), total cholesterol, triglycerides and total lipids were determined by colorimetric methods using commercial kits. Antioxidant capacity in plasma was determined using commercial kit produced by Biodiagnostic Company. Samples of liver from each treatment were prepared to determine liver parameters according to Folch *et al.* (1957). Liver cholesterol, LDL, triglycerides and total lipids were determined using suitable commercial kits. After measuring the egg

Table 2: Effect of strain, natural antioxidant and sulphate on the performance of Inshas and Dokki4 hens during the experimental period

Item	EN hen/period	EW (g)	EM (g)	FI g/hen/day	FC	WG (g)		
Strain effect								
Inshas	56.05±1.27	51.42±0.18	2882±64.66	96.08±0.60	3.21±0.06	148.02±19.44		
Dokki	60.70±1.52	47.92±10.22	2910±77.65	95.16±0.62	3.16±0.09	27.53±5.70		
Antioxidant effect								
Control	56.16±1.73	49.88±0.71	2800±92.79	95.75±0.86	3.30±0.12	118.15±21.00		
Thyme	60.06±2.03	49.77±0.66	2982±73.09	96.16±0.71	3.10±0.08	35.86±10.30		
Anise	58.91±1.83	49.36±0.73	2905±88.55	94.95±0.69	3.15±0.09	109.30±15.50		
Sulphate effect								
0	58.17±1.60	49.64±0.54	2885±76.47	96.26±0.56	3.22±0.08	78.33±10.30		
0.5	58.87±1.53	49.70±0.58	2906±66.18	94.98±0.62	3.15±0.07	97.21±12.76		
Source of variation								
			----- P value -----					
Strain	0.04	0.0001	NS	NS	NS	0.0003		
antioxidant	NS	NS	NS	NS	NS	0.0327		
Strain*antioxidant	NS	NS	NS	NS	NS	NS		
sulphate	NS	NS	NS	NS	NS	NS		
strain*sulphate	NS	NS	NS	NS	NS	NS		
antioxidant*sulphate	NS	NS	NS	NS	NS	NS		
strain*antioxidant*sulphate	NS	NS	NS	NS	NS	NS		
Treatments								
Strain	Type of antioxidant	Sodium Sulphate	EN (hen/period)	EW (g)	EM (g)	FI (g/day/hen)	FC	WG (g)
Inshas	C	0	59.57±2.02	51.03±0.23	3040±117.20	96.7±1.58	3.05±0.06	173.2 ^{ab} ±24.0
	T	0	53.52±3.82	51.73±0.56	2771±228.06	97.2±1.65	3.38±0.22	52.1 ^{bc} ±20.0
	A	0	55.02±3.22	51.33±0.07	2824±161.60	95.77±2.37	3.26±0.10	162.2 ^{ab} ±30.7
	C	0.5	52.45±4.55	52.28±0.42	2744±260.10	93.60±0.18	3.30±0.31	150.5 ^{ab} ±35.3
	T	0.5	57.92±3.62	51.13±0.53	2959±154.00	97.19±0.58	3.15±0.14	115.7 ^{ab} ±8.0
Dokki4	A	0.5	57.80±2.40	51.04±0.45	2951±148.70	95.92±1.79	3.13±0.21	235.7 ^{cd} ±5.5
	C	0	56.25±5.15	47.66±0.30	2679±228.00	98.14±0.43	3.54±0.31	81.5 ^{cd} ±6.3
	T	0	63.55±0.70	48.50±0.57	3081±2.37	95.17±0.42	2.96±0.01	-6.9 [±] 20.9
	A	0	61.15±6.25	47.61±0.78	2916±34.5	94.49±0.91	3.15±0.34	7.93 [±] 10.5
	C	0.5	56.37±2.77	48.55±0.59	2739±168.0	94.50±2.32	3.32±0.28	67.2 ^{cd} ±5.4
	T	0.5	65.25±0.90	47.74±0.11	3115±35.36	95.07±2.60	2.92±0.04	-16.7 [±] 20.4
	A	0.5	61.67±3.17	47.46±0.90	2930±206.0	93.60±0.23	3.08±0.20	31.8 ^{cd} ±5.6

C = control, T = thyme, A = anise, ^aMeans in the same column with different letters, differ significantly (p<0.05). Means±standard error

quality, 3 yolk samples from each treatment were separated from the broken eggs and extracted to determine yolk parameters according to Folch *et al.* (1957). Yolk cholesterol, LDL, triglycerides and total lipids were determined using suitable commercial kits. High Density Lipoprotein (HDL) concentration of each assayed sample (plasma, liver or yolk) was calculated by subtracting the LDL value from its total cholesterol. After 4 weeks from beginning of the experiment, a total number of 13440 eggs (70 eggs from each replicate/week) were incubated to evaluate the reproductive traits. Sodium Sulphate was supplied by the Egyptian Salt and Mineral Company. The thyme and anise were purchased from local market in Cairo. The statistical analysis was computed using the General Linear Models (GLM) procedure and the significant differences among treatments means were separated by Duncan's Multiple Range test, the procedure described in the SAS (SAS, 1990).

Results and Discussion

Production performance: The data in Table 2 showed that there was significant effect of strain on EN and EW. The data showed that the hens of Dokki4 recorded higher EN and lower EW than those of Inshas. The data in Table 2 indicated that there insignificant effect of

antioxidant on EW, EN, EM, FI and FC. However, significant effect of antioxidant only was observed on WG. The addition of thyme significantly decreased WG compared of hens fed control or anise diet. The sulphate effect on production performance found to be insignificant. Table 2 showed that with Dokki4, the addition of thyme or anise alone or in combination with sulphate to hens diets numerically increased EN and improved FC compared to hens fed control diet. These results disagree with El-Deeb *et al.* (2007) who found with quail that main effect of anise treatment significantly decreased egg laying rate by 10. 3% compared to control group. The beneficial effect of thyme with Dokki4 strain may be due to the phenolic compounds carvacrol and thymol present in thyme which considerably exhibit antimicrobial and antifungicidal activity (Basilico and Basilico, 1999). Also, it has been reported that dietary feeding essential oil extracted from herbs improved the secretion of digestive enzymes (Jang *et al.*, 2004). The data in Table 2 showed that the addition of thyme or anise alone or in combination with sulphate to basal diet decreased WG for local Dokki4 compared to hens fed control diet while anise+sulphate increased WG of Inshas hens. The anise seed itself is mildly estrogenic, this effect may substantiate the herbs use as a stimulant of sexual drive in female for milk production (Chevallier, 1996). The anise seed may increase the gain of hen by

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Table 3: Effect of strain, natural antioxidants and sulphate on some egg quality (yolk and shell) of Inshas and Dokki4 hens during the experimental period

Item			Yolk diameter (mm)	Yolk height (mm)	Yolk weight (g)	Yolk weight %	
Strain effect							
Inshas			38.63±0.21	17.78±0.17	17.72±0.18	32.78±0.31	
Dokki4			38.42±0.26	17.67±0.13	17.43±0.17	34.31±0.25	
Antioxidant effect							
Control			38.51±0.28	17.67±0.18	17.84±0.23	33.93±0.36	
Thyme			38.82±0.29	18.05±0.17	17.68±0.22	33.50±0.35	
Anise			38.25±0.29	17.46±0.18	17.20±0.19	33.20±0.37	
Sulphate effect							
0			39.10±0.23	17.53±0.15	17.52±0.17	33.52±0.30	
0.5			37.95±0.22	17.92±0.14	17.63±0.18	33.56±0.29	
Source of variation							
			----- P value -----				
strain			NS	NS	NS	0.0002	
antioxidant			NS	NS	NS	NS	
strain*antioxidant			0.02	0.04	NS	NS	
sulphate			0.0005	NS	NS	NS	
strain*sulphate			NS	0.01	0.0087	NS	
antioxidant*sulphate			NS	NS	NS	NS	
strain*antioxidant*sulphate			NS	NS	NS	NS	
Treatments							
Strain	Type of antioxidant	Sodium Sulphate	Yolk diameter (mm)	Yolk height (mm)	Yolk weight (g)	Yolk weight %	
Inshas	C	0	39.57 ^{ab} ±0.56	17.66 ^{ab} ±0.45	17.27 ^{ab} ±0.46	32.19 ^b ±0.61	
	T	0	39.14 ^{abc} ±0.44	18.71 ^a ±0.34	17.42 ^{ab} ±0.58	31.95 ^b ±0.93	
	A	0	38.21 ^{cd} ±0.52	17.17 ^b ±0.49	17.30 ^{ab} ±0.38	33.19 ^{ab} ±0.75	
	C	0.5	38.85 ^{abc} ±0.41	17.32 ^b ±0.36	18.37 ^a ±0.44	33.48 ^{ab} ±0.78	
	T	0.5	38.42 ^{abc} ±0.55	18.20 ^{ab} ±0.34	18.62 ^a ±0.32	33.49 ^{ab} ±0.65	
Dokki4	A	0.5	37.57 ^d ±0.53	17.60 ^{ab} ±0.40	17.33 ^{ab} ±0.45	32.35 ^b ±0.88	
	C	0	38.28 ^{cd} ±0.53	17.50 ^b ±0.22	17.72 ^{ab} ±0.43	35.05 ^a ±0.71	
	T	0	40.14 ^a ±0.63	17.07 ^b ±0.22	18.01 ^{ab} ±0.42	34.67 ^a ±0.55	
	A	0	39.28 ^{bc} ±0.69	17.07 ^b ±0.24	17.39 ^{ab} ±0.36	34.10 ^{ab} ±0.58	
	C	0.5	37.35 ^d ±0.64	18.21 ^{ab} ±0.39	18.00 ^{ab} ±0.55	34.98 ^a ±0.60	
T	0.5	37.57 ^{cd} ±0.57	18.21 ^{ab} ±0.38	16.65 ^b ±0.32	33.90 ^{ab} ±0.44		
A	0.5	37.92 ^{bc} ±0.52	18.00 ^{ab} ±0.29	16.80 ^b ±0.35	33.19 ^{ab} ±0.74		
Item							
			Yolk index	Yolk color	Shell weight (g)	Shell weight%	Shell thickness (mm)
Strain effect							
Inshas			46.15±0.52	7.94±0.05	5.31±0.05	9.83±0.09	0.390±0.003
Dokki4			46.22±0.51	7.92±0.02	5.09±0.06	10.02±0.09	0.404±0.003
Antioxidant effect							
Control			46.05±0.62	7.96±0.05	5.20±0.06	9.90±0.11	0.395±0.004
Thyme			46.76±0.67	7.89±0.05	5.31±0.06	10.07±0.10	0.403±0.004
Anise			45.80±0.60	7.94±0.04	5.10±0.08	9.81±0.12	0.393±0.004
Sulphate effect							
0			44.97 ^b ±0.48	7.88±0.05	5.17±0.05	9.89±0.09	0.395±0.003
0.5			47.40 ^a ±0.51	7.98±0.02	5.23±0.06	9.96±0.10	0.400±0.003
Source of variation							
			----- P value -----				
strain			NS	NS	0.0080	NS	0.005
antioxidant			NS	NS	NS	NS	NS
strain*antioxidant			0.01	NS	NS	NS	NS
sulphate			0.0005	NS	NS	NS	NS
strain*sulphate			0.006	NS	NS	NS	NS
antioxidant*sulphate			NS	NS	NS	NS	NS
strain*antioxidant*sulphate			NS	NS	NS	NS	NS
Treatments							
Strain	Type of antioxidant	Sodium Sulphate	Yolk index	Yolk color	Shell weight (g)	Shell weight %	Shell thickness (mm)
Inshas	C	0	44.73 ^{cd} ±1.26	8.14±0.14	5.15 ^{ab} ±0.12	9.61±0.18	0.382±0.008
	T	0	47.94 ^{ab} ±1.23	7.71±0.19	5.34 ^{abc} ±0.10	9.81±0.18	0.390 ^a ±0.008
	A	0	45.01 ^{bc} ±1.27	7.78±0.10	5.20 ^{bc} ±0.15	9.96±0.19	0.396 ^{bc} ±0.007
	C	0.5	44.67 ^{cd} ±1.08	7.85±0.09	5.45 ^a ±0.12	9.95±0.29	0.390 ^{bc} ±0.008
	T	0.5	47.54 ^{ab} ±1.31	7.65±0.1	5.46 ^{bc} ±0.15	9.78±0.16	0.390 ^{bc} ±0.009
Dokki4	A	0.5	47.04 ^{ab} ±1.39	8.10±0.09	5.29 ^{bc} ±0.13	9.90±0.30	0.392 ^{bc} ±0.008
	C	0	45.87 ^{bc} ±1.04	7.92±0.07	4.97 ^{bc} ±0.11	9.83±0.19	0.391 ^{bc} ±0.007
	T	0	42.66 ^a ±0.88	7.85±0.09	5.42 ^{bc} ±0.10	10.45±0.20	0.420±0.006
	A	0	43.62 ^{ab} ±0.96	7.85±0.09	4.95 ^{bc} ±0.17	9.71±0.30	0.389 ^{bc} ±0.011
	C	0.5	48.94 ^a ±1.32	7.92±0.07	5.25 ^{bc} ±0.14	10.20±0.18	0.416 ^{bc} ±0.006
T	0.5	48.67 ^{ab} ±1.37	8.00±0.10	5.03 ^{bc} ±0.13	10.26±0.26	0.412 ^{bc} ±0.006	
A	0.5	47.55 ^{ab} ±0.93	7.85±0.10	4.94 ^{bc} ±0.19	9.69±0.22	0.397 ^{bc} ±0.010	

C = control, T = thyme, A = anise, ** Means in the same column with different letters, differ significantly (P<0.05). Means±standard error

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Table 4: Effect of strain, natural antioxidant and sulphate on some egg quality (shape and albumin) of Inshas and Dokki4 hens during the experimental period

Item	Egg length (mm)	Egg breadth (mm)	Shape index (%)	Albumin height (mm)		
Strain effect						
Inshas	55.7 ^a ±0.22	41.58±0.13	74.63 ^b ±0.29	5.84±0.11		
Dokki4	53.9 ^b ±0.20	41.21±0.17	76.49 ^a ±0.39	6.05±0.11		
Antioxidant effect						
Control	55.10±0.30	41.42±0.15	75.25±0.35	5.98±0.15		
Thyme	54.82±0.28	41.60±0.24	75.98±0.53	5.91±0.12		
Anise	54.60±0.27	41.16±0.17	75.45±0.41	5.96±0.14		
Sulphate effect						
0	54.77±0.21	41.47±0.18	75.80±0.42	6.03±0.12		
0.5	54.91±0.25	41.32±0.13	75.32±0.29	5.87±0.10		
----- P value -----						
Source of variation						
strain	0.0001	NS	0.0003	NS		
antioxidant	NS	NS	NS	NS		
strain*antioxidant	NS	NS	NS	NS		
sulphate	NS	NS	NS	NS		
strain*sulphate	0.04	NS	NS	0.001		
antioxidant*sulphate	NS	NS	NS	NS		
strain*antioxidant*sulphate	NS	NS	NS	NS		
Treatments						
Strain	Type of antioxidant	Sodium Sulphate	Egg Length (mm)	Egg breadth (mm)	Shape index%	Albumin height (mm)
Inshas	C	0	55.07 ^{bc} ±0.62	41.85±0.36	76.08 ^{abc} ±0.79	5.54 ^c ±0.33
	T	0	55.78 ^{abc} ±0.50	41.71±0.32	74.81 ^b ±0.57	5.63 ^b ±0.31
	A	0	55.28 ^{bc} ±0.49	40.92±0.32	74.10 ^b ±0.85	5.50 ^c ±0.36
	C	0.5	56.92 ^a ±0.59	41.85±0.23	73.60 ^a ±0.64	6.16 ^{abc} ±0.21
	T	0.5	56.28 ^{ab} ±0.42	42.00±0.33	74.64 ^b ±0.62	6.02 ^{abc} ±0.19
Dokki4	A	0.5	55.21 ^{bcd} ±0.59	41.14±0.37	74.57 ^{bc} ±0.72	6.20 ^{abc} ±0.20
	C	0	54.21 ^{cde} ±0.52	40.85±0.25	75.44 ^{ab} ±0.78	6.71 ^a ±0.22
	T	0	54.21 ^{cde} ±0.51	42.07±0.78	77.72 ^a ±1.76	6.42 ^{bc} ±0.22
	A	0	54.07 ^{de} ±0.45	41.42±0.40	76.67 ^{ab} ±0.90	6.35 ^{abc} ±0.22
	C	0.5	54.21 ^{cde} ±0.42	41.14±0.32	75.90 ^{ab} ±0.44	5.50 ^c ±0.32
	T	0.5	53.00 ^d ±0.43	40.64±0.22	77.00 ^{ab} ±1.65	5.57 ^b ±0.22
	A	0.5	53.85 ^d ±0.61	41.14±0.31	76.47 ^{ab} ±0.72	5.78 ^b ±0.29
Item			Albumin weight (g)	Albumin weight %	Haugh unit %	
Strain effect						
Inshas			31.15 ^a ±0.39	57.38 ^a ±0.33	76.85 ^a ±0.88	
Dokki4			28.30 ^b ±0.26	55.65 ^a ±0.26	79.46 ^a ±0.80	
Antioxidant effect						
Control			29.68±0.50	56.16±0.41	78.00±1.08	
Thyme			29.83±0.40	56.41±0.37	78.01±0.92	
Anise			29.68±0.46	56.97±0.37	78.46±1.13	
Sulphate effect						
0			29.64±0.32	56.57±0.31	78.73±0.95	
0.5			29.81±0.41	56.46±0.32	77.58±0.74	
----- P value -----						
Source of variation						
strain		0.0001		0.0001		0.02
antioxidant		NS		NS		NS
strain*antioxidant		NS		NS		NS
sulphate		NS		NS		NS
strain*sulphate		NS		NS		0.0001
antioxidant*sulphate		NS		NS		NS
strain*antioxidant*sulphate		NS		NS		NS
Treatments						
Strain	Type of antioxidant	Sodium Sulphate	Albumin weight (g)	Albumin weight %	Haugh unit %	
Inshas	C	0	31.28 ^{ab} ±0.88	58.19±0.62	74.42 ^a ±2.56	
	T	0	31.76 ^a ±0.76	58.22 ^a ±0.92	75.14 ^a ±2.21	
	A	0	29.72 ^{abc} ±0.75	56.84 ^{ab} ±0.67	74.64 ^a ±3.05	
	C	0.5	31.29 ^{ab} ±1.23	56.53 ^{ab} ±0.93	79.14 ^{ab} ±1.58	
	T	0.5	31.67 ^a ±0.85	56.71 ^{ab} ±0.64	77.85 ^{ab} ±1.63	
Dokki4	A	0.5	31.21 ^{ab} ±1.28	57.74 ^a ±1.07	79.92 ^{ab} ±1.33	
	C	0	27.96 ^c ±0.82	55.11 ^b ±0.77	84.28 ^a ±1.42	
	T	0	28.45 ^b ±0.34	54.87 ^b ±0.59	82.07 ^a ±1.45	
	A	0	28.67 ^b ±0.52	56.18 ^{ab} ±0.52	81.85 ^a ±1.43	
	C	0.5	28.17 ^c ±0.68	54.80 ^b ±0.68	74.14 ^a ±1.83	
	T	0.5	27.42 ^c ±0.42	55.83 ^{ab} ±0.51	77.00 ^{ab} ±1.65	
	A	0.5	29.10 ^{ab} ±0.92	57.11 ^{ab} ±0.66	77.42 ^{ab} ±2.55	

C = control, T = thyme, A = anise, **Means in the same column with different letters, differ significantly (p<0.05). Means±standard error

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Table 5: Effect of strain, natural antioxidants and sulphate on some carcass parameters of Inshas and Dokki4 hens at the end of the experimental period (44 weeks of age)

Item	Dressed %	Carcass %	Heart %	Liver %	Gizzard %		
Strain Effect							
Inshas	90.38 ^b ±0.21	60.51±1.26	0.52±0.03	2.23 ^b ±0.08	1.94±0.07		
Dokki4	91.67±0.34	60.81±0.78	0.52±0.01	2.82±0.16	1.96±0.11		
Antioxidant effect							
Control	90.95±0.37	61.16±1.14	0.52±0.02	2.75±0.24	2.10±0.12		
Thyme	91.47±0.36	59.15±0.91	0.54±0.04	2.41±0.12	1.94±0.14		
Anise	90.75±0.45	61.52±1.49	0.50±0.03	2.42±0.14	1.83±0.07		
Sulphate effect							
0	91.07±0.35	60.70±1.22	0.53±0.02	2.66±0.16	2.02±0.11		
0.5	91.02±0.30	60.63±0.78	0.51±0.02	2.39±0.11	1.88±0.07		
Source of variation							
		----- P value -----					
strain	0.004	NS	NS	0.0007	NS		
antioxidant	NS	NS	NS	NS	NS		
strain*antioxidant	NS	NS	NS	NS	NS		
sulphate	NS	NS	NS	NS	NS		
strain*sulphate	NS	NS	NS	NS	NS		
antioxidant*sulphate	NS	NS	0.03	0.02	NS		
strain*antioxidant*sulphate	NS	NS	NS	NS	NS		
Treatments							
Strain	Type of anti-oxidant	Sodium Sulphate	Dressed %	Carcass %	Heart %	Liver %	Gizzard %
Inshas	C	0	90.36±0.26	62.00±1.59	0.51±0.08	2.45 ^b ±0.12	1.96±0.16
	T	0	90.29±0.34	58.05±2.58	0.41±0.04	2.36 ^b ±0.14	1.80±0.28
	A	0	89.57±0.68	63.10±1.82	0.58±0.14	1.88 ^b ±0.17	2.02±0.20
	C	0.5	89.69±0.84	58.27±1.32	0.53±0.04	2.27 ^b ±0.38	2.15±0.24
	T	0.5	90.78±0.12	60.46±1.35	0.76±0.06	2.21 ^b ±0.08	2.01±0.08
	A	0.5	91.18±0.20	60.46±1.75	0.43±0.04	2.20 ^b ±0.14	1.77±0.13
Dokki4	C	0	91.68±0.53	58.71±1.41	0.58±0.02	3.92±0.29	2.46±0.37
	T	0	92.69±0.05	59.15±2.17	0.55±0.03	2.48 ^b ±0.17	2.02±0.43
	A	0	91.35±1.53	63.19±0.35	0.52±0.03	2.84 ^b ±0.26	1.87±0.15
	C	0.5	92.06±0.43	64.72±2.50	0.47±0.01	2.34 ^b ±0.34	1.81±0.13
	T	0.5	91.88±0.78	59.37±1.43	0.53±0.07	2.51 ^b ±0.47	1.94±0.28
	A	0.5	90.38±1.05	59.70±0.69	0.47±0.07	2.82 ^b ±0.18	1.67±0.04

C = control, T = thyme, A = anise, *Means in the same column with different letters, differ significantly (p<0.05). Means±standard error

its estrogenic activity. The addition of sulphate to anise seed increased WG by 36% compared to hens fed control diet and this may be due to that sulphation enhances elimination of steroids from adrenal gland (Miyazaki *et al.*, 1969). The addition of thyme alone or with sulphate to Dokki4 hens significantly decreased WG compared to control diet. The variation of response to thyme between two strains may be due to variation in live body weight. Phenolic compounds (thymol and carvacrol) presence in thyme may be act as enzyme inhibitors (Fahey *et al.*, 1993) and consequently the response of lighter body weight like Dokii4 (1534 g) will be higher than heavier body weight like Inshas (1844g). For example it was found that the pure components of essential oils inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CO A) reductase activity (Crowell, 1999) which is a key regulatory enzyme in cholesterol synthesis. In this respect, McDaniel *et al.* (1981) reported that excessive body weight in broiler breeders hens reduced egg production.

Egg quality: There were significant differences between two strains in yolk weight%, shell weight, shell

thickness, while there was insignificant effect for antioxidant used in this study (Table 3). There was significant interaction between strain and antioxidant in yolk width, yolk height and yolk index. The sulphate decreased significantly yolk diameter while increased the yolk index. There was significant interaction between strains and sulphate in yolk height, yolk weight and yolk index. The data in Table 3 showed that addition of thyme alone or with sulphate increased shell weight% and shell thickness compared to hens fed control diet. Peebles and Brake (1987) found that egg shell weight and thickness were negatively correlated to the relative rate of water loss from eggs of strains hens between 30 and 64 week of age. Also, Bennett (1992) showed that breeder eggs are incubated, those with thin shells do not hatch. Since the thyme is known as antioxidant, the thyme may be improved the small environment in uterus (site of calcium deposition) and consequently increase shell weight and shell thickness. The data in Table 4 indicated that there was significant effect of strain on egg length, shape index, albumin weight, albumin weight% and Haugh unit while there were insignificant effects of antioxidant and sulphate. Also, there were significant

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Table 6: Effect of strain, natural antioxidants and sulphate on some internal organs of Inshas and Dokki4 hens at the end of the experimental period (44 weeks of age)

Item	Thymus %	Pancreas %	Ovary weight %	oviduct %	Oviduct length(cm)		
Strain effect							
Inshas	0.18±0.01	0.23±0.01	1.98±0.17	2.67±0.16	56.61±1.91		
Dokki 4	0.21±0.01	0.23±0.01	1.94±0.15	2.18±0.16	48.44±1.85		
Antioxidant effect							
Control	0.16 ^b ±0.01	0.25±0.01	1.85±0.21	2.14 ^a ±0.24	50.91±3.74		
Thyme	0.22 ^a ±0.01	0.23±0.01	2.18±0.25	2.82 ^a ±0.20	54.63±1.18		
Anise	0.21 ^{ab} ±0.01	0.22±0.01	1.88±0.12	2.34 ^{ab} ±0.14	52.23±1.75		
Sulphate effect							
0	0.20±0.01	0.24±0.01	1.79±0.17	2.34±0.09	52.22±1.72		
0.5	0.19±0.01	0.23±0.01	2.13±0.14	2.50±0.22	52.83±2.46		
Source of variation							
	----- P value -----						
strain	NS	NS	NS	0.01	0.002		
antioxidant	0.04	NS	NS	0.03	NS		
strain*antioxidant	NS	NS	NS	NS	NS		
sulphate	NS	NS	NS	NS	NS		
strain*sulphate	0.04	NS	NS	NS	NS		
antioxidant*sulphate	NS	NS	NS	NS	NS		
strain*antioxidant*sulphate	NS	0.04	0.02	NS	NS		
Treatments							
Strain	Type of antioxidant	Sodium Sulphate	Thymus %	Pancreas %	Ovary weight %	Oviduct %	Oviduct length(cm)
Inshas	C	0	0.12±0.02	0.22±0.04	1.20±0.15	2.10±0.24	44.00±5.29
	T	0	0.21±0.03	0.25±0.009	2.50±0.72	2.70±0.29	58.66 ^{ab} ±2.60
	A	0	0.21±0.05	0.25±0.003	1.54±0.12	2.73±0.12	60.33 ^{ab} ±1.45
	C	0.5	0.14±0.02	0.28±0.03	2.42±0.28	2.87±0.58	65.33±1.85
	T	0.5	0.20±0.02	0.23±0.06	2.65±0.31	3.53±0.01	59.50 ^{ab} ±2.50
Dokki 4	A	0.5	0.21±0.02	0.20±0.03	1.85±0.11	2.43±0.43	53.75 ^{ab} ±2.20
	C	0	0.18±0.01	0.27±0.01	2.32±0.23	2.23±0.04	51.00 ^b ±2.30
	T	0	0.24±0.01	0.22±0.02	1.49±0.54	2.33±0.18	52.00 ^{ab} ±3.00
	A	0	0.22±0.01	0.21±0.01	1.67±0.12	1.97±0.09	47.33 ^b ±0.88
	C	0.5	0.21±0.04	0.21±0.02	1.44±0.57	1.37±0.57	43.33±2.72
	T	0.5	0.21±0.04	0.23±0.009	2.25±0.15	2.95±0.58	50.00 ^b ±3.51
	A	0.5	0.18±0.03	0.25±0.03	2.46±0.28	2.22±0.13	47.00 ^b ±2.64
Source of variation							
	----- P value -----						
strain	NS	NS	NS	NS	NS	NS	NS
antioxidant	NS	NS	NS	NS	NS	NS	NS
strain*antioxidant	NS	NS	NS	NS	NS	NS	NS
sulphate	NS	NS	NS	NS	NS	NS	NS
strain*sulphate	NS	NS	NS	NS	NS	NS	NS
antioxidant*sulphate	NS	NS	NS	NS	NS	NS	0.02
strain*antioxidant*sulphate	NS	NS	NS	NS	0.02	NS	NS
Treatments							
Strain	Type of antioxidant	Sodium Sulphate	kidney %	Abdominal fat %	Proventriculus %	Spleen %	
Inshas	C	0	0.57±0.05	0.27 ^a ±0.03	0.55±0.02	0.17±0.01	
	T	0	0.57±0.10	0.15 ^b ±0.04	0.43±0.12	0.26±0.05	
	A	0	1.13±0.67	0.13 ^b ±0.04	0.63±0.09	0.24±0.04	
	C	0.5	0.60±0.16	0.28 ^a ±0.10	0.58±0.11	0.27±0.07	
	T	0.5	0.59±0.29	0.02 ^b ±0.01	0.61±0.05	0.19±0.02	
Dokki4	A	0.5	0.31±0.07	1.47 ^a ±0.67	0.40±0.03	0.21±0.02	
	C	0	0.55±0.05	0.22 ^a ±0.12	0.73±0.02	0.16±0.03	
	T	0	0.43±0.03	0.33 ^a ±0.03	0.54±0.05	0.24±0.05	
	A	0	0.44±0.08	0.15 ^b ±0.08	0.50±0.03	0.12±0.00	
	C	0.5	0.43±0.06	0.20 ^b ±0.06	0.50±0.01	0.22±0.02	
	T	0.5	0.42±0.02	0.24 ^b ±0.01	0.54±0.12	0.13±0.01	
	A	0.5	0.48±0.02	0.27 ^b ±0.12	0.60±0.11	0.18±0.05	

C = control, T = thyme, A = anise, ^a^bMeans in the same column with different letters, differ significantly (p<0.05). Means±standard error

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Table 7: Effect of strain, natural antioxidants and sulphate on some plasma parameters of Inshas and Dokki4 hens at the end of the experimental period (44 weeks of age)

Item	Antioxidant Capacity		Ca mg/dl	Pmg/dl	LDL mg/dl	HDL mg/dl	
	mml/l						
Strain Effect							
Inshas	0.47±0.04		12.43±0.29	4.10±0.33	62.60 ^b ±4.90	29.84 ^b ±2.48	
Dokki4	0.52±0.03		12.54±0.31	4.540.43	70.90 ^a ±3.63	35.29 ^a ±1.80	
Antioxidant Effect							
Control	0.39 ^a ±0.03		11.99±0.39	3.61±0.33	71.80 ^a ±6.34	34.88 ^a ±3.28	
Thyme	0.53 ^{ab} ±0.06		13.03±0.28	5.01±0.58	60.03 ^b ±3.24	29.18 ^b ±1.67	
Anise	0.56 ^a ±0.03		12.43±0.37	4.25±0.39	70.59 ^a ±6.00	34.85 ^a ±2.69	
Sulphate effect							
0	0.51±0.04		11.93 ^b ±0.26	4.28±0.33	60.47 ^b ±3.09	29.00 ^b ±1.55	
0.5	0.48±0.04		13.04 ^a ±0.28	4.34±0.44	73.55 ^a ±4.79	36.47 ^a ±2.41	
Source of variation							
----- P value -----							
strain	NS		NS	NS	0.03	0.005	
antioxidant	0.05		NS	NS	0.02	0.02	
strain*antioxidant	NS		NS	NS	0.0002	0.0002	
sulphate	NS		0.007	NS	0.0008	0.0002	
strain*sulphate	NS		NS	NS	NS	NS	
antioxidant*sulphate	NS		NS	NS	0.04	0.02	
strain*antioxidant*sulphate	NS		NS	NS	0.04	NS	
Treatments							
Strain	Type of antioxidant	Sodium Sulphate	Antioxidant Capacity mml/l	Ca mg/dl	P mg/dl	LDL mg/dl	HDL mg/dl
Inshas	C	0	0.23±0.09	11.48±0.62	3.99±0.40	51.51 ^a ±5.61	24.24 ^a ±4.54
	T	0	0.61±0.07	11.81±0.58	4.22±0.73	55.56 ^a ±7.57	25.29 ^a ±2.77
	A	0	0.52±0.04	11.69±1.22	3.53±0.30	59.20 ^{cd} ±0.98	27.85 ^{ab} ±0.46
	C	0.5	0.41±0.02	13.28±0.10	3.00±0.75	52.16 ^b ±5.24	24.43 ^a ±1.05
	T	0.5	0.53±0.20	13.71±0.06	5.06±1.31	59.83 ^{cd} ±7.72	29.46 ^{cd} ±3.80
	A	0.5	0.52±0.09	12.59±0.22	4.41±1.13	103.96 ^{cd} ±2.55	51.20 ^a ±1.25
Dokki4	C	0	0.45±0.04	11.59±0.50	3.68±0.67	77.45 ^{bc} ±7.64	38.14 ^b ±3.76
	T	0	0.51±0.14	13.03±0.16	6.00±2.04	58.42 ^{cd} ±6.65	28.73 ^{cd} ±2.23
	A	0	0.70±0.05	11.96±0.49	4.87±0.91	56.30 ^a ±0.26	27.73 ^{ab} ±0.13
	C	0.5	0.46±0.03	11.63±1.31	3.58±1.40	92.78 ^{ab} ±1.69	45.69 ^{ab} ±0.83
	T	0.5	0.46±0.15	13.56±0.52	5.10±1.38	66.30 ^{cd} ±6.30	33.24 ^{cd} ±3.66
	A	0.5	0.50±0.03	13.48±0.63	4.17±0.80	69.26 ^{cd} ±5.71	35.68 ^a ±2.94
Source of variation							
----- P value -----							
strain		0.01		NS		NS	
antioxidant		0.02		0.0001		0.0001	
strain*antioxidant		0.0002		NS		NS	
sulphate		0.0005		NS		NS	
strain*sulphate		NS		0.0005		NS	
antioxidant*sulphate		0.03		NS		NS	
strain*antioxidant*sulphate		0.04		0.02		NS	
Treatments							
Strain	Type of antioxidant	Sodium Sulphate	Total cholesterol mg/dl	Triglyceride mg/dl	Total lipids g/dl		
Inshas	C	0	75.75 ^a ±9.51	462.3 ^a ±41.9	15.00 ^a ±1.92		
	T	0	80.85 ^a ±10.30	251.2 ^a ±12.36	6.81 ^a ±0.68		
	A	0	87.06 ^a ±1.44	491.8 ^a ±41.8	11.16 ^{ab} ±1.80		
	C	0.5	76.60 ^a ±7.83	606.4 ^a ±5.93	16.44 ^a ±3.59		
	T	0.5	89.30 ^a ±11.5	381.73 ^b ±31.90	8.89 ^b ±0.72		
	A	0.5	155.17 ^a ±3.81	488.57 ^b ±8.60	12.24 ^{ab} ±2.47		
Dokki4	C	0	115.60 ^b ±11.4	669.61 ^a ±22.20	17.04 ^a ±2.78		
	T	0	87.15 ^a ±4.88	336.10 ^{cd} ±9.79	8.89 ^b ±1.59		
	A	0	84.04 ^a ±0.39	456.65 ^a ±63.56	14.07 ^a ±0.34		
	C	0.5	138.48 ^{ab} ±2.53	462.30 ^{cd} ±41.9	15.00 ^{ab} ±1.92		
	T	0.5	99.55 ^{cd} ±9.96	310.95 ^{cd} ±22.14	8.98 ^b ±1.01		
	A	0.5	104.95 ^{cd} ±8.65	409.11 ^b ±22.10	11.01 ^{ab} ±0.42		

C = control, T = thyme, A = anise, ^aMeans in the same column with different letters, differ significantly (p<0.05). Means±standard error

interaction between strain and sulphate in egg length, albumin height and Haugh unit%. It was observed from the data presented in Table 4 that addition of sulphate to hen diet increased albumin height and Haugh unit % in Inshas strain while decreased it in Dokki4 strain and the difference may be due to variation between the two strains. In this respect, Silversides and Scott (2001) showed that major factors on albumen quality are the strain and age of a laying hen, eggshell quality and storage time and conditions.

Carcass parameters and internal organs: Significant effect of strain in dressing%, liver weight% while antioxidant and sulphate had no significant effect on carcass parameters (Table 5). There was significant interaction between antioxidant and sulphate in heart and liver.

It was observed that there were significant differences between different treatments on liver weight % (Table 5). The addition of thyme and anise tended to decrease the liver weight % compared to control diet and this may be due to the effect of essential compounds presence in these additive on lipid metabolism. It was found that the pure components of essential oils inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CO A) reductase activity (Crowell, 1999) which is a key regulatory enzyme in cholesterol synthesis. Data in Table 6 showed that there was significant strain effect on oviduct weight % and oviduct length while, antioxidant affect thymus and oviduct weight %. Since the thyme and anise contain many compounds known as immunostimulate, the thymus weight % increased as a result of these compounds. Also, the feed additive used in this study tended to increase the oviduct weight % may be as a result of decreasing weight gain (Table 2). However, Renema *et al.* (1999) reported that carcass lipids made up to 6.3% of less reproductively developed in low body weight birds compared to 10.1% in the high body weight birds. There was significant interaction between strain and sulphate in thymus weight %. Significant interaction between antioxidant and sulphate in spleen weight % was observed. There was significant interaction between strain, antioxidant and sulphate in pancreas weight %, ovary weight %, oviduct length and proventriculus weight %. Significant differences between treatments in oviduct length (Table 6). With Inshas hens, the antioxidant tended to increased oviduct length while, they did not with Dokki4 hens. It was observed significant differences between experimental treatments in abdominal fat weight %. Hens of Inshas strain fed Anise+sulphate recorded the highest value of abdominal fat compared to other treatments (Table 6) and these results in harmony with the data of weight gain (Table 2).

Blood constituents: Table 7 showed that there were insignificant differences between two strain in plasma

antioxidant capacity, Ca, P, triglycerides and total lipids while there were significant differences in LDL, HDL and total cholesterol. There was significant effect for antioxidants used in this study on plasma antioxidant capacity. The hens fed control diets recorded the lowest value of plasma antioxidant capacity while hens fed anise diets recorded the highest value. These results agree with Cao *et al.* (1998) who found with human that the increased serum antioxidant capacity after the treatments of strawberries and spinach and indicated the possible absorption of phenolic compounds in these diets. There was significant effect of antioxidant on plasma LDL, HDL, total cholesterol, triglyceride and total lipids. The addition of thyme significantly decreased those parameters compared to hens fed control diet while anise decreased only the triglyceride and total lipids. Lee *et al.* (2003), found that dietary carvacrol (the compound presence in thyme) significantly lowered plasma triglycerides and phospholipids by 12 and 7%, respectively and indicating that dietary carvacrol, but not thymol may have more impact on *de novo* lipogenesis than on cholesterol biosynthesis in their study. On the other hand, Case *et al.* (1995) found that feeding of thymol at a dietary concentration of 150 ppm to Leghorn chickens for 21 day reduced serum cholesterol by 9%. Anise is also lowered the triglycerides and total lipids significantly compared to control diet. However, most of essential oil is known to be altering lipid metabolism. Previous studies have shown that hyperlipidemia increases the plasma levels of oxygen free radicals (Prasad and Kalra, 1993) and produce oxidized compounds such as malondialdehyde. From previous discussion it may be concluded that the decreasing plasma lipid by thyme and anise may be the reason of increasing plasma antioxidant capacity of hens fed their diets.

There was significant interaction between strain and antioxidant in LDL, HDL and total cholesterol. Addition of sulphate significantly increased the plasma calcium and these results can be explained by findings of Lent and Wideman (1994) who found that supplemented hens diet by ammonium sulphate had significantly higher absolute urinary Ca excretion rates. There was significant effect of sulphate in plasma LDL, HDL and total cholesterol. However, Nockels (1973) showed that addition of sulphate to hens ration in the presence of adequate endogenous ascorbate may enhance cholesterol mobilization from tissue such as muscle and promoted its excretion into egg. The sulphate may increase mobilization of cholesterol and consequently increased its level in plasma. There was significant interaction between strain and sulphate in plasma triglycerides and also significant interaction between antioxidant and sulphate in plasma LDL, HDL and total cholesterol. Table 7 showed that there was significant interaction between strain, antioxidant and sulphate in

Table 8: Effect of strain, natural antioxidants and sulphate on liver parameters of Inshas and Dokki4 hens at the end of the experimental period (44 weeks of age)

Item	LDL (mg/g)	HDL (mg/g)	Total cholesterol (mg/g)	Triglyceride (mg/g)	Total lipid (mg/g)		
Strain effect							
Inshas	75.66±4.53	39.22±2.50	114.8±7.03	452.5±6.47	243.9±7.20		
Dokki	70.25±4.36	38.56±2.43	108.82±6.77	449.8±8.14	244.2±7.10		
Antioxidant effect							
Control	86.93±4.21	46.74±2.31	133.67±6.48	479.8±4.47	275.5±3.98		
Thyme	55.25±2.52	28.76±1.11	84.01±3.60	422.1±4.66	215.4±4.46		
Anise	76.70±4.70	41.18±2.51	117.88±7.19	451.5±7.66	241.1±6.02		
Sulphate effect							
0	70.53±4.42	37.83±2.38	108.36±6.78	442.1±7.76	239.8±7.37		
0.5	75.38±4.48	39.96±2.52	115.35±7.00	460.2±6.21	248.2±6.77		
Source of variation							
----- P value -----							
strain	NS	NS	NS	NS	NS		
antioxidant	0.0001	0.0001	0.0001	0.0001	0.0001		
strain*antioxidant	0.006	0.001	0.003	0.0013	NS		
sulphate	NS	NS	NS	0.0007	NS		
strain*sulphate	NS	NS	NS	NS	NS		
antioxidant*sulphate	0.003	0.002	0.002	0.02	NS		
strain*antioxidant*sulphate	0.001	0.0004	0.001	NS	NS		
Treatments							
Strain	Type of antioxidant	Sodium Sulphate	LDL (mg/g)	HDL (mg/g)	Total cholesterol (mg/g)	Triglycerides (mg/g)	Total lipid (mg/g)
Inshas	C	0	95.51±6.91	49.43±3.50	144.94±10.41	475.19 ^{abc} ±8.39	280.12 ^a ±12.74
	T	0	54.23 ^b ±3.22	27.09 ^a ±1.69	81.32 ^{bc} ±4.80	410.70 ^a ±4.80	213.36 ^a ±5.27
	A	0	69.23 ^b ±3.37	35.63 ^{bc} ±1.68	104.86 ^{bc} ±5.05	446.94 ^{abc} ±7.34	230.63 ^{cd} ±7.05
	C	0.5	69.76 ^b ±8.66	36.34 ^{bc} ±4.81	106.10 ^b ±13.46	464.07 ^{bc} ±3.92	266.29 ^{bc} ±3.18
	T	0.5	63.68 ^b ±5.41	32.56 ^{cd} ±2.56	96.24 ^{bc} ±7.98	434.44 ^{abc} ±8.64	215.79 ^d ±10.54
	A	0.5	101.58 ^a ±1.65	54.31 ^a ±0.61	155.89 ^a ±2.24	483.79 ^{bc} ±6.04	257.26 ^{abc} ±18.47
Dokki4	C	0	89.31 ^a ±4.07	49.99±1.88	139.31 ^a ±5.95	484.71 ^{ab} ±5.62	272.86 ^a ±4.31
	T	0	47.29 ^a ±2.93	26.58 ^a ±1.66	73.87 ^a ±4.58	412.07 ^a ±10.04	206.44 ^a ±13.80
	A	0	67.61 ^a ±2.97	38.24 ^a ±1.41	105.86 ^a ±4.38	423.19 ^a ±14.46	235.76 ^{bc} ±3.08
	C	0.5	93.12 ^a ±6.68	51.20 ^a ±2.45	144.33 ^a ±9.11	495.29 ^a ±7.47	283.02 ^a ±8.53
	T	0.5	55.79 ^b ±4.88	28.82 ^a ±2.02	84.61 ^{bc} ±6.88	431.34 ^{abc} ±6.71	226.36 ^{cd} ±2.65
	A	0.5	68.37 ^b ±7.09	36.54 ^{ab} ±4.10	104.92 ^{bc} ±11.19	452.35 ^{bc} ±7.76	240.94 ^{bc} ±14.13

C = control, T = thyme, A = anise, *Means in the same column with different letters, differ significantly (p<0.05). Means±standard error

plasma LDL, total cholesterol and triglycerides. The data in Table 7 indicated that there were significant differences between experimental treatments in LDL, HDL, total cholesterol, triglycerides and total lipids. As we expected the Inshas hens fed anise+sulphate recorded significantly higher value of plasma LDL, HDL, total cholesterol compared to hens fed control diet and this is not the same with Dokki4. These result can be explained as a result of anise estrogenic activity which increased by sulphate. The addition of thyme to Inshas hens diet decreased triglycerides and total lipids by 45. 60 and 54. 6%, respectively. Compared to thyme addition of anise to Inshas hens failed to decreased triglyceride and decreased total lipid by only 25. 6%. The differences between two antioxidants may be due to different structure between their active compounds and their effect on pass way of lipid synthesis.

Liver parameters: The data in Table 8 showed that there was significant effect for antioxidant on all liver parameters. The addition of thyme to hens diets significantly decreased LDL, HDL, total cholesterol, triglycerides and total lipids in liver compared to hens fed control diet and this can be explained as a result of

its effect on lipid synthesis. Anise addition also significantly decreased all liver parameters compared to hens fed control diet. However, the pure components of essential oils inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase activity (Crowell, 1999) which is a key regulatory enzyme in cholesterol synthesis. These results agree with those obtained by Hassan *et al.* (2007) who found that addition of 2% fenugreek to Japanese quail diet decreased the liver cholesterol, LDL, HDL and total lipids content compared to control group.

There was significant interaction between strain and antioxidant in liver LDL, HDL, total cholesterol and triglycerides. The interaction can be explained as a result that addition of anise+sulphate to Inshas strain (Table 8) increased these parameters while decreased them with Dokki4 strain. As we discussed before the addition of sulphate may increased the estrogenic activity of anise. There was significant effect for sulphate on liver triglycerides. The addition of sulphate increased the triglycerides in the liver. Also there was significant interaction between antioxidant and sulphate in all liver parameters and this can be explain as a result of increasing the response to anise by sulphate compared

Table 9: Effect of strain, natural antioxidants and sulphate on yolk parameters of Inshas and Dokki4 hens at the end of the experimental period (44 weeks of age)

Item	LDL (mg/g)	HDL (mg/g)	Total cholesterol (mg/g)	Triglycerides (mg/g)	Total lipid (mg/g)		
Strain Effect							
Inshas	11.59±0.63	4.16±0.22	15.76±0.85	269.17±12.58	290.42±9.70		
Dokki	11.33±0.67	4.12±0.26	15.46±0.92	287.98±19.72	322.00±11.08		
Antioxidant effect							
Control	13.0 ^a ±0.77	4.65 ^a ±0.32	17.66 ^a ±1.10	316.56 ^a ±16.29	333.90 ^a ±2.73		
Thyme	9.79 ^b ±0.59	3.52 ^b ±0.20	13.31 ^b ±0.79	225.40 ^b ±17.86	268.23 ^b ±8.50		
Anise	11.60 ^{ab} ±0.73	4.26 ^{ab} ±0.26	15.86 ^{ab} ±0.99	293.70 ^{ab} ±17.31	317.80 ^{ab} ±16.77		
Sulphate effect							
0	10.62 ^a ±0.52	3.84±0.17	14.46 ^a ±0.69	284.3±17.45	304.40±12.10		
0.5	12.31±0.70	4.45±0.27	16.76±0.98	272.7±15.7	308.80±10.05		
Source of variation							
			----- P value -----				
strain	NS	NS	NS	NS	0.008		
antioxidant	0.01	0.02	0.01	0.006	0.0002		
strain*antioxidant	NS	NS	NS	NS	0.03		
sulphate	NS	NS	0.04	NS	NS		
strain*sulphate	NS	NS	NS	NS	NS		
antioxidant*sulphate	NS	NS	NS	NS	NS		
strain*antioxidant*sulphate	NS	NS	NS	NS	NS		
Treatments							
Strain	Type of antioxidant	Sodium Sulphate	LDL (mg/g)	HDL (mg/g)	Total cholesterol (mg/g)	Triglyceride (mg/g)	Total lipid (mg/g)
Inshas	C	0	12.62 ^{bc} ±0.30	4.43±0.10	17.05 ^{ab} ±0.41	301.53±8.21	328.8 ^{bc} ±9.22
	T	0	8.12 ^a ±1.18	3.00±0.40	11.12 ^a ±1.61	216.72±20.5	250.3 ^a ±31.13
	A	0	11.14 ^{bc} ±1.17	4.05±0.46	15.20 ^{ab} ±1.63	290.22±7.71	272.9 ^{bc} ±23.57
	C	0.5	12.06 ^{bc} ±1.04	4.24±0.36	16.30 ^{ab} ±1.41	305.30±22.8	333.6 ^{ab} ±3.14
	T	0.5	12.01 ^{bc} ±1.19	4.22±0.41	16.24 ^{ab} ±1.61	228.90±15.7	271.6 ^{bc} ±17.13
	A	0.5	13.61 ^{ab} ±2.44	5.03±0.90	18.64 ^{ab} ±3.35	272.30±28.8	285.0 ^{bc} ±8.50
Dokki4	C	0	12.60 ^{bc} ±1.48	4.42±0.52	17.03 ^{ab} ±2.00	336.20±20.3	339.2 ^{bc} ±2.46
	T	0	9.46 ^b ±0.70	3.32±0.24	12.78 ^b ±0.94	264.70±46.4	276.8 ^b ±9.88
	A	0	9.80 ^b ±0.11	3.81±0.04	13.61 ^b ±0.16	296.7±30.36	358.6±32.35
	C	0.5	14.75 ^a ±1.80	5.51±0.20	20.26 ^a ±2.00	323.1±20.2	333.9 ^{ab} ±6.07
	T	0.5	9.57 ^b ±0.72	3.54±0.26	13.11 ^b ±0.98	215.28±11.3	274.1 ^b ±5.72
	A	0.5	11.84 ^{bc} ±0.76	4.16±0.26	16.00 ^{ab} ±1.02	315±22.50	354.5±38.25

C = control, T = thyme, A = anise, ^{a-d}Means in the same column with different letters, differ significantly (p<0.05). Means±standard error

to hens fed thyme+sulphate. Also, there was significant interaction between strain, antioxidant and sulphate on liver LDL, HDL and total cholesterol. The data in Table 8 indicated that there were significant differences between treatments in all liver parameters. As we expected the hens of Inshas strain fed anise+sulphate recorded the highest values of all liver parameters while, it is not the same with Dokki4.

Yolk parameters: As shown in Table 9 there were insignificant differences between the two strains in yolk LDL, HDL total cholesterol and triglycerides while there was significant difference in total lipids. Dokki4 hens recorded higher value than that of Inshas hens. Turk and Barnett (1971) found that eggs from broiler breeders contained significantly more cholesterol than did eggs laid by commercial layer strain. There was significant effect of antioxidant in all yolk parameters. The hens fed thyme significantly recorded the lowest values of yolk LDL, HDL and total cholesterol. As we discussed before the pure compounds of essential oils inhibit hepatic 3-hydroxy-3-methyl coenzyme A (HMG-COA) reductase activity (Crowell, 1999) which is a key regulatory enzyme in cholesterol synthesis and consequently a

hypocholesterolemic effect of essential oil can be expected (Lee *et al.*, 2004). Also, there was significant effect of antioxidant on yolk triglyceride and total lipid parameters and the hens fed the thyme significantly recorded values were lower compared to hens fed control diet. These results agree with those found by Hassan *et al.* (2007) who found that addition of 2% fenugreek to Japanese quail diet significantly decreased the yolk cholesterol, LDL, HDL and lipids compared with control group. There was significant interaction between strain and antioxidant in yolk total lipid. It was observed that while anise decreased the level of yolk total lipid in Inshas strain, it increased in Dokki strain and this may be related to its mildly estrogenic effect which differ between the two strains.

There was significant effect of sulphate on yolk LDL and total cholesterol. These results agree with Nockels (1973) who found that sulphate ingestion increased the total egg cholesterol significantly and he showed that sulphate in presence of adequate endogenous ascorbate may enhance cholesterol mobilization from tissue such as muscle and promoted its excretion into egg. Data in Table 9 showed the effect of different treatments on yolk parameters. There were significant

Table 10: Effect of strain, natural antioxidants, sulphate on reproductive performance of Inshas and Dokki4 hens during the experimental period

Item	Fertility (%)	Hatchability (%)	Hatchability of fertile eggs (%)	Hatched Chick weight (g)		
Strain effect						
Inshas	93.27 ^b ±0.56	77.63 ^b ±1.08	81.06 ^b ±1.93	34.83 ^a ±0.15		
Dokki	95.35 ^a ±0.64	84.17 ^a ±0.95	89.29 ^a ±1.06	32.14 ^b ±0.17		
Antioxidant effect						
Control	93.92±0.73	80.20 ^b ±1.16	85.21±1.83	33.51±0.27		
Thyme	95.58±0.68	84.18 ^a ±1.22	87.57±1.86	33.57±0.23		
Anise	93.43±0.82	78.32 ^b ±1.45	82.76±2.35	33.39±0.28		
Sulphate effect						
0	94.70±0.60	80.91±1.14	85.04±1.92	33.48±0.20		
0.5	93.92±0.64	80.89±1.00	85.31±1.37	33.50±0.22		
Source of variation						
----- P value -----						
strain	0.0100	0.0001	0.0004	0.0001		
antioxidant	NS	0.0021	NS	NS		
strain*antioxidant	NS	NS	NS	NS		
sulphate	NS	NS	NS	NS		
strain*sulphate	0.006	0.001	0.03	NS		
antioxidant*sulphate	0.02	NS	NS	0.02		
strain*antioxidant*sulphate	NS	NS	NS	NS		
Treatments						
Strain	Type of antioxidant	Sodium Sulphate	Fertility (%)	Hatchability (%)	Hatchability of fertile eggs (%)	Hatched Chick weight (g)
Inshas	C	0	95.00 ^{abcd} ±1.42	77.97 ^c ±2.97	79.38 ^{bc} ±5.61	34.65 ^a ±0.39
	T	0	91.42 ^d ±0.97	77.04 ^c ±3.07	79.82 ^{bc} ±5.65	35.36 ^a ±0.28
	A	0	91.25 ^a ±1.67	71.46 ^a ±2.73	76.56 ^c ±6.04	34.76 ^a ±0.33
	C	0.5	92.67 ^{bcd} ±1.36	80.15 ^{bc} ±2.14	85.60 ^{abc} ±3.24	35.25 ^a ±0.33
	T	0.5	95.89 ^{abc} ±1.15	82.23 ^{abc} ±1.75	85.51 ^{abc} ±2.61	34.32 ^a ±0.34
	A	0.5	93.39 ^{bcd} ±1.14	76.96 ^c ±2.69	79.50 ^{bc} ±4.91	34.67 ^a ±0.43
Dokki4	C	0	96.42 ^{ab} ±1.23	83.36 ^{abc} ±1.96	90.20 ^{ab} ±2.43	31.73 ^b ±0.36
	T	0	97.85 ^a ±0.89	88.72 ^a ±1.49	93.45 ^a ±1.28	32.62 ^b ±0.33
	A	0	96.25 ^{ab} ±1.00	86.93 ^{ab} ±1.92	90.84 ^{ab} ±2.15	31.77 ^b ±0.41
	C	0.5	91.60 ^c ±1.48	79.32 ^c ±2.14	85.65 ^{abc} ±1.81	32.42 ^b ±0.48
	T	0.5	97.14 ^{ab} ±1.29	88.75 ^a ±1.95	91.49 ^{ab} ±2.27	31.97 ^b ±0.32
	A	0.5	92.85 ^{bcd} ±2.22	77.94 ^c ±2.95	84.11 ^{abc} ±3.98	32.35 ^b ±0.61

C = control, T = thyme, A = anise, ^{a-d}Means in the same column with different letters, differ significantly (p<0.05). Means±standard error

differences between treatments in yolk LDL, total cholesterol and total lipid. As we expected the natural antioxidant decreased yolk LDL, HDL, total cholesterol and triglycerides compared to hens fed control diet except Inshas hens fed anise+sulphate.

Reproductive performance: The data in Table 10 showed that there was significant effect for strain in fertility parameters. Since Dokki4 strain is lighter than Inshas, the fertility and hatchability of it will be higher compared to Inshas. Hocking and Bernard (2000) suggested that fertility can be maintained at high levels in naturally-mated flocks of broiler breeders with adequate control of body weight. As shown in Table 10 there was significant interaction between strain and sulphate. It was observed that addition of sulphate to hens diets tended to increased fertility in Inshas strain while tended to decrease it in Dokki4 strain. There was significant interaction between antioxidant and sulphate. The addition of sulphate to anise seed numerically decreased the fertility in Dokki4 strain and this can be explained as a result of its mildly estrogenic effect. In

this respect, El-Deeb *et al.* (2007) found that the addition of Anise seed to quail diet significantly reduced the fertility. As we discussed before that sulphate may increase the estrogenic activity because sulphation enhances elimination of steroids from adrenal gland (Miyazaki *et al.*, 1969). However, Pappas *et al.* (2006) showed that poor fertility might be because the phospholipids of avian spermatozoa are characterized by high proportions of arachidonic and docosatetraenoic fatty acid, which are very susceptible to oxidation. There was significant effect of strain on hatchability, the Dokki4 hens recorded higher value compared to Inshas hens.

The egg of Dokki4 strain found be smaller than egg of Inshas (Table 2) and consequently has lower heat production. French (1997) showed that large eggs also face more difficulties to remove the surplus heat from the egg.

Also, there was significant effect of antioxidant on hatchability. The addition of thyme to hens diets significantly increased the hatchability of eggs compared to hens fed control diets or anise diets. The improve in hatchability by thyme can be explain as a result of its

effect on decreasing plasma total lipid (Table 7) and consequently decreased the lipid and oxidized compounds pass to egg. Botsoglou *et al.* (1997) showed that antioxidant constituents of thyme into the hen through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into the yolk. Also, the antioxidants in thyme may transfer to eggs. In this respect, Galobart *et al.* (2001) found that eggs from the carnosic acid (from dietary rosemary extract) treatment showed a delay in the iron-induced lipid oxidation and indicated that carnosic acid may effectively act as an antioxidant in eggs when high enough doses are used in the hen diets. Thyme also increased antioxidant capacity and decreased LDL in plasma (Table 7) and consequently decreased the sources of free radical. However, Liaurado *et al.* (1997) reported a 5.6% improvement in hatchability by adding 6 mg/Kg canthaxanthin into a commercial broiler diet. In this respect, Peebles and Brake (1985) found that vitamin C at 50 and 100 mg/kg significantly improved egg production and hatchability during the post peak period. Also, in this parameter there was significant interaction between strain and sulphate. Sulphate tended to increased hatchability with Inshas strain, while tended to decrease it with Dokki4 except with thyme (increase it from 86.93 to 88.75%).

Compared to control of Inshas strain, the addition of thyme did not alter the hatchability while anise decreased it by 8.34% compared to hens fed the control diet. Addition of sulphate to thyme increased the hatchability by 6.65% compared to control diet while in Dokki4 hens the addition of thyme alone increased it by 6.42% compared to hens fed control diet. Addition of anise to Dokki4 hens increased hatchability by 4.28% compared to hens fed the control diet. The addition of sulphate to thyme increased the hatchability of Dokki4 hens by 6.46% compared to hens fed control diet. The data in Table 10 showed that hens of Inshas strain need sulphate to increase the response to thyme and to decrease the bad effect of anise seed. Sulphate is the conjugated material in pass ways metabolite of thyme and anise. Williams (1959) reviewed the earlier literature which showed that thymol was excreted as glucuronide and ethereal sulphate conjugates in rabbits, dogs and man. Le Bourhis (1970) reported that 35 to 50% and 4 to 5% of the anethole (the active compound in anise seed) given to rats, rabbits and humans were excreted as conjugates of anisic acid and p-hydroxybenzoic acid, respectively and the conjugated material included glucuronides, sulphates and glycine derivatives. Bartelt and Kirinya (1976) found that liver slices from geese, ducks and chickens contained high sulphate conjugation enzyme activities.

In contrast with Dokki4 hens, the sulphate did not increase the response to thyme and decrease the

beneficial effect of anise seed. These results can be explained by the fact that chick embryo development is associated with an accumulation of polyunsaturated fatty acids in tissue lipids (Speake *et al.*, 1998) making them susceptible to lipid peroxidation (Surai, 1999a). Antioxidant compounds presence in thyme deposited into yolk (Krause and Ternes, 1999) and consequently increase the adaptation mechanism to deal with overproduction of free radicals and increased the hatchability. If the birds with heavy weight like Inshas strain (1844 g) theoretically will increase free radical production and will oxidize the antioxidant before reach to egg. Some studies estimated that 2 to 4 % of total oxygen consumed by mitochondria is converted to reactive oxygen species (Boveris and Chance, 1973; Chance *et al.*, 1979; Turrens and Boveris, 1980). Pappas *et al.* (2005) found that selenium was observed and deposited became less efficient with age and the demands for selenium for antioxidant system and immune response increased. In this case the heavy birds need the sulphate ion to conjugate with antioxidant and escape it to egg. Conversely, when the bird is lighter like Dokki4 (1534 g) is produced a lower free radical and the phenolic compounds can be escaped rapidly to egg and in this case it did not need sulphate. In this respect, Ninfali *et al.* (2005) suggested that not all of the ingested phenolics can reach the plasma, but those that escape in blood stream lead to a significant increase in total plasmatic antioxidant capacity, both when they are in the free a glyconic form and when they are glucuronide or sulphate conjugated form.

On the other hand, the thyme addition decreased the deposition of total lipid in yolk (Table 9) and consequently decreased the substrate that produce free radical. In this respect, Botsoglou *et al.* (2005) found that lower value of malondialdehyde in eggs from the hens fed Saffron or α -tocopherol as compared to controls and they suggested that possible transfer of antioxidant constituents of saffron into the hen through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into yolk. We hypothesis that natural antioxidants could be used in proportion to live bodyweight and the response to sulphate depended on the strain and type of natural antioxidant. Further studies are needed to elucidate the mechanism of working these natural antioxidants.

Also, in hatchability of fertile eggs there was significant effect of strain and significant interaction between strain and sulphate.

There was significant effect of strain on hatched chick weight and significant interaction between antioxidant and sulphate. It was observed that sulfate tended to increase hatched chick weight when it added to anise and conversely when it added to thyme.

Conclusion

- 1) Addition of thyme or anise to laying hens diets numerically increased egg number and improved feed conversion.
- 2) Addition of thyme or anise increased antioxidant capacity in blood plasma while decreased LDL, HDL, total cholesterol, triglyceride and total lipids in blood plasma, liver and egg yolk.
- 3) Addition of thyme tended to improve fertility and hatchability while anise tended to decrease these parameters.
- 4) The response to sulphate depended on the strain and type of natural antioxidant.
- 5) The combination of thyme and sulphate is the most successful additive for improving hatchability under the condition of this study.
- 6) Further studies must be carried out to study the possibility of using natural antioxidants in commercial broiler breeders diets.

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