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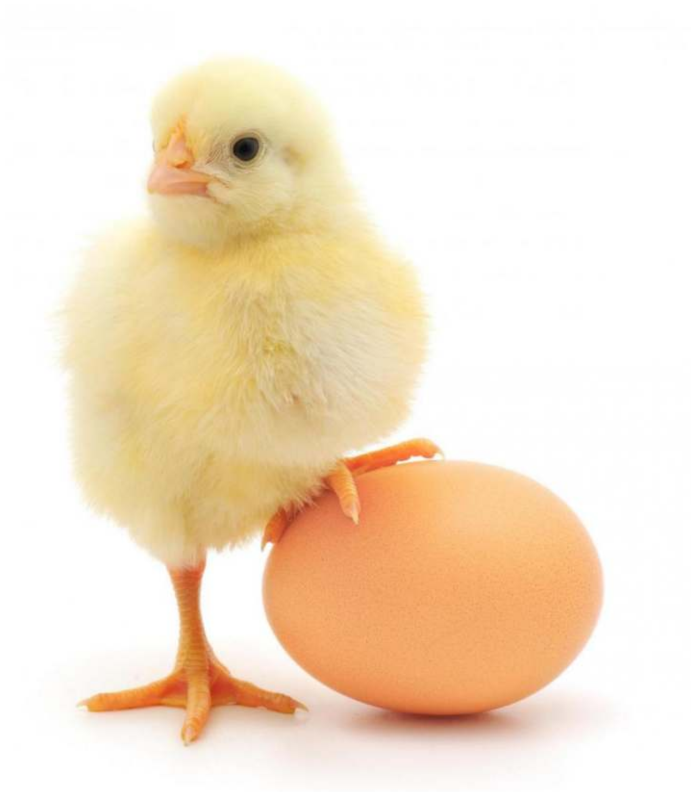

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Evaluation of Some Natural Antioxidant Sources in Broiler Diets: 1-Effect on Growth, Physiological, Microbiological and Immunological Performance of Broiler Chicks

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Abstract: This study was conducted to evaluate the efficiency of aqueous extract of ginger root (GAE), aqueous extract of beetroot (BAE) and tomato puree (TP), as natural antioxidant sources, in broiler diets during summer season. Chicks fed on corn-soybean meal basal diets in three phases feeding system. Basal diets were formulated to contain whole strain nutritional requirements including 50 IU of vitamin E/kg. There were two control groups the first fed on basal diet (Con) and supplemented with either 50 IU/kg vitamin E (E-100, to be the second control group), or supplemented with GAE, BAE or TP at levels of 0.5 or 1% from 1 to 40 d of age. The total phenols content (in Gallic acid equivalent) of GAE and BAE were 44 and 31 µg/L, respectively and lycopene content in TP was 155 mg/kg. The environmental temperature and humidity surrounding birds during the experimental period ranged from 35 to 41°C and 30 to 45%. Growth performance, some plasma constituents and viral and bacterial immunity status at 40 d of age were recorded. The results indicated that, among examined natural additives, using BAE improved the overall body weight gain, while adding GAE to broiler diets decreased feed consumption significantly. However the worst feed conversion ratio recorded by TP group, that group recorded the best antioxidant status including plasma total antioxidant capacity and malondialdehyde. Generally, level 1% of supplementation increased the consumed feed and depressed the total bacteria count of intestine at 40 d of age. On the other side, titer of immunity against respiratory viral Diseases (ND and IB), hemoglobin, red and white blood cells increased by adding GAE to diets. Neither antioxidant source, level nor their interactions could change lipids or cholesterol compounds in plasma. The overall results indicated that the best overall broiler performance during summer season was obtained by using GAE at level of 1% as natural antioxidant.

Key words: Broiler, antioxidant, ginger, beetroot, tomato, summer season, performance

INTRODUCTION

During normal metabolism, the antioxidant defense system, natural and synthetic antioxidants and the antioxidant enzymes, can mitigate free radicals are produced. These radicals cause several adverse effects on body if they are present in excessive levels (oxidative stress) under stressful environments such as heat exposure (Sies, 1991). Oxidative stress has been regarded as one of the major factors negatively affecting performance of birds in the Condensed poultry industry (Lin *et al*., 2006) and as a main factor in the pathogenesis of several serious diseases (Kris-Etherton *et al*., 2004). Therefore, supplementation of synthetic antioxidants (e.g., alpha-tocopheryl acetate or butylated hydroxy toluene) to relieve the oxidative stress has become a common practice in the poultry industry. Recently Ahn *et al*. (2002) showed that, use of plant extracts as natural antioxidants has gained increasing interest because of the global trend of restriction in use of synthetic substances (Zhang *et al*., 2009). It has been reported that heat stress increases lipid oxidative stress peroxidation and depresses growth in birds (Sahin and Kucuk, 2003). Sahin *et al*. (2013) showed that, although exposing to high environmental temperatures can lead to oxidative stress associated with a reduced antioxidant status, reflected by increased oxidative damage and lowered plasma concentrations of antioxidants, using antioxidants in poultry feed including phytochemicals can alleviate these negative effects of high environmental temperatures.

Ginger (*Zingiber officinale*) has been widely used as a condiment and as a herbal medicine to treat a wide range of disorders (Ali *et al*., 2008). Ginger roots contain a number of biologically active components which reported to be phenylpropanoid-derived compounds including gingerols and shogaols (Kundu *et al*., 2009). These compounds exert varying biological activities, including antioxidant (Nakatani, 2000; Rababah *et al*., 2004), antimicrobial (Sasidharan and Menon, 2010) and various pharmacological effects (Ali *et al*., 2008; Ling *et al*., 2010). Chrubasik *et al*. (2005) presented a review suggesting that the preparation method of ginger product affected its clinical efficacy. When ginger is dried gingerol undergoes a dehydration reaction forming shogaols, which are about twice as pungent as gingerol (Ling *et al*., 2010).

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Ginger has been used in poultry diets in many forms such as dried ginger powder (Awadein *et al*., 2012; Malekizadeh *et al*., 2012), essential oil (Jang *et al*., 2007) and aqueous extract (Sudarshan *et al*., 2010) and showed different enhancements affects such as, improving feed conversion ratio by addition of ginger at 0.25% to broiler diets (Onu, 2010). Also, Zhang *et al*. (2009) reported increased activities of antioxidant enzymes, glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) and reduced level of Malondialdehyde (MDA) in broilers fed on diet supplemented with ginger at the rate of 5 g/kg.

Betanines or betalains are natural dye extracted from beetroot to use in different food industries. Recent studies have shown that betalains have antioxidant, antimicrobial and antiviral activity (Zhong *et al*., 2005; Pedreno and Escribano, 2001). Although, some researcher reported that the bioavailability of betalains is at least as high as flavonoids, which are well-accepted as natural antioxidants (Romer *et al*., 2010), using betalains as feed additive has been limited because some factors, such as temperature and pH, influence on the pigment stability (Socaciu, 2008). So betalains is exposed to degradation immediately after extraction and the degradation is accelerated by raising the pH, temperature and water (Sturzoiu *et al*., 2011). Recently, some studies modified the extraction and storage condition of beet root extract to get high concentration and more stable betalains (Socaciu, 2008).

Tomato and tomato products are natural sources of antioxidant components (lycopene, ascorbic acid, phenolics and flavonoids (Abushita *et al*., 1997; Agarwal and Rao, 1998), whereas Lycopene is the most important one. Lycopene, a fat soluble carotenoid, is a precursor of beta-carotene (Sandmann, 1994; Atasoy, 2012) and has an effective free radical scavenging activity at least twice that of β-carotene (Di Mascio *et al*., 1989) and a potent antioxidant that has been shown to play critical role in cancer prevention (Bunghez *et al*., 2011) by lowering oxidative damage to DNA in cancerous cells (Stacewicz-Sapuntzakis and Bowen, 2005). Some workers used isolated lycopene, tomato or tomato products in poultry diets as antioxidant source and reported positive effects on growth performance and lipid peroxidation (Sahin *et al*., 2008), while others could not detect clear effect of lycopene on immune response of laying hens (Oslon *et al*., 2008). Another group of investigators found that inclusion of tomato by product (tomato pomace) at level 30% of male chickens improved seminal quality and decreased lipid peroxidation (Mangiagalli *et al*., 2010; Saemi *et al*., 2012). Some reports determined increased availability of lycpene in tomato products which exposed to relatively high temperature during preparation process of tomato products such as paste and puree than fresh tomato (Anese *et al*., 1999; Agarwal *et al*., 2001; Capanoglu *et al*., 2008).

This study aimed to examine the effect of three natural antioxidant sources, ginger root aqueous extract (GAE), beetroot aqueous extract (BAE) and tomato puree (TP), on growth, physiological, microbiological and immunological performance of broiler chicks during summer season.

MATERIALS AND METHODS

This work has done in Animal Production Research Institute (APRI) and with collaboration with Regional Center for Food and Feed (RCFF), ARC, Egypt.

Three hundred and twenty 1-d old unsexed Arbor Acres broiler chicks were individually weighed and divided into 8 treatments of 4 replicates each (10 chicks each). Chicks fed on corn-soybean meal basal diets which met the strain requirements during starting (1-10 d), growing (11-24 d) and finishing (25-40 d) periods (Table 1). There were two control diets the first was control basal diet (Con) which contained 50 IU of vit. E/kg of diet and supplemented with either 50 IU/kg vitamin E as alpha-tocopherol acetate (E-100, to be the second control diet) or one of the natural antioxidant additives (GAE, BAE and TP) at levels of 0.5 or 1.0% from 1 to 40 d of age. All birds were kept under similar management conditions. The environmental temperature and humidity surrounding birds were recorded daily during the experimental period (Table 2).

Table 1: Composition and calculated analysis of control basal diets (Con)

	Starter (1-10 day)	Grower (11-24 day)	Finisher (25-40 day)
Composition (per 100 Kg)			
Yellow corn	52.28	59.05	63.19
Soybean meal (44% CP)	34.00	26.70	22.50
Corn gluten (60% CP)	6.00	7.00	6.30
Soy bean oil	3.00	3.00	4.00
Di-calcium phosphate	1.84	1.67	1.59
Lime stone	1.43	1.20	1.10
L-Lysine HCl	0.32	0.31	0.28
Dl-Methionine	0.26	0.20	0.17
Sodium chloride	0.24	0.24	0.24
Sodium bicarbonate	0.23	0.23	0.23
Vitamins premix*	0.10	0.10	0.10
Minerals premix**	0.30	0.30	0.30
Total	100.00	100.00	100.00
Calculated analysis (%)			
Crude protein	23.17	21.25	19.04
Metabolizable energy (Kcal/Kg)	3100	3110	3207
Ether extract	5.63	5.08	6.88
Crude fiber	3.80	3.45	3.22
Calcium	1.04	0.90	0.84
Av. Phosphorus	0.50	0.45	0.43
Lysine	1.44	1.24	1.09
Methionine	0.68	0.60	0.54
Methionine+cystine	1.06	0.95	0.86
Sodium	0.15	0.16	0.17

*Supplied per kg of diet: Vit. A, 11000 IU, Vit. D3, 5000 IU, Vit. E, 50 mg, Vit K3, 3 mg, Vit. B1, 2 mg, Vit. B2 6 mg, B6 3 mg, B12, 14 mcg, Nicotinic acid 60 mg, Folic acid 1.75 mg, Pantothenic acid 13 mg and Biotine 120 mcg

**Supplied per kg of diet: Choline 600 mg, Copper 16 mg, Iron 40 mg, Manganese 120 mg, Zinc 100 mg, Iodine 1.25 mg and Selenium 0.3 mg.

Table 2: Environmental temperatures and relative humidity during experimental period

Period	Temperature°C		Relative humidity (%)	
	Minimum	Maximum	Minimum	Maximum
Starting (1-10 day)	37	39	20	55
Growing (11-24 day)	36	41	30	45
Finishing (25-40 day)	36	37	30	35
Overall period (1-40 day)	36	41	20	55

Antioxidant sources: Aqueous extract of ginger and beetroot were prepared in Poultry Nutrition Department Labs before starting the growth trail while TP was obtained from local commercial company.

GAE: It was prepared according to the method reported by Kishk and El Sheshetawy (2010) to reach the maximum free radical scavenging activity (94.4%) and 0.94 protecting factor, by extracting the dried ginger powder with water (0.72:100, W:V) at 60°C/24.5 min. Total phenols contents of the prepared GAE was determined using Folin-Ciocalteu (FC) assay according to the method of Wright *et al.* (2000) and Atoui *et al.* (2005) and was 44 µg/mL in Gallic acid equivalent.

BAE: It was prepared according the method of Sturzoiu *et al.* (2011) by extract the fresh beetroot with water (1:5 W:V) in acidic media using ascorbic acid (1g/L)+citric acid (2g/L) at 25°C/3 min. Total phenols contents of the prepared BAE determined by method of Wright *et al.* (2000) and Atoui *et al.* (2005) and was 31 µg/L in Gallic acid equivalent.

TP: The determined value of lycopene content in TP was 155 mg/Kg using procedures described by Bunghez *et al.* (2011) using UV-Vis technique.

Growth traits: Live body weights and feed consumption of chicks were recorded at 10, 24 and 40 days of age, then live body weight gains and feed conversion ratios were calculated.

Physiological traits

Slaughtering and Blood sampling: At the end of the experimental period (40 d of age), 4 birds/treatment (bird/replicate), around the average body weight were slaughtered and three blood samples were collected from each bird in heparinized tubes (2 complete blood sample and 1 plasma sample/bird). One complete blood sample/slaughtered bird was used to determine blood hematological parameters, while the second sample sent directly to the regional veterinary lab to examine the immunological status. The heparinized plasma samples kept at -20°C until the time of chemical analyses.

Plasma chemical constituents: One plasma sample/bird was used to determine Total Lipids (TL) and Total Cholesterol (TC), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Triglycerides (TG), Total antioxidant capacity (TAOC) and Malnodialdehyde (MDA) by colorimetric methods using analytical kits produced by Biodiagnostic Company.

Immunological response: At 40 d of age one whole blood sample/bird (4 samples/treatment) were used to examined the immune response to Newcastle Disease Virus (NDV) and Infectious Bronchitis Virus (IBV) by measuring titer against these viruses using preventing from hemangontinasion method and using commercial Elisa Kits, respectively. These examinations were carried out in Reference Laboratory for Veterinary Quality Control on Poultry Production, Egypt.

Microbiological traits: At the End of the experiment small intestine of slaughtered birds (4 birds/treatment) were collected and bacteriologically examined for detection of pathogenic bacteria, i.e., *Escherichia Coli, Campylobacter* and *Salmonella* according to Collins *et al.* (1998), Oosterom *et al.* (1983) and Ellis *et al.* (1976), respectively. Also count of total bacteria and *Staphylococcus aureus* in small intestine were carried out according to Gouda (2002).

Statistical analysis: Data of GAE, BAE and TP treatments were analyzed by using two way analysis of variance to detect the effect of antioxidant source and level. Data of all experimental treatments, including Con and E-100, were analyzed by using one way analysis of variance to detect the differences between them. Variables showed significant differences at F-test ($p = 0.05$) were compared to each other's using Duncan's Multiple Range Test (Duncan, 1955). The statistical procedures were computed using SAS (1997).

RESULTS AND DISCUSSION

Growth performance: The recorded environmental temperatures and relative humidity % during starting, growing and finishing periods, Table 2, showed that there were permanent exposing to high temperature during the overall experimental period and this continuous stress had effect on the chicks' performance and their antioxidant system. Results of broiler growth performance in Tables 3 and 4 showed that there were clear reduction in final body weight and feed consumption during the experimental period, compared to the ideal growth performance of Arbor Acres broiler chicks reported in strain catalog. This result is commonly due to exposure chicks to high environmental temperature, while using of natural antioxidant sources either at 0.5 or 1% enhance growth performance significantly. Among examined sources of antioxidant, chicks fed diets supplemented with BAE recorded the highest values of final live body weight (1731 g),

Table 3: Effect of ginger aqueous extract (GAE), beetroot aqueous extract (BAE) and tomato puree (TP) on body weight (BW) and body weight gain (BWG) of broilers

	Body weight (g)			Body weight gain (g)			
	10 d	24 d	40 d	1-10 d	11-24 d	25-40 d	1-40 d
Main factors							
Antioxidant source							
GAE	170^{b}	764	1677^{b}	129^{b}	594	913^{ab}	1636^{b}
BAE	183^{a}	787	1731^{a}	141^{a}	604	944^{a}	1689^{a}
TP	173^{b}	761	1656^{b}	132^{b}	588	895^{b}	1615^{b}
Supplemental level							
0.5%	178	768	1674	136	590	906	1632
1.0%	173	773	1702	132	600	929	1661
P-value							
Antioxidant	0.0037	0.1998	0.0048	0.0071	0.5207	0.0480	0.0054
Level	0.1292	0.7107	0.1445	0.1270	0.3910	0.1587	0.1450
Interactions	0.0080	0.0029	0.0138	0.0071	0.0075	0.2251	0.0135
Mean of P-SE±	1.67	6.53	9.89	1.67	5.7	8.32	9.88
Treatments							
Con	166^{c}	690^{d}	1535^{d}	124^{c}	524^{c}	845^{c}	1493^{d}
E-100	170^{bc}	742^{c}	1660^{bc}	128^{bc}	572^{b}	918^{ab}	1618^{bc}
GAE 0.5	166^{c}	730^{cd}	1627^{c}	125^{c}	564^{b}	896^{bc}	1585^{c}
GAE 1.0	175^{bc}	797^{ab}	1728^{ab}	134^{abc}	622^{a}	931^{ab}	1687^{ab}
BAE 0.5	187^{a}	803^{a}	1722^{ab}	145^{a}	612^{a}	919^{ab}	1681^{ab}
BAE 1.0	179^{ab}	770^{abc}	1740^{a}	137^{ab}	591^{ab}	970^{a}	1698^{a}
TP 0.5	181^{ab}	771^{abc}	1674^{abc}	140^{a}	590^{ab}	903^{bc}	1633^{abc}
TP 1.0	165^{c}	552^{c}	1638^{bc}	124^{c}	586^{ab}	887^{bc}	1597^{c}
Mean of P-SE±	1.41	5.85	8.76	1.41	5.19	7.35	8.76
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0032	0.0001

Table 4: Effect of ginger aqueous extract (GAE), beetroot aqueous extract (BAE) and tomato puree (TP) on feed intake and feed conversion ratio of broilers

	Feed Intake (g)				Feed Conversion Ratio			
	Starter	Grower	Finisher	Overall	Starter	Grower	Finisher	Overall
Main factors								
Antioxidant source								
GAE	167^{b}	1081^{b}	1396^{b}	2581^{c}	1.29^{c}	1.75	1.55^{b}	1.59^{b}
BAE	197^{a}	1025^{ab}	1478^{a}	2700^{a}	1.39^{a}	1.74	1.60^{ab}	1.61^{b}
TP	173^{b}	1038^{a}	1459^{a}	2671^{b}	1.31^{b}	1.79	1.65^{a}	1.66^{a}
Supplemental level								
0.5%	184^{a}	1020^{b}	1405^{b}	2609^{b}	1.34^{a}	1.76	1.57	1.61
1.0%	174^{b}	1034^{a}	1484^{a}	2692^{a}	1.32^{b}	1.76	1.63	1.63
P-value								
Antioxidant	0.0001	0.0161	0.0001	0.0001	0.0001	0.4697	0.0368	0.0064
Level	0.0297	0.0094	0.0001	0.0001	0.0001	0.8791	0.0793	0.1573
Interactions	0.0068	0.0001	0.0001	0.1724	0.0001	0.5595	0.0264	0.0237
Mean of Pooled SE±	2.37	3.09	5.84	6.69	0.003	0.017	0.015	0.0098
Treatments								
Con	180^{bc}	1011^{b}	1320^{d}	2510^{d}	1.45^{a}	1.99^{a}	1.62^{ab}	1.68^{ab}
E-100	160^{d}	1041^{a}	1345^{d}	2545^{d}	1.25^{g}	1.87^{ab}	1.49^{b}	1.59^{c}
GAE 0.5	162^{d}	984^{c}	1385^{c}	2531^{d}	1.30^{e}	1.77^{bc}	1.57^{b}	1.61^{c}
GAE 1.0	171^{cd}	1053^{a}	1406^{bc}	2631^{c}	1.28^{f}	1.73^{c}	1.54^{b}	1.57^{c}
BAE 0.5	203^{a}	1037^{a}	1432^{b}	2672^{b}	1.40^{b}	1.72^{c}	1.59^{b}	1.60^{c}
BAE 1.0	191^{ab}	1013^{b}	1523^{a}	2723^{a}	1.39^{c}	1.77^{bc}	1.61^{b}	1.63^{bc}
TP 0.5	186^{bc}	1039^{a}	1398^{c}	2623^{c}	1.33^{d}	1.79^{bc}	1.57^{b}	1.62^{c}
TP 1.0	161^{d}	1036^{a}	1522^{a}	2719^{a}	1.30^{e}	1.79^{bc}	1.73^{b}	1.71^{a}
Mean of Pooled SE±	2.00	2.83	5.65	6.63	0.003	0.017	0.014	0.008
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0032	0.0060	0.0003

body weight gain (1689 g) and feed consumption (2700 g) at the end of experimental period (40 days of age) compared to GAE and TP groups, while calculated feed conversion ratio of GAE group was (1.59) as significant (p = 0.0064) as that calculated for BAE group (1.61) because GAE group consumed the lowest quantity of feed (2581 g) during the experimental period. On the other hand the calculated feed conversion ratio of TP group was the worst value (1.66). Adding antioxidant sources at levels of 1.0 % to broiler diets increased feed consumption during the experimental period significantly (p = 0.0001). Results of one way analysis of variance between all experimental treatments showed that, live body weight, body weight gain and feed conversion ratio of broiler chicks were improved significantly by adding any source of antioxidant except those of chicks fed diet supplemented with 1% TP. Using GAE at level 1.0% in broiler diets resulted in the best feed conversion ratio (1.57), without significant different with the calculated value of Vit.E-100 group which was 1.59 during the overall experimental period.

The aforementioned researches and reviews proved the adverse effects of high environmental temperature on nutrients digestibility, growth, health, welfare and survival and product quality of poultry (Mashaly *et al*., 2004; Khan *et al*., 2012a,b; Sahin *et al*., 2013) and the ability of some antioxidant sources to reduce these problems (Sahin *et al*., 2008, 2011). The reported improvement of growth performance had observed previously by using ginger or tomato powder as a source of antioxidant. Onu (2010) found that inclusion of ginger in broiler diets at level 0.25% increased the final body weight and improved feed conversion ratio, also Tekeli *et al*. (2011) reported the same trend of growth when using ginger at 120, 240 or 360 ppm in broiler diets. In other researches, no significant improvements were detected in body weight, feed intake or feed conversion ratio by using ginger in broiler diets within range between 0.5 and 4.5% (Ademola *et al*., 2009; Zhang *et al*., 2009; Kehinde *et al*., 2011). Also, Sahin *et al*. (2008) reported increased growth performance of quail exposed to high ambient temperature when fed on diets supplemented with graded levels of tomato powder (0.0, 2.5 and 5%) and the improvement was correlated with the supplementing level. The increasing in consumed feed of natural antioxidant supplemented groups in this study was explained previously by improvement of palatability and quick digestive effect of the natural products by increase secretion and activity of gastrointestinal enzymes (Zhang *et al*., 2009; Zhao *et al*., 2011).

Physiological performance: The presented results of Physiological Performance in Table 5 showed that neither antioxidant source nor supplemented levels had an effect on plasma lipid profile of broiler chicks at 40 days of age. The determined values of plasma TL of chicks in Con and E-100 groups were lower than those of other groups. Results of plasma antioxidant status clearly showed that both supplemental antioxidant source and level had significant effect on plasma TAOC, while supplemental levels did not change plasma MDA determined values. The best results obtained by using TP as natural antioxidant source and this improvement increased by increasing the level of supplementation from 0.5 to 1%. Among experimental treatments, Con group showed the worst antioxidant status, while E-100 group was as significant as TP groups. Adding GAE to broiler diets increased Hb, RBCs, WBCs and Lymphocytes (L) and decreased Heterophil (H) of chicks significantly than determined values for BAE and TP groups. Using antioxidants at level of 1.0% decreased H and H/L determined values and increased L values significantly. Among all experimental treatments, birds of Con group showed significant reduction of Hb, RBCs, WBCs and L values, while significant increase of H and H/L determined values were obtained compared to some other treatments.

Some researchers could not detect significant change in blood lipid profile (Nasiroleshami and Torki, 2010) or blood hematological parameters (Ademola *et al*., 2009; Kehinde *et al*., 2011) by using ginger in broiler diets. On the other hand, some workers recorded reduction of blood TC, LDL or HDL when used ginger extract at levels 0.4 and 0.6% to drinking water for broilers (Saeid *et al*., 2010), when used ginger in broiler diets at levels of 0.1 and 0.2% (Mohamed *et al*., 2012) or when used ginger in layer diets at levels 1 and 3% (Malekizadeh *et al*., 2012). The increase of RBCs in birds fed on BAE diets was in agreement of those obtained in a study on distribution of betalin pigment in red blood cells and the resistance of the cells to ex vivo induced oxidative hemolysis in humans. Results of micromolar basis supported the concept that betalin may offer antioxidative protection to the cells (Tesoriere *et al*., 2005). Ademola *et al*. (2009) reported increase of serum WBCs of broiler fed on diet contains ginger at 1, 1.5 and 2%. Khan *et al*. (2012) showed in his review that heat stress reduce number of WBCs and the H/L ratio has been used as a reliable indicator of stress in birds (Mashaly *et al*., 2004), also McFarlane and Curtis (1989) supported the view that the H/L increased under heat stress in broiler chicks. So the obtained significant increase in WBCs and reduction of H and H/L values in experimental groups compared with Con group indicating that these treatments are effective ways to reduce the harmful effect of heat stress on chick's immunity and GAE is the best source of antioxidant when used at 1% of broiler diet.

The reported enhancement of the antioxidant status and reducing plasma MDA level by using antioxidants confirmed the previous results of using antioxidant phytochemicals as ginger (Zhang *et al*., 2009; Akbarian *et al*., 2011; Saied *et al*., 2011) and tomato products (Sahin *et al*., 2008, 2011), which showed decreased concentrations of serum and liver MDA and increased the activities of antioxidant enzymes (GSHPx and SOD). Sadeghi *et al*. (2012) reported that inclusion of ginger (0.75%) in broiler diets resulted in improvement of TAOC and decreased plasma MDA of challenged and unchallenged broiler chicks with *Salmonella*. The effect of diet supplemented with red beet leafs on antioxidant status of plasma and tissue was investigated in mice by Lee *et al*. (2009). Their results showed that levels of antioxidants (glutathione and beta-carotene) and the activities of antioxidant enzyme (glutathione peroxidase) in plasma were considerably increased, suggesting that antioxidant defenses were improved by red beet leafs diet and increased resistance of lymphocyte DNA to oxidative damage.

However most of previous researches could not detect the smode of action of antioxidant phytochemicals

(Zhang *et al*., 2009; Khan *et al*., 2012) reported that active substances in ginger (phenols and ketone derivatives) enhanced serum antioxidants by slowing the processes of oxidation of the feed (Zhao *et al*., 2011), while Sahin *et al*. (2013) reported more specific pathway by which lycopene (tomato) and some phytochemicals can enhance the antioxidant status of birds when exposed to stress factors in their review. They reported that exposure to heat stress (as stress factor) enhanced expression of inflammatory transcription nuclear factor (NF-kB) and suppressed expression of nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor in hepatic cells. These transcription factors affect on more than 200 genes to overcome oxidative stress. Also they reported that lycopene can interfere with multiple cell-signaling pathways in stressed poultry and elicit antioxidant effects through inhibiting NF-kB expression and activating Nrf2 expression so enhanced expression of several phase-II detoxifying enzymes as well as other antioxidant enzymes/protein then reduce concentration of MDA as a result (Lian and Wang, 2008, Gupta *et al*., 2010; Sahin *et al*., 2008-2013). Ling *et al*. (2010) proved the efficacy of 6-shogaol of ginger in inhibition breast cancer cell invasion by reducing matrix metalloproteinase-9 expression via blockade of NF-kB activation in human. Unfortunately, there were no available researches about using beet root products in poultry diets to discuss.

Immunological status: The determined values of blood titer against NDV and IBV of broiler chicks at 40 day of age, Table 6, showed that using natural antioxidant sources improved immunological status of broiler chicks kept under high environmental temperature. Chicks fed diet supplemented with GAE recorded the highest significant values of titer against NVD (9.01) and IBV (8.31) followed by values of BAE group (8.60 and 7.66, respectively), while TP chicks recorded the lowest values (8.11 and 7.51, respectively). Increasing supplemental level of antioxidant sources from 0.5% to 1.0% did not change the immunological status against viruses of either ND or IB. Generally, Con group showed the worst immunological status compared to other groups whereas the measured titer values were 7.33 and 6.5 for NDV and IBV, respectively. These results were in the same trend of blood hematological parameters presented in Table 5 and confirmed the reported conclusion by McFarlane and Curtis (1989) and Mashaly *et al*. (2004) about the adverse effect of heat stress on chicken immunity and reduction of antibody synthesis through increasing inflammatory cytokines and depressing T-helper cytokines, which are very important for antibody production. Published reviews of Khan *et al*. (2012 a,b) showed the ability of some antioxidant substances (ginger, vitamins C and E) to reduce the adverse effect of heat stress on chicks immunity. However Kausar *et al*. (1999) recorded increased means titer in primary and secondary responses against NDV of broiler chicks by using carminative mixture containing ginger at the dose rate of 4 mL/L of drinking water, Olson *et al*. (2008) did not detect any effect of using tomato lycopene at levels between 65 and 840 mg/kg diet on parameters of immune response (inflammatory, cutaneous basophile hypersensitivity or 1° and 2° antibody response) of laying hens. Azhir *et al*. (2012) studied the effect of adding ginger root powder at levels 5 and 10 g/kg diet on humoral immunity of broiler chicks against NDV using HI test (Prevention from Hemangontinasion). Their results showed considerable increase of HI titer of chicks fed diet supplemented with 10 g ginger powder/kg diet compared to control unsupplemented group at 35 day of age. The superior determined titer against NDV and IBV values of GAE groups was in match with the blood hematological parameters presented in Table 5 and confirming the effectiveness of GAE to decrease the hazardous effects of free radicals and heat stress on broiler immunity.

Microbiological Status of Intestine:

Results of bacteriological examination of small intestine (Table 6) showed absence of *Salmonella* and *E. Coli* in all experimental samples while *Campylobacter* were detected. Both supplemental antioxidant source and level had significant effect on the count of total bacteria in small intestine. The highest significant count of total bacteria recorded for TP source group and supplementation level of 0.50%. On the other hand, count of *Staphylococcus aureus* in small intestine did not affected by using supplemental antioxidant sources at 0.5 or 1.0% of diet. Among all experimental treatments, Con group recorded the highest values of both count of total bacteria and *Staphylococcus aureus* in small intestine compared to other treatments so indicating that adding antioxidant source to broiler diet had antimicrobial effect as mentioned before by Lee *et al*. (2004). The antibacterial effect of aqueous extract of ginger oil on count of *Staphylococcus aureus, Salmonella* and *E. Coli* when applied in ratio 1:150, 1:250 and 1:500 to chicken meat reported by Sudrashan *et al*. (2010). Also Awadein *et al*. (2012) observed decreased 17 % of *Salmonella* colonization in intestine of challenged Mandara laying hens with *Salmonella* when fed on diet containing 1% dried ginger root powder. Lee *et al*. (2004) reported that although microflora in digestive tract could be beneficial, it can adversely affect the host, if it is not properly controlled. It is well-known that non-starch polysaccharides present in cereals stimulate growth of microflora (Smits and Annison, 1997), leading to low growth performance. Gut microflora can hydrolyze conjugated bile salts (Feighner and Dashkevicz, 1987) which limits fat digestion (Krogdahl, 1985). Shanoon *et al*. (2012)

Table 5: Effect of ginger aqueous extract (GAE), beetroot aqueous extract (BAE) and tomato puree (TP) on blood hematological parameters and plasma constituents of broilers

	Plasma Lipid Profile (mg/dL)					Plasma antioxidant status		Blood hematological parameters[1]						
	TL	TC	HDL	LDL	TG	TAOC (mmol/L)	MDA (nmol/mL)	Hb (g/dL)	PCV (%)	RBC ($x10^6/mm^3$)	WBC ($x10^4/mm^3$)	H (%)	L (%)	H/L
Main Factors														
Antioxidant source														
GAE	721	157	63.6	71.7	84.6	0.46^b	5.08^a	12.6^a	31.1	3.10^a	4.16^a	26.7^b	68.6^a	0.39^b
BAE	735	166	64.9	84.8	83.4	0.45^b	4.96^a	11.2^b	29.7	3.02^a	3.61^b	30.5^a	64.8^b	0.47^a
TP	748	169	57.2	94.2	88.9	0.73^a	2.37^b	10.7^b	28.7	2.88^b	3.91^{ab}	29.04^a	65.6^b	0.44^a
Supplemental level														
0.5%	743	167	62.7	84.5	80.6	0.42^b	4.01	11.4	29.3	3.00	3.82	29.7^a	65.1^b	0.46^a
1.0%	727	162	61.1	82.7	90.6	0.67^a	4.26	11.6	30.5	3.01	3.97	27.7^b	67.7^a	0.41^b
P-value														
Antioxidant	0.8166	0.4358	0.3560	0.1535	0.8196	0.0429	0.0933	0.0001	0.1548	0.0051	0.0095	0.0037	0.0004	0.0012
Level	0.6524	0.5470	0.7237	0.8396	0.2002	0.0701	0.1784	0.2694	0.2076	0.8595	0.2349	0.0242	0.0010	0.0079
Interactions	0.8006	0.9650	0.8101	0.5641	0.6053	0.0418	0.0504	0.5827	0.8538	0.0514	0.7681	0.6031	0.3482	0.6446
Mean of Pooled SE±	14.8	3.52	2.11	4.48	3.51	0.09	0.51	0.19	0.49	0.03	0.07	0.52	0.53	0.11
Treatments														
Con (-)	495^b	158	69.4	73.9	73.2	0.31^b	7.1^a	10.0^d	27.7	2.67^d	3.42^c	34.3^a	59.8^d	0.57^a
E-100	595^b	154	51.6	88.5	71.0	0.81^a	2.36^c	11.9^{ab}	29.3	2.97^{bc}	3.77^{ab}	30.9^{bc}	63.5^c	0.48^b
GAE 0.5	742^a	159	65.3	66.8	84.5	0.32^b	4.76^b	12.6^a	30.3	3.02^{abc}	4.05^{ab}	28.2^c	66.8^b	0.42^c
GAE 1.0	701^a	155	61.8	76.7	84.5	0.61^{ab}	5.40^{ab}	12.5^a	30.0	3.17^a	4.27^a	25.2^d	70.5^a	0.35^d
BAE 0.5	728^a	168	66.9	85.6	77.6	0.31^b	4.80^b	11.0^{bcd}	29.0	3.10^a	3.50^c	31.3^{bc}	64.1^c	0.49^b
BAE 1.0	742^a	165	62.9	84.2	89.3	0.59^{ab}	5.13^{ab}	11.5^{bc}	30.5	2.95^{bc}	3.72^{bc}	29.6^{bc}	65.4^{bc}	0.45^{bc}
TP 0.5	759^a	173	55.9	101.1	79.7	0.62^{ab}	2.46^c	10.5^{cd}	28.5	2.87^c	3.90^{bc}	29.5^{bc}	64.3^c	0.46^{bc}
TP 1.0	738^a	165	58.0	87.2	98.0	0.82^a	2.27^c	10.9^{cd}	29.0	2.90^c	3.92^{bc}	28.5^{bc}	67.0^b	0.43^c
Mean of Pooled SE±	22.18	3.15	1.83	3.68	3.15	0.08	0.46	0.18	0.41	0.03	0.06	0.53	0.58	0.01
P-value	0.0099	0.8518	0.2319	0.4548	0.4848	0.0743	0.0172	0.0001	0.2530	0.0002	0.0258	0.0001	0.0001	0.0001

Table 6: Effect of ginger aqueous extract (GAE), beetroot aqueous extract (BAE) and tomato puree (TP) on immunity against NDV and IBV and intestine microbiology status of broilers

	Titer against NDV	Titer against IBV	Intestine bacteria count		Intestine bacteria detection		
			Total (x10^6)	*Staph. aureus* (x10^4)	*Campylobacter*	*Salmonella*	*E. coli*
Main factors							
Antioxidant source							
GAE	9.01^{a}	8.31^{a}	7.1^{b}	35	+	-	-
BAE	8.60^{b}	7.66^{b}	10.6^{b}	14	+	-	-
TP	8.11^{c}	7.51^{b}	22.8^{a}	110	+	-	-
Supplemental level							
0.5%	8.48	7.81	17^{a}	100	+	-	-
1.0%	8.68	7.85	9.1^{b}	6	+	-	-
P-value							
Antioxidant	0.0018	0.0017	0.0176	0.2467			
Level	0.2228	0.7649	0.0485	0.0673			
Interactions	0.9553	0.8085	0.4498	0.3431			
Mean of Pooled SE±	0.11	0.10	2.6	27			
Treatments							
Con	7.33^{d}	6.53^{d}	210.0^{a}	3900^{a}	+	-	-
E-100	8.20^{bc}	7.66^{bc}	10.0^{b}	50.0^{b}	+	-	-
GAE 0.5	8.90^{a}	8.23^{ab}	8.3^{b}	70.0^{b}	+	-	-
GAE 1.0	9.13^{a}	8.40^{a}	6.0^{b}	1.5^{b}	+	-	-
BAE 0.5	8.53^{abc}	7.70^{bc}	15.0^{b}	26.0^{b}	+	-	-
BAE 1.0	8.67^{ab}	7.63^{bc}	6.1^{b}	1.8^{b}	+	-	-
TP 0.5	8.00^{c}	7.50^{c}	30.0^{b}	210.0^{b}	+	-	-
TP 1.0	8.23^{bc}	7.53^{c}	15.0^{b}	14.0^{b}	+	-	-
Mean of Pooled SE±	0.12	0.13	17	3.1			
P-value	0.0003	0.0002	0.0094	0.0012			

subjected broiler chicks to oral dose of ginger essential oil at levels 20 and 40 mg/kg/day for 7 weeks. Their results showed significant ($p<0.01$) reduction in colony forming units of *Staphylococci* spp., compared with the control group. It is clear that controlling the microflora could positively influence birds' performance and that feed supplements with anti-microbial activity are potential alternatives to antibiotics. This explanation conjugated the best results recorded for GAE and BAE and their lower count of total bacteria.

In conclusion the obtained results in this study proved the efficacy of examined natural antioxidant in enhancement growth, physiological, microbiological and immunological performance of broiler chicks during summer season. Generally, GAE showed more efficacy than BAE and TP, so it is recommended to use as natural effective antioxidant at level 1% to broiler diets during summer season.

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