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EFFECT OF VITAMIN C, VITAMIN E OR BETAINE ADDITION ON ALLEVIATION OF HEAT STRESS IMPACTS ON GROWING RABBITS

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ABSTRACT

This study was conducted to evaluate the effects of vitamin C (VC), vitamin E (VE) or betaine (BET) dietary supplementation on growth performance, nutrients digestibility and antioxidant status of growing male rabbits under heat stress conditions. A total of 48 weaned New Zealand male rabbits with 641±15.77 g average body weight and 5 weeks of age was randomly divided to four experimental groups (12 rabbits per group), each was fed a basal diet without any supplementation (control group) or supplemented with 500 mg VC (2^{nd} group), 200 mg VE (3^{rd} group) or 1000 mg BET (4^{th} group)/kg diet for 8 weeks. Throughout the experiment, the overall mean of temperature humidity index (THI) was 84.67± 0.35, reflecting a state of severe heat stress. Results of growth performance showed that rabbits receiving VE, VC or BET had significantly higher live body weight (LBW), daily body weight gain (DBWG), and reduced water intake and feed conversion rate (FCR) as compared to the non-supplemented group. In particular, BET supplemented growing rabbits showed significantly marked improvement of the digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and nitrogen free extract (NFE) and nutritive values compared to the non-supplemented group. All tested additives evoked significant improvement of antioxidant status, lipid peroxidation, white blood cells (WBCs) and lymphocyte count. Conclusively, the results revealed that VE, VC and BET could be useful as an economic efficient and natural dietary additives protecting against the negative impacts of heat stress in growing rabbits to maintain performance and antioxidant status.

Key words: Rabbits, heat stress, vitamin C, vitamin E, betaine, growth performance, digestibility, oxidative stress.

INTRODUCTION

Demands for animal proteins are increasing as world population increases. To meet such demand other alternative for the traditional meat source for production is mandated. Due to their high reproductive capabilities, raising rabbits is a good potential source to fulfill demand for animal proteins (Daader *et al*., 2016). In tropical and subtropical regions, heat stress is the major constraint on animal productivity, especially rabbits, because of its drastic impacts on animal health and growth (Selim *et al*., 2005). In particular, in Egypt, the climate is characterized by a long hot period (from May to October) and short mild one (from December to March (Hassan *et al*., 2011).

Recent studies have shown that heat stress induced negative effect could be possibly the result of the increased production of oxygen-derived free radicals and oxidative stress (Sahin and Kucuk, 2003; Liu *et al*., 2011). Hence, rations enriched with natural antioxidants could be used to alleviate the negative effects of high climatic conditions in animals (Tuzcu *et al*., 2008).

Vitamin C (VC), ascorbic acid, is an essential micronutrient required for normal metabolic functioning of the body (Carr and Frei, 1999). In particular, VC could guard against oxidative stress damage through its free-radical scavenging activity (Lee, 2002). Vitamin E (VE), Alpha-tocopherol, is a highly effective

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natural antioxidant that protects cellular membranes against oxidative damage (Morrissey *et al.*, 1994).

Betaine (BET), N, N, N-trimethylglycine, is a naturally occurring compound with osmoprotective properties, which is vital in the nervous, immune, renal and cardiovascular systems (Kidd *et al.*, 1997). In mammals, BET conserves osmotic equilibrium and maintains the tertiary structure of macromolecules in the kidney and other tissues (Yancey and Burg, 1990; Weik *et al.*, 1998). Also, it is an important source for methyl group required for the formation of methionine and S-adenosylmethionine (SAM) (Lever *et al.*, 2004). However, betaine is not present in large quantities in animal feedstuffs (Wang *et al.*, 2004).

The objective of this study was to investigate the effect of dietary supplementation of VC, VE or BET as a natural antioxidant on growth performance, digestibility and antioxidant status of growing rabbits to alleviate the negative effects of heat stress conditions.

MATERIALS AND METHODS

The study was conducted in Rabbit Research Farm, Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. The study was initiated in June 2015 and continued for 8 weeks.

A total of 48 growing New Zealand White (NZW) rabbits (male, 5 wk of age, and 641±15.77 g LBW) were purchased from the Laboratory Animal Farm at Zagazig University. The rabbits were divided into 4 simillar groups (12 rabbits per group) and fed the basal diet without any additive (control group, C), or supplemented with 500 mg vitamin C (Microvit® C Promix 1000, Adisseo, France) /kg diet (VC group), 200 mg vitamin E (Microvit® E Promix 50, Adisseo, France)/kg diet (VE group), and 1000 mg betaine (Betafine®, Adisseo, France) /kg diet (BET group).

The basal diet was formulated to cover the recommended nutrient requirements of growing rabbits according to NRC (1977). The formulation and chemical analysis of the basal-diet are shown in Table 1. All additives were pre-mixed with 1 kg of each diet and successively mixed into the remaining diet to obtain the homogenous inclusion level.

During the experimental period, the rabbits were housed individually in galvanised wire cages (35×35×60 cm). All animals were kept under the same managerial and hygienic conditions. Urine and feces on the rabbitry floor were removed every morning. Rabbits were acclimated one week prior to use in any study herein. Throughout the experimental period of 8 weeks, each cage contained a feeder and potteries to provide free access to feed and fresh water, respectively. The feed and water was offered individually *ad libitum* and refilled at 8:30 am and 2:30 pm daily.

Throughout the experimental period, ambient temperatures and relative humidity were measured in the rabbitry using automatic thermo-hygrometer (OF 14:140, H 10 – 99%; TFA Dostmann GmbH + Co. KG, Wertheim, Germany) twice a day at 8:30 am and 14:30 pm. Inside the rabbitry unit. The THI was calculated according to Livestock and Poultry Heat Stress Indices (LPHSI, 1990) as the following equation:

$$THI = db°F - [(0.55-0.55RH)(db°F - 58)]$$

Where, db° F is dry bulb temperature in Fahrenheit degrees, and RH is the relative humidity as a percentage. The THI values obtained were then categorized as follows: < 82= absence of heat stress, 82 - < 84 = moderate heat stress, 84 - < 86 = severe heat stress and 86 and more = very severe heat stress.

Feed and water intake were recorded weekly by weighing the remained amounts of feed and measuring the amount of water then subtracting them from the offered before putting the new ones.

All rabbits were individually weighed at the beginning of the experiment and at weekly intervals throughout the experimental period. Live body weight was done in the morning before having access to feed and water at 08:30 am. Daily body weight gain was calculated and feed conversion ratio was calculated according to Berger and Halver (1987) based on the following equation: Feed conversion ratio (FCR) = Feed intake / body weight gain. Economic efficiency (EE) was calculated as described by Attia *et al.* (2014) using the input–output analyses.

Table 1. Formulation and chemical analysis of the basal-diet fed to rabbits

Item	Amount
Ingredients (g/kg)	
Alfalfa hay	330
Barley grain	250
Wheat bran	250
Soybean meal	150
Sodium chloride	5
limestone	10
Minerals mixture*	1.5
Vitamins mixture**	1.5
DL- Methionine	2
Total	1000
Chemical analysis (% on DM basis)	
Organic matter	90.82
Crude protein	18.20
Crude fiber	12.17
Ether extract	3.26
Nitrogen free extract	57.19
Ash	9.18
Calculated values	
Calcium (%)	0.91
Phosphorus (%)	0.54
Calcium/Phosphorus	1.68

* Each 1.5 kg contains: manganese 80 mg, zinc 60 mg, iron 30 mg, copper 4 mg, iodine 0.5 mg, selenium 0.1 mg and cobalt 0.1 mg.

** Each 1.5 kg contains: vitamin A 12000000 IU, vitamin D_3 3000000 IU, vitamin E 10000 mg, vitamin K_3 2000 mg, vitamin B_1 1000 mg, vitamin B_2 5000 mg, vitamin B_6 1500 mg, vitamin B_{12} 10 mg, Biotein 75 mg, folic acid 1000 mg, nicotinic 30000 mg and pantothenic acid 10000 mg.

At the last week of the experimental period, 4 rabbits from each group were individually housed in metabolic cages for 7 days digestibility trial. The daily collected feces from each animal was weighed, then all the feces were bulked, 50% subsample was taken, oven dried at 60°C for 24 hour and stored for laboratory analysis. Feed and feces samples were chemically analyzed according to AOAC (2000). Total digestible nutrients (TDN) were calculated according to Cheeke *et al.* (1982) as follows:

$$TDN (\%) = DCP (\%) + DCF (\%) + DNFE (\%) + (DEE (\%) \times 2.25)$$

The digestible energy (DE) values (Kcal/Kg diet) of the experimental diets were calculated according to Schieman *et al.* (1972) as follows:

DE (Kcal/Kg diet) = 5.28 DCP (g) + 9.51 DEE + 4.2 (DCF+DNFE).

Where, DCP = digestible crude protein, DEE = digestible ether extract, DCF = digestible crude fiber and DNFE = digestible nitrogen free extract.

At the slaughter time, two separate blood samples were collected from each rabbit; 2.5 ml was collected into an EDTA tube for use in hematological evaluations. A 10 ml second sample was taken into a glass tube (without EDTA) and left for 20 min at room temperature to coagulate; after centrifugation at 3000 rpm for 10 min, the generated serum was isolated and placed at -20°C until used in the biochemical assays outlined below. In the aliquot contained EDTA (1 mg/ml), determinations of total red blood cells (RBCs), packed cell volume (PCV), hemoglobin (Hb), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBCs), lymphocytes and heterophiles were carried out according to the method of Grindem (2011) using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy).

Oxidative status was assessed by evaluation of the enzymatic antioxidant biomarker; catalase (CAT) according to Aebi (1984). Reduced glutathione (GSH) determinations were made using the protocol described by Beutler *et al.* (1963). Lipid peroxidation was evaluated through measurement of malondialdehyde (MDA) content in the tissues according to Uchiyama and Mihara (1978).

The differences among treatments were statistically analyzed with a one-way ANOVA test in a completely randomized design as the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where μ= the overall mean, T_i = the fixed effect of treatment, e_{ij} = residual error. The significant differences among means were compared using Duncan's new multiple-range test (Duncan, 1955).

RESULTS AND DISCUSSION

In the current study, the overall mean of ambient temperature, relative humidity and temperature humidity index (THI) were 90.37 ± 0.34°F, 67.84 ± 0.76% and 84.67 ± 0.35, respectively which reflecting a state of severe heat stress.

Growth Performance

The effects of vitamin C (VC), vitamin E (VE), or betaine (BET) on growth performance parameters including average daily feed intake (ADFI), final live body weight (FLBW), daily body weight gain (DBWG), feed conversion ratio (FCR), and mortality rate (MR) of rabbits reared under heat stress conditions are presented in Table 2. Initially, a notable retardation of the growth performance of the non-supplemented heat stressed growing rabbits was clear at the end of experiment.

High ambient temperature decreases growth performance, possibly because of excessive reactive oxygen species that oxidize and destroy cellular biological molecules, inhibit some ATPase activities and finally cause a variety of impairments to intestinal tissues (Liu *et al.*, 2011). Additionally, (Ondruska *et al.*, 2011) reported that feed intake; feed conversion ratio and body weight gain of growing NZW rabbits were negatively affected when rabbits were exposed to heat stress.

In contrast, throughout the experimental period, rabbits receiving VC, VE or BET had significantly ($P < 0.05$) higher FLBW by 10.7, 12.2 and 11.4% respectively, compared to the non-supplemented group. Addition of VC, VE or BET to rabbit diets significantly ($P < 0.05$) improved the DBWG during the period between 5-9 weeks of age and insignificantly improved the DBWG during the period between 9-13 weeks of age. However, throughout the overall period, addition of VC, VE or BET had significantly higher DBWG by 17.4, 19.4 and 18%, respectively, compared to the control group.

Nevertheless, not significant differences were recorded in ADFI between all experimental groups along the experimental period. On the other hand, supplementation of rabbit diets with VC, VE or BET evoked a significant decrease in FCR by 14.0, 13.5, and 13.8%, respectively compared to the non-supplemented group. The present results are in

Table 2. Effect of tested diets on growth performance and mortality rate of NZW growing rabbits

	Experimental diet				Sig.
	Control	Vitamin C	Vitamin E	Betaine	
Live body weight (g)					
5 wk	646±32	636±30	639±32	643±37	NS
9 wk	1194±43	1343±40	1363±51	1319±45	NS
Final (13 wk)	1822^{b}±46	2017^{a}±45	2044^{a}±29	2030^{a}±54	*
Body weight gain (g)					
5-9 wk	19.59^{b}±0.86	25.24^{a}±1.09	25.87^{a}±1.07	24.15^{ab}±1.32	**
9-13 wk	22.41±0.76	24.08±0.79	24.30±1.34	25.38±0.87	NS
Overall (5-13 wk)	21.00^{b}±0.53	24.66^{a}±0.61	25.08^{a}±0.64	24.77^{a}±1.06	***
Daily feed intake (g)					
5-9 wk	72.46±1.15	74.35±4.28	80.30±5.17	76.64±4.97	NS
9-13 wk	122.2±3.2	121.2±3.2	118.9±2.7	123.7±2.9	NS
Overall (5-13 wk)	94.53±3.31	95.35±4.40	98.76±3.05	96.05±6.07	NS
Feed conversion ratio (g feed/ g gain)					
5-9 wk	3.71^{a}±0.06	2.95^{b}±0.17	3.10^{b}±0.20	3.17^{ab}±0.17	*
9-13 wk	5.45^{a}±0.16	5.04^{b}±0.13	4.89^{b}±0.13	4.87^{b}±0.11	***
Overall (5-13 wk)	4.50^{a} ±0.14	3.87^{b}±0.19	3.94^{b}±0.12	3.88^{b}±0.19	***
Mortality rate (%)	16.67	8.33	16.67	16.67	NS

Means in the same row bearing different letters differ significantly (P < 0.05). NS = Not significant, * P <0.05, ** P <0.01 and *** P <0.001.

harmony with those of Arafa *et al.* (2012), who used a 120 mg of VE in growing rabbit diets reared under moderate heat stress conditions and Attia *et al.* (2009) who used 250 mg of VE in diet of slow-growing heat stressed chicks. Similarly, BET enhanced the growth performance in heat stressed pigs (Campbell *et al.*, 1995), ducks (Wang *et al.*, 2004), chickens (Zhan *et al.*, 2006), and geese (Su *et al.*, 2009). The improvement of growth performance achieved with VE and VC could be related to their inhibitory role on the production of both prostaglandins and the enzymes involved in gluco-corticoids production, corticosterone which negatively affects growth of stressed animals (Dalólio *et al.*, 2015; Hajati *et al.*, 2015). It has been also postulated that the improved performance results from a decrease in protein-derived gluconeogenesis (Pardue *et al.*, 1985). The improved performance due to BET supplementation might be attributed to several factors including donation of methyl groups (Kidd *et al.*, 1997) beside increasing growth hormone and insulin-like growth factor-I (Huang *et al.*, 2007). Here, it has been noted that, among different experimental groups, VC supplemented rabbits exhibited the lowest mortality rate. Similarly, (Ismail *et al.*, 1992) reported that a daily dose of 25 mg VC to breeding does reared in hot season in Egypt (23-32ºC) was able to reduce the incidence of still births and kit mortality during the first week of live. This could be related to its role in defending against pathogenic virus and bacteria and enhancing the immune system (Elghaffar *et al.*, 2000).

Water intake

In the current trial, control group recorded the highest daily water intake when estimated by different unit especially in ml/g feed intake as shown in Table 3. This finding is compatible with the outcome of Badr (2015) in rabbit does.

This could be a way through which rabbits compensate water loss in respiratory vaporization (Habeeb *et al*., 1993). On the contrary, compared to control rabbits, supplementation of VC, VE, or BET evoked a significant ($P < 0.001$) reduction by 19, 18 and 33% in daily water intake (ml/g feed intake) of heat stressed growing rabbits at 5-13 weeks of age. This reduction could be possibly related to the ameliorative effect of the tested additives on animal's rectal, ear and skin temperatures.

Digestibility and Nutritive Values

Herein, in the non-supplemented rabbits, a marked dawdling effect of severe heat stress condition on the digestibility of DM, OM, CP and NFE was evident. This could be explained by the fact that the blood flow to the gastrointestinal tract is reduced during heat stress condition so nutrient digestibility is generally suppressed (Pearce, 2011). On the other hand, data in Table 4 showed that the digestibility of DM, OM, CP and NFE of growing rabbits under severe heat stress were significantly ($P < 0.05$ and $P < 0.01$) increased in response to BET supplementation compared to the non-supplemented group. Furthermore, the improvement of digestion coefficients of DM, OM, CP and NFE were reflected on the enhancement of nutritive values (DE, TDN and DCP). VE evoked a significant increase in the digestibility of CP and DCP in heat stressed growing rabbits, while OM and DM were significantly increased in VC supplemented group.

Overall, among the tested additives, BET only evoked a significant improvement in nutritive values with respect to the control group. It was suggested that such favorable effect of BET could be linked to its potency in stabilizing cell membranes through interaction with membrane phospholipids and to reduce fecal water content and accordingly increase the digestibility of several nutrients (Klasing *et al.*, 2002). Additionally, BET has been shown to enhance intestinal immunity and improve gut health and function (Metzler-Zebeli *et al.*, 2009).

Hematological Parameters

The effects of dietary supplementation with VC, VE or BET on hematological parameters (erythrograms, and leukograms) are shown in Table 5. The erythrograms revealed that mean values of RBCs, Hb, MCV, MCHC, and platelets count were not significantly differ in all supplemented rabbits compared to those in the control one. In BET or VC groups, both WBCs and lymphocytes values were significantly ($P < 0.01$) higher than in control one.

But, the former values showed a trend toward an increase in VE supplemented group, but were not differ significantly from those seen in control rabbits. Such elevation in WBCs and lymphocytes counts might be due to activation of gut associated lymphoid tissue in response to the diet supplemented with the tested additives. Several previous studies have confirmed the positive effects of dietary BET in improving cell-mediated immune responsiveness *via* increasing nitric oxide release form heterophils and macrophages in rats (Zhang *et al.*, 1996) and poultry (Farooqi *et al.*, 2005). Moreover, Wintergerst *et al.* (2006) and Pekmezci (2010) confirmed that supplementation of VC or VE was found to improve components of the human immune system such as natural killer cell activities and lymphocyte proliferation.

Antioxidant and Lipid Peroxidation Status

During exposure to heat stress, ample of oxygen-derived free radicals is generated causing oxidative damage of macromolecules (Sahin and Kucuk, 2003). Additionally, catecholamines and corticosteroids are released in excess inducing lipid peroxidative damage (Bahrami *et al.*, 2012). Hence, in the current study, to clarify the underlying mechanism of the tested feed additives in ameliorating heat stress impacts in growing rabbits, antioxidants and lipid peroxidation assays were performed. Fig. 1 demonstrate the effects of VC, VE or BET supplementation on the enzymatic antioxidant

Table 3. Effect of tested diets on daily water intake of NZW growing rabbits

Item	Control	Vitamin C	Vitamin E	Betaine	Sig.
Daily water intake as;					
ml /head					
5-9 wk	246 ± 9.5	230 ± 13.2	239 ± 12.5	213 ± 10.4	NS
9-13 wk	387^{a}± 12.3	327^{b}± 14.4	358^{ab}± 12.8	285^{c}± 9.7	***
5-13 wk	315^{a}± 10.1	278^{bc}± 12.8	294^{ab}± 12	249^{c}± 8.9	**
ml/Kg body weight					
5-9 wk	253 ±14.83	222 ±11.09	232 ±16.08	211±10.39	NS
9-13 wk	252^{a} ±10.09	173^{bc} ±17.34	204^{b} ±10.59	165^{c} ±5.78	***
5-13 wk	251^{a} ±11.67	197^{b} ±11.46	220^{ab} ±11.90	192^{b} ±8.46	**
ml/g feed intake					
5-9 wk	3.92^{a}± 0.16	3.53^{ab}± 0.32	3.29^{ab}± 0.23	2.95^{b}± 0.16	*
9-13 wk	3.85^{a}± 0.19	2.75^{c}± 0.08	3.13^{b}± 0.10	2.27^{d}± 0.08	***
5-13 wk	3.88^{a}± 0.16	3.14^{b}± 0.19	3.19^{b}± 0.14	2.61^{c}± 0.11	***

Means in the same row bearing different letters differ significantly (P < 0.05). NS= Not significant * P <0.05, ** P <0.01 and *** P <0.001.

Table 4. Effect of tested diets on digestibility and nutritive values of NZW growing rabbits

	Experimental diet				Sig.
	Control	**Vitamin C**	**Vitamin E**	**Betaine**	
Nutrient digestibility (%)					
Dry matter	64.12^{c}± 0.83	66.80^{ab}± 0.27	66.03^{bc}± 1.02	68.96^{a}± 0.76	**
Organic matter	64.99^{c}± 0.85	67.63^{ab}±0.28	67.26^{abc}±1.07	69.55^{a}± 0.79	*
Ether extract	66.91± 2.46	65.59± 2.51	67.01± 1.36	69.47± 1.09	NS
Crude protein	73.81^{b}± 0.51	75.47^{ab}± 0.23	75.74^{a} ± 0.83	77.11^{a} ± 0.53	*
Crude fiber	34.32± 2.15	39.35± 1.16	36.67± 2.21	41.62± 1.31	NS
NFE	68.59^{b}± 0.90	71.27^{ab}± 0.32	71.09^{ab}± 1.07	73.09^{a}± 0.99	*
Nutritive values					
DCP (%)	13.44^{b}± 0.09	13.74^{ab}± 0.04	13.79^{a}± 0.15	14.04^{a}± 0.10	*
TDN (%)	61.75^{b}± 0.75	64.09^{ab}±0.22	63.81^{ab}± 1.00	65.99^{a}± 0.76	*
DE (Kcal) #	2740^{b}± 32.51	2842^{ab}± 9.82	2831^{ab}±43.64	2925^{a}± 32.91	*

Means in the same row bearing different letters differ significantly (P < 0.05). NS = Not significant, * P <0.05 and ** P <0.01 # calculated according to Schieman *et al*. (1972)

Table 5. Hematological parameters as affected by vitamin C, vitamin E or betaine supplementation

	Experimental diet				Sig.
	Control	**Vitamin C**	**Vitamin E**	**Betaine**	
Erythrogram					
RBCs ($10^6/mm^3$)	4.27±0.32	4.80±0.32	4.53±0.36	4.63±0.08	NS
Hb (g/dl)	10.15±0.41	11.00±0.43	10.20±0.32	10.63±0.09	NS
PCV (%)	28.68±2.35	32.98±2.46	30.13±2.29	30.28±0.94	NS
MCV(Fl)	67.19±1.12	68.65±1.88	66.62±0.37	65.31±1.23	NS
MCHC (%)	35.76±1.54	33.65±1.42	34.25±1.79	35.20±1.09	NS
Platelets	225±16	317±27	255±19	231±20	NS
Leukogram					
WBCs ($10^3/mm^3$)	2.89^c±0.39	6.29^a ±0.99	3.43^{bc} ±0.22	4.95^{ab} ±0.27	**
Lymphocyte ($10^3/mm^3$)	2.68^c±0.33	5.57^a±0.75	3.25^{bc}±0.22	4.57^{ab} ±0.17	**
Heterophile ($10^3/mm^3$)	0.06±0.02	0.36±0.23	0.06±0.01	0.12±0.05	NS

Means in the same row bearing different letters differ significantly (P < 0.05). NS = Not significant, ** P <0.01.

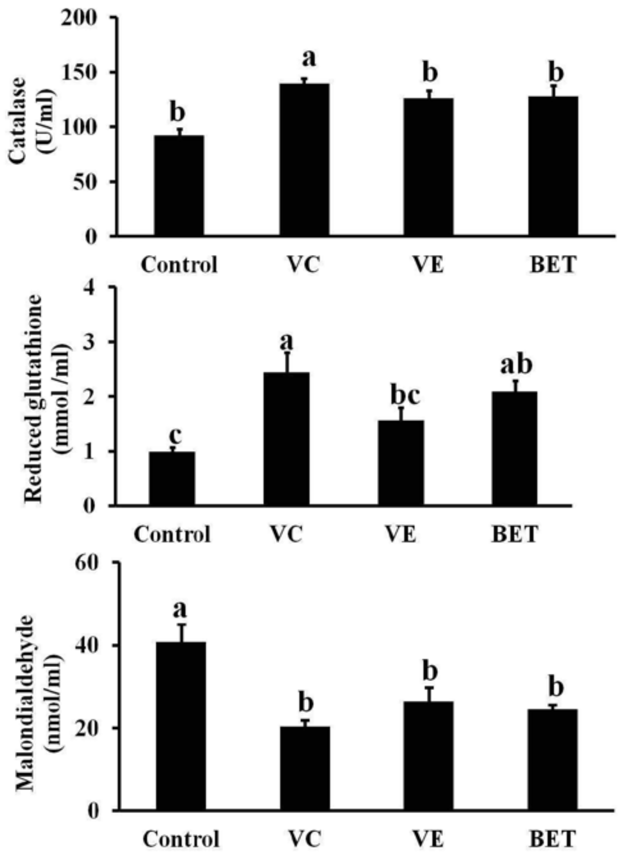

Fig. 1. Effect of vitamin C, vitamin E, or betaine on oxidative status of NZW growing rabbits

(*i.e.* CAT), non-enzymatic antioxidant (*i.e.* GSH) and lipid peroxidation biomarker (*i.e.* MDA) of severe heat stressed growing rabbits. Primarily, a sharp decline in reduced glutathione content and catalase activity concomitanted with a rise in the MDA level was evident in the control rabbits as a result of heat stress induced oxidative stress and lipid peroxidation.

Controversy, CAT activity and GSH contents were significantly increased in VE, VC or BET supplemented rabbits compared with those in control ones. In contrast, MDA level was significantly ($P< 0.01$) depleted in all tested additives relative to the control ones. VC and VE, as soluble nature, participate actively in the structure of organic compounds because it is situated at the membrane level, minimizing oxidative damage and the peroxidations of fatty acids and phospholipid components (Bou *et al.*, 2009; Hajati *et al.*, 2015). On the other hand, the methyl donor property of BET could be partially responsible for its antioxidant activity (Alirezaei *et al.*, 2015).

Noteworthy, BET showed a significant improvement in growth performance, digestibility and antioxidant status with potency similar to that of VC and VE. Similarly, in slow-growing chicks, supplementation of BET at 1g/kg and VC at 250 mg/kg diet was equally potent for partial amelioration of heat stress effects on their performance (Qota *et al.*, 2008).

BET, as an osmolyte and a methyl group donor, may maintain the thermo neutral state of the animal through reducing heat-induced inhibition of osmotic equilibrium and maintaining the tertiary structure of macromolecules in the kidney and other tissues (Lever *et al.*, 2004; Huang *et al.*, 2007).

Economical Feed Efficiency

With respect to the price of kilogram diet of the control group, addition of VE, VC or BET markedly elevates the later price (Table 6). However, the relative revenue was increased by 17, 20, and 23% with addition of BET, VC, or VE, respectively. Hence the economical feed efficiency in this study showed that the using BET, VC, or VE in the growing rabbit diets was more economical than the non-supplemented diets.

Conclusion

Based on the data presented above, it could be concluded that supplemental dietary BET, VC, or VE enhanced growth performance and nutrients digestibility, stabilized the normal oxidative balance, and elevated the cell-mediated immunity of rabbits exposed to heat stress. Thus, from both health and an economic point of view, several benefits might be gained by adding these additives to the diet of commercial rabbits under heat stress conditions.

Table 6. Input-output analysis and economic efficiency of diets supplemented with the tested feed additives

	Experimental diets			
	Control	**Vitamin C**	**Vitamin E**	**Betaine**
Total feed intake (g)	5294	5340	5531	5379
Price/Kg feed (LE)	2.40	2.50	2.44	2.55
Total feed cost (LE)	12.71	13.35	13.50	13.72
Body weight gain (g)	1176	1381	1405	1387
Total revenue (LE*)	28.22	33.14	33.72	33.29
Net revenue (LE)	15.51	19.79	20.22	19.57
Economic efficiency (EE)**	1.22	1.48	1.50	1.43
Relative economic efficiency (%)	100	121	123	117

* The price was calculated according to the local market price which was 24 LE/Kg rabbit live weight. ** EE= net revenue/ total feed cost

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تأثير إضافة فيتامين ج، فيتامين هـ أو البتايين على تخفيف أثر العبء الحرارى على الأرانب النامية

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أجريت هذه الدراسة لتقييم أثر إستخدام كل من البتايين و فيتامين ج و هـ كإضافات غذائية علي الأداء الإنتاجي وهضم العناصر الغذائية و مضادات التأكسد في الأرانب النامية المعرضة للعبء الحراري الشديد، تم استخدام عدد ٤٨ من ذكور الأرانب النيوزلاندية البيضاء عمر ٥ اسابيع متوسط وزن ٦٤١±١٥.٧٧جراماً، قسمت الأرانب إلي أربع مجموعات متجانسة (١٢ أرانب لكل مجموعة)، تم تغذية المجموعة الأولي علي عليقة مقارنة بدون إضافات (كنترول) بينما أضيف كل من فيتامين ج، هـ أو البتايين بمعدل ٥٠٠ و ٢٠٠ و١٠٠٠ مجم/كجم من العليقة على التوالي لباقي المجموعات لمدة ٨ اسابيع.أظهرت النتائج حدوث تحسن معنوي لوزن الجسم الحي ومعدل النمو اليومي مع نقص ملحوظ في كمية المياه المستهلكة يوميا ومعدل التحويل الغذائي في الأرانب التي تغذت علي العلائق المعاملة بالإضافات الغذائية مقارنة بتلك المغذاة علي عليقة الكنترول، أظهرت الأرانب المغذاة علي العلائق المحتوية علي البتايين تحسنا معنويا ملحوظا في معاملات الهضم لكل من المادة الجافة، المادة العضوية، البروتين الخام والمستخلص الخالي من النيتروجين مقارنة بتلك المغذاة على عليقة المقارنة، أحدثت جميع الإضافات المستخدمة تحسنا ملحوظا في مؤشرات الضغط التأكسدي ودرجة تأكسد الدهن و عدد خلايا كرات الدم البيضاء والخلايا الليمفاوية.وإجمالياً فإن إضافة البتايين أو فيتامين ج أو هـ إلى علائق الأرانب النامية المتعرضة للعبء الحراري الشديد أظهرت تأثيرات إيجابية على الأداء الإنتاجي والعائد الاقتصادي ومضادات التأكسد بالجسم.

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