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EFFECTS OF DIETARY BETAINE SUPPLEMENTATION ON GROWING RABBITS UNDER HEAT CONDITIONS.

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The objective of the present study is to determine the effects of dietary betaine supplementation on growth performance, carcass characteristics, rectal temperature, respiration rate and immune response of growing rabbits under high ambient temperature. Ninety New Zealand White (NZW) rabbits weaned at 6 weeks of age, were randomly divided into five experimental treatments (n=18) and were provided with water and a commercial pelleted basal diet ad libitum. Animals were fed the basal diet supplemented with 0 (control), 250, 500, 750 and 1000 mg betaine /kg diet for 12 continuous weeks. The average daily temperature and relative humidity inside the house reached about 30.3±0.9 °C and 76.2±2.5 %, respectively.Results obtained showed that under heat stress conditions, inclusion of betaine in the growing rabbit diets increased the final body weight and hot carcass weight (P≤ 0.05) by 20 and 34.7 %, respectively, and improved (P≤ 0.05) the feed conversion by 12% as the level of betaine increased from 0 to 1000 mg / kg in rabbit diets . Betaine supplementation ameliorated some of the adverse effects of heat stress on immune response, rectal temperature and respiration rate. The inclusion of 1000 mg betaine/kg in the growing rabbit diets markedly enhanced the humoral and cellular immune responses being about two-fold its corresponding value of controls and significantly reduced rectal temperature and respiration rate. Serum T3, T4, total protein, globulin and total lipids were significantly increased (P≤ 0.05) while serum glucose concentration was significantly decreased due to dietary betaine. Digestibility coefficients of CP, EE and NFE were increased (P≤0.05) by 12.8, 10.7 and 10.6% in rabbits feed with 1000 mg/ kg as compared with those fed control diet . In conclusion, supplementation of betaine in growing rabbits diets enhanced growth performance and carcass weight, however, stabilized the normal physiological balance, and elevated the humoral and cell-mediated immunity as well as reduced rectal temperature and respiration rate in growing rabbits exposed to heat stress.

Keywords: Betaine, stress, performance, carcass, immunity, digestibility.

Rabbits are very sensitive to heat stress, as they have few functional sweat glands and have difficulties in eliminating body heat when the environmental temperature is high. Exposing rabbits to high ambient temperatures (above 30°C) impaired the growth performance (Fekry, 1989; Marai *et al.*, 2002), decreased feed consumption, body weight and weight gains (Chiericato *et al.*, 1995; Marai *et al.*, 1996 and 2001) however, increased water consumption (Marai *et al.*, 2001 and 2002). In heat stressed rabbits, both respiration rate and pulse rate are increased (Naqvi *et al.*, 1995; Marai *et al.*, 2002). Moreover, exposing rabbits to high environmental temperature resulted in disturbing the normal physiological balance of the animal's body temperature, hormonal and water balances (Daader et al., 1989; Habeeb *et al.*, 1989 and1997). Under heat stress, plasma T3 and T4 concentrations and the immunity responsiveness were also decreased (Boiti *et al.*, 1992; Amici *et al.*, 2000; Mustafa *et al.*, 2008). Siegel (1995) documented that thermal stressors have been shown to reduce concentrations of circulating antibodies and suppress cell-mediated immunity resulting in reducing the fitness or survival leading to increasing the mortality.

Betaine, or glycine betaine, is a naturally occurring compound, which widely used, in animal nutrition. Betaine has methyl donor and osmoprotective properties, which are essential in the nervous, immune, renal and cardiovascular systems (Kidd *et al.*, 1997). Betaine supplementation significantly improved body weight gain and feed conversion in chickens (Hassan *et al.*, 2005; Zhan *et al.*, 2006), ducks (Wang *et al.*, 2004) and pigs (Campbell *et al.*, 1995). Klasing *et al.* (2002) observed that betaine increased villi height and presumably absorptive area. Also, a positive effect of dietary betaine on carcass characteristics and fat distribution was noticed in avian species and mammals (Virtanen and Rosi, 1995; Wang *et al.*, 2004; Huang *et al.*, 2006). Moreover, betaine was involved to enhance immune response by (Zhang *et al.*, 1996; Klasing *et al.*, 2002; Hassan *et al.*, 2005). However, Zhang *et al.* (1996) recorded that betaine may be contribute in regulating cytokines production by liver macrophages (Kupffer cells) via inhibiting the prostaglandin synthesis in rats.

A number of experiments showed that the addition of betaine to the diets in particular improves performance under stress conditions that affect cell osmolarity including coccidiosis infection (Klasing *et al.*, 2002) and heat stress (Teeter *et al.*, 1999; Zulfiki *et al.*, 2004; Farooq *et al.*, 2005). Also, Wang *et al.*, (2004) suggested that dietary betaine supplementation seems to be important to improve the resistance to stress. Dietary betaine (0.3 g/kg) has been shown to decrease heat stress in poultry (Zulkifli *et al.*, 2004). Since betaine can have a positive effect on carcass yield and quality in both poultry (Wang *et al.*, 2004) and pigs (Huang *et al.*, 2006). The addition betaine to feed may help to overcome the negative effects of heat stress on performance and carcass quality (Kidd *et al.*, 1997 and Metzler-Zebeli *et al.*, 2009). These positive effects of betaine may be also related to the reduction of body temperature in broiler chickens fed diet with betaine under heat stress conditions

(Belay *et al.*, 1992 and Zulfiki *et al.*, 2004). Therefore, the objective of the present study is to determine effects of dietary betaine supplementation on growth performance, carcass characteristics, rectal temperature, respiration rate, blood serum constituents, digestibility and immune response of growing rabbits under high ambient temperature.

MATERIALS AND METHODS

The present experiment was carried out in Sakha Research Farm, Animal Production Research Institute, Ministry of Agriculture, Egypt, during the period from July to September 2009. (The hot climate in Egypt).

Animals, diets and housing

A total of 90 NZW rabbits, weaned at 6 weeks of age with an average initial body weight, 739.74 ± 35.30, g were randomly distributed to five experimental treatments (18 rabbits) each then divided into 3 replicates of six rabbits each. The basal diet was formulated to be isonitrogenous (17.0 %) and isocalorie (2500 Kcal DE / Kg diet), and to satisfy the nutrient requirements of growing rabbits according to the Agriculture Ministry Decree (1996) recommendation. Animals were fed the basal diet supplemented with 0 (control), 250, 500, 750 and 1000 mg betaine /kg diet throughout the whole experiment which lasted for 12 continuous weeks. Betaine was provided as Betafin®-BP (betaine anhydrous/pharmaceutical grade, Finnfeeds Finland Ltd).

Rabbits were housed individually in stainless steel individual cages (35x35x60 cm) provided with feeders and automatic nipple drinkers. The building was open-air, naturally ventilated and provided with sided electric fans. All rabbits were kept under the same managerial, hygienic and environmental conditions . Diets were offered to rabbits *ad libitum* and fresh water was available all the time .The averages of ambient temperature, relative humidity and temperature humidity index (THI) inside the building were 30.3±0.9 °C, 76.2±2.5% and 29.1, respectively, which means severe heat stress, according to Marai *et al.* (2002) there is severe heat stress when THI is higher than 28.9. The THI was calculated according to Marai *et al.* (2001):

$$THI = db℃ - [(0.31 - 0.031\ RH) \times (db℃ - 14.4)]$$

Where, db℃ is dry bulb temperature in Celsius degrees, and RH is the relative humidity as a percentage. All the experimental animals were healthy and clinically free from internal and external parasites and were kept under the same management and hygienic conditions. All experiments were performed in accordance with institutional guidelines concerning animal use.

Measurements

The rabbits were individually weighed at the beginning of the experiment and then weekly. Weighing was carried out before offering the morning meal (once

week) at 8.00 h and live body gain weight was calculated weekly. Feed consumption and feed conversion were determined.

Respiration rate and rectal temperature were recorded once a week at 9.00-11.00 h for each animal. The respiration rate was recorded by counting the flank movements per minute by using a hand counter. The rectal temperature was measured by using a clinical thermometer inserted into the rectum for 2 minutes at depth of 4 cm.

At the last week of the experiment, digestibility trial was conducted using 20 rabbits (four rabbits from each treatment group), which were housed individually in metabolism cages that allow faeces and urine separation. The preliminary period continued for 7 days and the collection period extended for 5 days. Feed and feces were daily recorded quantitatively for chemical analysis according to AOAC (2000)

At the termination of the experiment , 4 rabbits from each treatment were randomly taken kept off feed for 12 hours, weighed and slaughtered for carcass traits and meat analysis. Slaughter procedure and carcass analysis were carried out as described by Blasco and Ouhayoumn (1996). After complete bleeding, pelt, viscera and tail were removed and the carcass and some carcass components were weighed. Giblets weight (liver, kidneys, heart and spleen) and carcass measurements were obtained and their proportion to the live body weight was calculated. Dressing percentage was calculated by dividing the dressed meat weight by preslaughter weight and expressed as a percentage. Meat chemical analyses including crude protein (CP), ether extract (EE), crude fiber (CF) and ash were determined according to AOAC (2000).

Blood samples were collected at slaughter to determine blood components. Serum was separated by centrifugation at 5900g for 10 min and frozen at -20 ℃ until analysis. Blood serum triiodothyronine (T3), thyroxin (T4), total proteins, albumin, total lipids, total cholesterol, glucose, sodium, potassium, calcium, phosphorus and activity of serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase (ALP) were colormetrically determined using commercial kits purchased from Bio-diagnostic, EGYPT, following the same steps as described by manufactures. However, globulin was calculated by the difference between total proteins and albumin.

Total antibody titres against sheep red blood cells (SRBCs) were measured in 4 rabbits selected randomly in each treatment at 12 weeks of age. Humoral immunity is an organism's antibody response to pathogens. Antibody response to a T-dependent (both T- and B-cells must recognize antigen) antigen was assessed by measuring serum antibody titre following a primary intramuscular injection of 0.5 ml of a 40% SRBCs suspension (in phosphate-buffered saline) in the right biceps femoris muscle. Antiserum to SRBCs was collected 7 days after challenge. Antibody titres to SRBCs were measured using a microhaemagglutination assay by the method of Wegmann and Smithies (1966).

Delayed type hypersensitivity responses were measured in another 4 rabbits selected randomly in each treatment at 12 weeks of age. To determine cell-mediated immunity, the lymphoproliferative responses to phytohemagglutinin-P (PHA-P), a lectin from *Phaseolus vulgaris* (Sigma Chemical, cat. 61765), was measured in the right ear of each animal following the procedure of Stadecker *et al.* (1977). Fifty ?g of PHA-P in 0.1 ml of sterile saline was injected intradermally in the right ear of each animal. The left ear sites (as negative controls) were injected with sterile saline only. Ear thickness of each rabbit was measured to the nearest 0.01mm using a constant-tension dial micrometer (Mitutoyo Co., Tokyo, Japan) just before the injection and again at 24 h post-PHA-P injections. The response to PHA-P mitogen was calculated in millimeters as the difference between the increment of thickness of right ear post PHA-P injection and increment of thickness of left ear post saline injection (Stadecker *et al.*, 1977).

Table 1: Composition and chemical analysis of the experimental diet

Ingredients	(%)
Clover hay (12 % CP)	22.50
Barley grain	27.25
Wheat bam	28.90
Soybean meal (44% CP)	15.00
Molasses	3.00
Limestone	0.70
Di-calcium phosphate	1.70
Sodium chloride	0.50
Mineral-vitamin premix [1]	0.30
DL-Methionine	0.15
Total	**100.00**
***Calculated analysis*[2]:**	
Crude protein %	17.00
Ether extract %	2.994
Digestible energy (Kcal / Kg)	2500
Crude fiber %	12.00
NDF %[m]	36.81
ADF %[n]	20.38
Hemicellulose %[o]	16.43
Calcium %	1.09
Total phosphorus %	0.80
Methionine %	0.409
Lysine %	0.718
DE : CP	147.06

[1] **One kilogram of premix contained** : Vit. A, 150,000 IU; Vit. E, 100 mg; Vit. K3, 21 mg; Vit. B1, 10 mg; Vit. B2, 40 mg; Vit. B6, 15 mg; pantothenic acid, 100 mg; Vit. B12, 0.1 mg; niacin, 200 mg; folic acid, 10 mg; biotin, 0.5 mg; choline chloride, 5,000 mg; Fe, 0.3 mg; Mn, 600 mg; Cu, 50 mg; Co, 2 mg; Se, 1 mg; and Zn, 450 mg.

[2] According to feed composition Tables for Animal and Poultry Feedstuffs used in Egypt (2001).

[m][n][o] Calculated according to Cheeke (1987). [m] % NDF = 28.924 + 0.657 (% CF)

[n] % ADF = 9.432 + 0.912 (% CF) [o] % Hemicellulose = % NDF - % ADF

Statistical Analysis

The differences among treatments were statistically analyzed with one-way ANOVA test in a completely randomized design using Statistical Packages for the Social Sciences (SPSS®, 2001). The significant differences among means were compared using Duncan's new multiple-range test (Duncan, 1955). Data presented as percentages were transformed to the corresponding arcsine values before performing the statistical analysis. $P \leq 0.05$ was set as the limit of significance.

RESULTS AND DISCUSSION

1-Growth performance:

Results concerning effects of dietary betaine supplementation on growth performance of growing rabbits which presented in (Table 2), indicated that supplemented diet with betaine had a positive effect on growth performance of growing rabbits during a period of high ambient temperature. Under high ambient temperature, final body weight and total gain were increased ($P \leq 0.05$) by 20 and 30 %, respectively, in the rabbits fed diet with 1000 mg / kg as compared with these fed control diet (0 mg /kg). Feed intake increased ($P \leq 0.05$) from 83.7 to 96 g /d as the level of betaine increased from 0 to 1000 mg / kg in rabbit diets . Also, feed conversion improved ($P \leq 0.05$) by 12 % in the rabbits fed diet containing 1000 mg / kg as compared with those fed control diet . According to our knowledge, no reports of similar research in rabbits have been published; however, the results from the current trial are in agreement with several studies in chickens (Hassan *et al.*, 2005; Zhan *et al.*, 2006), ducks (Wang *et al.*, 2004) and pigs (Campbell *et al.*, 1995). These authors concluded that betaine supplementation significantly improved body weight gain and feed conversion. The improvement of growth performance due to betaine supplementation might be attributed to several reasons; i.e., donating of methyl groups (Kidd *et al.*, 1997), increasing growth hormone and insulin-like growth factor-I (Huang *et al.*, 2006), enhancing intestinal immunity (Klasing *et al.*, 2002) and improving gut health and function (Kettunen *et al.*, 2001; Metzler-Zebeli *et al.*, 2009). Moreover, betaine has been shown to stabilize cell membranes through interaction with membrane phospholipid (Rudolph *et al.*, 1986), and to reduce fecal water content and increase the digestibility of several nutrients (Virtanen, 1995). These properties could be reduce intestinal membrane damage, dehydration, diarrhea and mal-digestion and/or absorption which leading to improve gut health and consequently enhance the ability of the animals to withstand coccidial infection (Kettunen *et al.*, 2001). Therefore, betaine may have contributed to the improved performance of rabbits directly, by partial inhibition of coccidial invasion and development, and indirectly by supporting the intestinal structure and function. It is important to mention that, in the present experiment, there was no apparent occurrence of coccidiosis syndrome.

Table 2: Effect of dietary betaine supplementation on growth performance in growing rabbits under high ambient temperature.

Items	Betaine (mg/kg)					Sig
	0	250	500	750	1000	
Initial body weight (g)	735.5± 6.8	747.8± 5.7	738.5± 9.5	740.9± 9.5	735.7± 5.7	NS
Final body weight (g)	2110.3± 24.4^{d}	2233.6±21.5^{c}	2243.3± 28.8^{c}	2418.5± 23.1^{b}	2529.1± 21.5^{a}	*
Daily gain (g)	16.37±0.1^{d}	17.35±0.1^{c}	18.40± 0.1^{c}	20.0±0.1^{b}	21.35± 0.2^{a}	*
Total gain (g)	1375 ± 11.6^{c}	1486± 5.8^{d}	1545± 17.3^{c}	1678 ± 11.5^{b}	1793 ± 14.8^{a}	
Daily feed intake (g)	83.67±1.2^{b}	84.33± 0.9^{b}	84.66± 1.4^{b}	94.66± 1.7^{a}	96.03± 1.0^{a}	*
Feed conversion	5.11± 0.05^{a}	4.78±0.04^{b}	4.64± 0.08^{b}	4.69±0.08^{b}	4.5± 0.1^{b}	*
Viability % (6 to 18 wks)	83.3	87.5	88.9	94.4	100	

^{a,b,c,d} Means followed by different letters in the same row differed significantly.
NS= Non significant, * P<0.05.

2- Carcass traits and chemical composition of meat :

Data shown in (Table 3), indicated that betaine supplementation significantly increased hot carcass weight and kidney weight by 34.7 % and 37.9 % , respectively, in the rabbits fed diet with 1000 mg / kg as compared with these fed control diet . These results are in correspondence with the previous reports of Virtanen and Rosi (1995). Wang *et al.* (2004) Huang *et al.* (2006) who revealed a positive effect of dietary betaine on carcass characteristics in avian species and in mammals. Wang *et al.* (2004) indicated that betaine supplementation significantly increased breast meat yield in ducks significantly. In contrast to these results, Esteve-Garcia and Mack (2000) found relatively small and non-significant responses to betaine supplementation increasing breast yield of broiler chickens. As shown in Table 3, dietary betaine supplementation increased CP ($P \leq 0.05$) and decreased EE ($P \leq 0.05$) of meat. These responses were similar to observations by Esteve-Garcia and Mack (2000) who observed that betaine supplementation increased protein level in the muscles. They also concluded that betaine could enhance lipid catabolism via its role in carnitine synthesis leading to low carcass fat deposition. Conversely, Garcia *et al.* (2000) and Hassan *et al.* (2005) revealed that betaine addition had no significant effect on DM, CP, EE, and ash of meat.

3- Some physiological parameters :

Data concerning the effect of betaine supplementation on rectal temperature and respiration rate are illustrated in Figure 1(A, B), respectively. It is noteworthy to observe that both rectal temperature and respiration rate were decreased significantly in a dose-dependent manner when dietary betaine was increased. Interestingly, betaine supplementation to growing rabbit diets ameliorated some of the adverse effects of heat stress on rectal temperature and respiration rate. These results are coincident with those of Belay *et al.* (1992) and Zulfiki *et al.* (2004), who stated that body temperature of broiler chickens was decreased when betaine was added to the feed under heat stress conditions. In Egypt, the climate is characterized by a long hot

Table 3: Effect of dietary betaine supplementation on carcass characteristics and chemical composition of meat in growing rabbits under high ambient temperature.

Items	Betaine (mg/kg)					Sig
	0	250	500	750	1000	
Live body weight (g)	2100±28.8^{cd}	2200±37.7^{c}	2250±36.4^{c}	2400±35.3^{b}	2520±33.0^{a}	*
Hot carcass weight(g)	1239.3± 29.1^{d}	1374.6± 8.1^{c}	1388.3± 27.0^{c}	1510.0± 21.2^{b}	1669.6± 27.6^{a}	*
Dressing (%)	59.00± 0.5	58.00± 1.1	58.50± 0.8	58.67± 1.4	58.0± 1.7	NS
Liver (g)	83.80± 3.2	84.27± 1.1	83.53± 2.8	84.20± 1.3	85.57± 1.4	NS
(%)	3.91± 0.2	3.83± 0.0	3.74± 0.2	3.52± 0.1	3.67± 0.4	NS
Kidney (g)	21.00± 0.5^{b}	23.47± 1.0^{b}	23.37± 1.8^{b}	25.33± 0.6^{ab}	28.97± 1.6^{a}	*
(%)	1.00±0.04^{b}	1.07± 0.07^{ab}	1.05±0.14^{ab}	1.06±0.06^{ab}	1.16±0.05^{a}	*
Heart (g)	7.30± 0.1	7.50± 0.1	7.57± 0.1	7.70± 0.1	8.13± 0.1	NS
(%)	0.35± 0.01	0.34± 0.02	0.34± 0.02	0.32± 0.02	0.32± 0.01	NS
Lung (g)	14.00± 1.63	15.00± 2.8	15.10± 1.1	15.60± 0.3	16.0± 0.5	NS
(%)	0.66± 0.07	0.69± 0.15	0.66± 0.02	0.65± 0.05	0.64± 0.04	NS
Head (g)	236.7± 2.4	226.0± 3.0	233.3± 14.5	244.7± 6.6	253.3± 19.2	NS
(%)	11.27± 0.2	10.28± 0.2	10.36± 0.2	10.22± 0.3	10.10± 0.4	NS
Chemical composition of meat (%DM basis):						
DM	30.00± 1.5	30.17±1.7	30.13± 2.2	30.43± 3.3	31.17± 2.3	NS
CP	63.16± 1.7^{b}	64.50± 1.0^{ab}	63.63± 1.7^{b}	64.43± 1.7^{ab}	67.63± 1.1^{a}	*
EE	24.93± 0.8^{a}	24.20± 1.2^{a}	24.00± 1.2^{ab}	23.83± 0.9^{ab}	23.00± 0.8^{b}	*
Ash	4.19± 0.1	4.33± 0.1	4.00± 0.3	3.99± 0.1	4.00± 0.2	NS

^{a,b,c,d} Means followed by different letters in the same row are differed significantly.
NS= Non significant, * P≤0.05

period (from May to October) and short mild one (from December to March). In hot period, rabbits have difficulty in elimination body heat due to their unfunctional sweat glands (Marai *et al.*, 1996). Different physical and physiological methods have been used to alleviate the heat load in heat stressed animals. The physical methods used are such as sheltering, air conditioning, zone air cooling, drinking cool water, using wet or iced sacks in the cages, spray or sprinkling the roofs and floor with tap water and shearing (Habeeb *et al.*, 1997). However, in all cases rabbits must be kept dry, since wet coats are predisposing causes for pneumonia and respiratory troubles (Marai *et al.*, 1996) which have led to the investigation of a number of dietary agents which might alleviate the adverse effects of temperature. It is well known that body temperature and respiration rate are increased following exposure to high (>30°C) environmental temperatures (Naqvi *et al.*, 1995; Habeeb *et al.*, 1997; Marai *et al.*, 2001, 2002). In this respect, due to its ability in reducing body temperature and consequently respiration rate, betaine might be supplemented to the growing rabbit diets during hyperthermic stress in order to alleviate some of the adverse effects of heat stress.

From another point of view, the increase in body temperature due to the exposure to ambient temperatures above the thermal comfort zone has a negative impact on animal performance via decreasing feed intake, body weight gain and the resistance to disease and increasing the feed conversion ratio (Habeeb *et al.*, 1997; Marai *et al.* 2001; Bani *et al.*, 2005). The decrease in feed consumption is due to

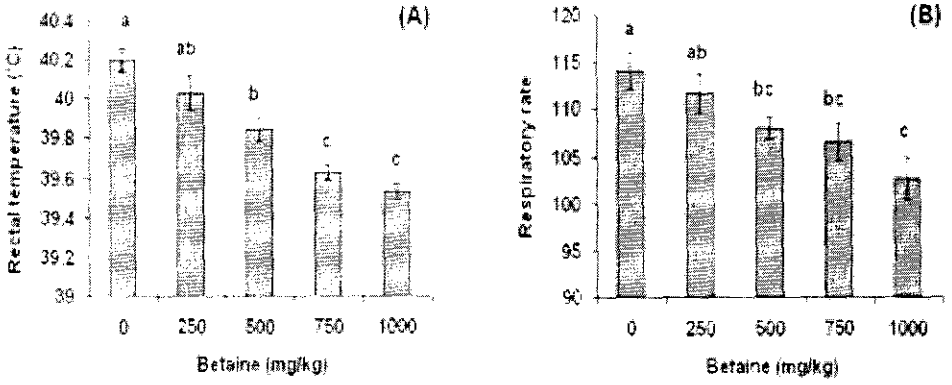

Figure 1: Effect of dietary betaine supplementation on rectal temperature (A) and respiratory rate (B) in growing rabbits under high ambient temperature. Values are expressed as means± standard error; means with different letters are significantly ($P \leq 0.05$) different from each other.

impairment of appetite as a result to stimulation of the peripheral thermal receptors by the environmental temperature to transmit suppressive nerve impulses to the appetite center in the hypothalamus that causes that phenomenon (Marai *et al.* 2002). Since betaine had a significant effect in reducing body temperature which consequently participated in enhancing feed intake and body weight as well as carcass yield (Table 2& 3), it could be assumed that dietary betaine supplementation might help to overcome the negative effects of heat stress on growth performance and carcass yield. This assumption is in agreement with Kidd *et al.* (1997) who concluded that the addition of betaine to feed or drinking water might help to overcome the negative effects of heat stress on performance and carcass quality.

4- Immunity :

The influences of dietary betaine treatments on humoral and cellular immunity are graphically presented in Figure 2(A and B), respectively. It is interesting to note that dietary betaine supplementation improved both humoral immunity (measured by antibody titres against SRBCs) and cell-mediated immunity (indicated by PHA-P induced proliferative response) as compared with control ($P \leq 0.05$). Under heat stress conditions, the inclusion of 1000 mg betaine/kg in the growing rabbit diets markedly enhanced the humoral immune response being about two-fold its corresponding value of controls (Figure 2A). Not surprising that the same trend was observed in cellular immune response (Figure 2B). These results are in accordance with some published work (Kettunen *et al.*, 2001; Klasing *et al.*, 2002; Hassan *et al.*, 2005) meanwhile contradict others (e.g., Tsiagbe *et al.*, 1987). In fact,

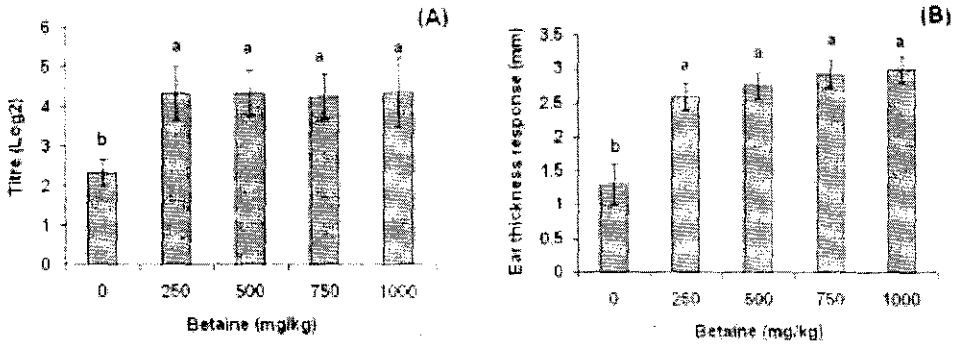

Figure 2: Effect of dietary betaine supplementation on antibody titre (A) and change of ear thickness response to PHA-P (B) in growing rabbits under high ambient temperature. Values are expressed as means± standard error; means with different letters are significantly (P<0.05) different from each other.

according to our knowledge, information on the effect of betaine on immunity in rabbits is scarce, however; several studies documented the positive effects of dietary betaine in improving immunity in rats (Zhang *et al.*, 1996) and poultry (Klasing *et al.*, 2002; Hassan *et al.*, 2005; Farooq *et al.*, 2005). Also, Farooq *et al.* (2005) reported that betaine supplementation improved the immune response in birds exposed to heat stress. Likewise, Hassan *et al.* (2005) noted that betaine addition at 0.072 and 0.144% significantly increased antibody titre against SRBCs. Similarly, Klasing *et al.* (2002) proved that betaine is involved in enhancing cell-mediated immune responsiveness via increasing nitric oxide release form heterophils and macrophages. Moreover, Zhang *et al.*, (1996) indicated that, in rats, betaine is involved in regulating cytokines (e.g., tumor necrosis factor-α, TNF-α) production by liver macrophages (Kupffer cells) via inhibiting the prostaglandin synthesis, suggesting a new role of organic osmolytes (especially betaine) in modulating immune function. Furthermore, Cosquer *et al.*, (2004) elucidated that glycine betaine possesses antibacterial activities including 15 Gram-positive and Gram-negative bacteria. Presence of these properties in betaine may explain the enhancement of immune system activity. This means that dietary betaine might be affected the pathogenic bacteria. Taken together, the previous studies obviously indicated that dietary betaine supplementation plays a vital role in improving the immune response and, indeed, getting such benefits in growing rabbits is a vital necessity especially under stressful conditions.

5- Blood serum metabolites :

With respect to the effect of dietary betaine supplementation on blood serum constituents in growing rabbits under high ambient temperature, it could be observed

that serum T3, T4, total protein, globulin and total lipids were significantly increased ($P \leq 0.05$) when betaine was supplemented (Table 4). The positive effect of betaine in serum total protein and globulin confirmed the improvement in humoral and cellular immunity (Figure 2 A and B). The increase in serum total protein and globulin due to betaine supplementation could be associated with its ability as a methyl group donor which is fairly consistent in protein metabolism (Kidd *et al.*, 1997). These results are in correspondence to the report of Hassan *et al.* (2005) who postulated that inclusion of betaine (0.072 to 0.144%) in the chick diets significantly increased serum total protein. Also, Matthews and Southern (2000) reported that betaine had increased ($P < 0.07$) average plasma total protein of coccidiosis-infected chicks. Additionally, in the current study (Table 4), betaine supplementation increased the activity of metabolic hormones (T3 and T4) in growing rabbits under heat stress conditions. Similar observations were obtained by Huang *et al.* (2006) who stated that serum T3 and T4 levels in pigs fed betaine were elevated by 57.95% and 51.80%, respectively. In reality, normal thyroid hormones levels are necessary for adequate development, maintenance and function of both the antibody- and cell-mediated immune responses (Cremaschi *et al.*, 2000 & Klecha *et al.*, 2000). Cremaschi *et al.* (2000) indicated that thyroid hormones can enhance the antibody production in mice. Moreover, Chandratilleke *et al.* (1994) proved that T3 is involved in enhancing interleukin-2 production in birds. Moreover, as shown in Table (4), betaine supplementation stabilized the normal mineral balance (including sodium, potassium, calcium and phosphorus) in growing rabbits under hyperthermia condition. Since, hyperthermia resulted in disturbing the normal physiological balance of the animal's body, particularly mineral and water balances (Fekry, 1989) and betaine has osmoprotective properties (Kidd *et al.* 1997), it could be assumed that betaine might

Table 4: Effect of dietary betaine supplementation on blood serum constituents in growing rabbits under high ambient temperature.

Items	Betaine (mg/kg)					Sig
	0	250	500	750	1000	
T3 (ng/ml)	1.06± 0.08^{b}	1.37± 0.09^{a}	1.33± 0.08^{a}	1.51±0.11^{a}	1.43± 0.11^{a}	*
T4 (µg/dl)	3.43±0.10^{c}	3.88± 0.14^{b}	4.00± 0.11^{b}	4.51± 0.12^{a}	4.46± 0.12^{a}	*
Total protein (g/dl)	5.42± 0.11^{b}	5.80±0.12b^{a}	5.93± 0.11^{a}	6.00± 0.13^{a}	6.00± 0.09^{a}	*
Albumen (g/dl)	3.04± 0.16	3.07± 0.09	3.15± 0.12	3.13± 0.09	3.16± 0.11	NS
Globulin (g/dl)	2.38± 0.06^{b}	2.73± 0.09^{a}	2.78± 0.07^{a}	2.93± 0.03^{a}	2.84± 0.04^{a}	*
Total lipids (mg/dl)	2.67± 0.13^{b}	3.22± 0.13^{a}	3.30± 0.13^{a}	3.40± 0.13^{a}	3.29± 0.13^{a}	*
Cholesterol (mg/dl)	91.20± 0.12	91.16± 0.03	91.24± 0.09	89.90± 0.06	91.10± 0.12	NS
Glucose (mg/dl)	147.33± 4.67^{a}	120.0± 7.32^{b}	115.0± 8.66^{b}	113.2±5.77^{b}	112.3± 5.79^{b}	*
GOT (IU/l)	33.66± 0.66^{a}	30.0± 2.88^{ab}	28.1± 1.73^{ab}	29.5±1.73^{ab}	27.0± 1.73^{b}	*
GPT (IU/l)	26.00± 1.53	26.3± 1.53	24.2± 2.31	24.1± 2.31	23.16± 1.73	NS
ALP (IU/l)	22.5± 1.26^{a}	18.63± 1.32^{b}	16.0± 1.31^{bc}	14.33±1.1^{bc}	13.7± 0.78^{c}	*
Sodium (mg/100 ml)	145± 2.88^{a}	140± 1.16^{ab}	136± 2.31^{b}	125± 2.89^{c}	118± 1.15^{d}	*
Potassium (mg/100 ml)	4.3± 0.17^{d}	5.2± 0.20^{c}	5.5± 0.29^{c}	5.6± 0.35^{c}	5.8± 0.17^{a}	*
Calcium (mg/100 ml)	8.2± 0.35^{c}	9.0± 0.58^{bc}	10.8± 0.46^{a}	9.7± 0.12^{ab}	10.5± 0.29^{a}	*
Phosphorus (mg/100 ml	4.6± 0.35^{c}	6.8± 0.15^{b}	7.0± 0.29^{b}	7.9± 0.26^{a}	8.0± 0.28^{a}	*

^{a,b,c,d} Means followed by different letters in the same row are differed significantly.

NS= Non significant, * P≤0.05.

play an essential role in stabilizing the normal mineral balance during heat stress conditions. Therefore, it could be speculated that, under stress conditions, dietary betaine may be able to enhance the activity of thyroid hormones and stabilize the normal mineral balance which consequently might participate in improving the growth performance and immune response. In these circumstances, the presence of betaine may have a very important role in restoring the negative effects of heat stress in growing rabbits.

Betaine supplementation significantly decreased serum glucose concentration linearly in a dose-dependent manner (Table 4). It is well established that under stress conditions, glucose uptake of the cells is suppressed and the level of serum glucose is increased in order to serve adequate amount of glucose to the sensitive organs such as heart and brain (Lilleloj, 1992). In the current study, the declining in serum glucose concentration may be ascribed to the higher level of growth hormone and insulin in rabbits fed betaine. This assumption comes in harmony with Huang *et al.* (2006) who documented that dietary betaine reduced serum glucose concentration due to elevating of the circulating insulin concentration in pigs. In the present study, liver functions as judged by liver enzymes (*e.g.* GOT, GPT and ALP) were significantly decreased by dietary betaine supplementation (Table 4). It is important to mention that, according to Okerman (1994), the values obtained in current trail are within the normal ranges of these enzymes in rabbits. Marai *et al.* (2002) reviewed that serum transaminase activities (GOT and GPT) are increased during the hot summer in rabbits. Therefore, it could be noted that betaine supplementation helped in keeping serum transaminase enzymes within the normal ranges during heat stress conditions.

6- Nutrients digestibility :

As shown in Table 5, digestibility coefficients for CP, EE, and NFE were significantly improved by 12.8, 10.7 and 10.6 % , respectively, in the rabbits fed diet with 1000 mg / kg as compared with those fed control diet . Augustine *et al.* (1997) reported that betaine increased the apparent digestibility of lysine, protein and fat in broilers. Moreover, betaine has been shown to stabilize cell membranes through interaction with membrane phospholipid and mucosal structure leading to increase the digestibility and absorption of several nutrients (Virtanen, 1995; Kettunen *et al.*, 2001). Recently, Metzler-Zebeli *et al.* (2009) concluded that there is a growing evidence that betaine is involved in supporting intestinal growth, structure and function, thereby improving the nutrient digestibility.

Based on the data presented above, it could be concluded that supplemental dietary betaine enhanced growth performance and carcass weight, stabilized the normal physiological balance, and elevated the humoral and cell-mediated immunity as well as reduced rectal temperature and respiration rate when growing rabbits were subjected to heat stress. Thus, several benefits might be gained by adding betaine to the commercial rabbit diets under heat stress conditions.

Table 5: Effect of dietary betaine supplementation on digestibility coefficients in growing rabbits under high ambient temperature.

Digestibility (%)	Betaine (mg/kg)					Sig
	0	250	500	750	1000	
DM	64.10±3.06	63.59±2.96	65.39±2.88	65.09±2.86	65.70±2.39	NS
CP	65.93±0.88^{b}	68.00±1.45^{b}	71.00±1.21^{ab}	73.13±3.66^{a}	74.36±2.03^{a}	*
EE	70.23±1.16^{b}	73.56±2.89^{ab}	74.36±2.4^{ab}	75.66±3.33^{ab}	77.73±4.33^{a}	*
CF	24.39±2.18	24.56±1.20	25.06±1.45	24.96±1.29	25.36±2.03	NS
NFE	70.93±0.88^{b}	74.63±1.45^{ab}	75.13±1.26^{ab}	74.56±2.25^{ab}	78.8±2.52^{a}	*

^{a,b} Means followed by different letters in the same row are differed significantly.
NS= Non significant, * P≤0.05.

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تأثير اضافة البيتاين على الأرانب النامية تحت الظروف الحارة

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تهدف الدراسة لتحديد تأثير أضافة البيتاين على النمو – صفات الذبيحة – درجة حرارة المستقيم – معدل التنفس – والأستجابة المناعية للأرانب النامية تحت ظروف الحرارة العالية . تم أستخدام ٩٠ أرنب نيوزيلندى سفطوم عمر ٦ أسابيع وقسمت عشوائيا الى خمس معاملات (كل معاملة ١٨ أرنب) وتم أمدادها بالماء والعلف للأستهلاك الحر . تم تغذية الأرانب على العليقة الأساسية مضاف اليها صفر (عليقة مقارنة)- ٢٥٠- ٥٠٠ – ٧٥٠ – ١٠٠٠ مليجرام بيتاين / كجم علف لمدة ١٢ أسبوع . كان المتوسط اليومى للحرارة والرطوبة النسبية داخل العنبر حوالى ٣٠,٣ +٠,٩ درجة مئوية و ٧٦,٢+٢,٥ % على التوالى . نستخلص أن البيتاين فى علائق الأرانب النامية تحت ظروف الأجهاد الحرارى يزيد وزن الجسم النهائى ووزن الذبيحة بمعدل ٢٠ -± ٣٤,٧ % على التوالى ويحسن الكفاءة التحويلية بمعدل ١٢ % بزيادة مستوى البيتاين من صفر الى ١٠٠٠ مليجرام / كجم فى علائق الأرانب . من الواضح أن أضافة البيتاين تخفف التأثيرات الضارة للأجهاد الحرارى على المناعة – درجة حرارة المستقيم ومعدل التنفس . وجود ١٠٠٠ مليجرام بيتاين /كجم فى علف الأرانب النامية يزيد الأستجابة المناعية بحوالى ضعفين قيمة مجموعة المقارنة وتقلل معنويا درجة حرارة المستقيم ومعدل التنفس . زادت معنويا قيم السيرم من البروتين الكلى – الجلوبيلين – الليبيدات الكلية بينما تركيز الجلوكوز فى السيرم انخفض معنويا بأضافة البيتاين . زادت معاملات هضم البروتين الخام – الدهن الخام – المستخلص الخالى من الأثير بمعدل ١٢,٨- ١٠,٧- ١٠,٦ % فى الأرانب التى تغذت على ١٠٠٠ ملجم بيتاين / كجم علف بالمقارنة بمجموعة المقارنة . نستخلص من ذلك أن أضافة البيتاين يحسن النمو ووزن الذبيحة ويؤدى الى ثبات التوازن الفسيولوجى الطبيعى ويرفع المناعة ويقلل درجة حرارة المستقيم ومعدل التنفس فى الأرانب النامية تحت ظروف الأجهاد الحرارى .