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Impact of Betaine Supplementation on the Growth Performance, Tonic Immobility, and Some Blood Chemistry of Broiler Chickens Fed Normal and Low Energy Diets During Natural Summer Stress

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Abstract

The current study was conducted to investigate the effect of graded levels of betaine supplementation to the normal and low energy diets of broiler chickens during summer season on growth performance, tonic immobility (TI), economic value and some blood biochemical parameters. Three hundred one days-old broiler chicks were used. The chicks were divided into 6 groups (each of 50 chicks) and each group was 5 replicated (2x3 factorial design). The first, second and third groups were fed normal energy diet (NED) with 0, 1 and 2g betaine/kg diet (air dry basis) for 35 days, respectively. While, the fourth, fifth and sixth groups were fed low energy diet (LED) with 0, 1 and 2g betaine/kg diet (air dry basis) for 35 days, respectively. The experiment was carried out during summer season. Birds freely access to water and feed. Results showed no significant difference of growth performance between NED and LED groups. Betaine supplementation (2g/kg diet) to both NED and LED increased significantly ($p<0.05$) the body weight (BW) (1880.61 and 1787.66g, respectively), body weight gain (BWG) (1822.22 and 1728.77g, respectively), feed intake (FI) (3027.36 and 3136.42g, respectively), relative growth rate (RGR) (187.93 and 187.24, respectively) and TI (92.83 and 71.83s, respectively) with no significant difference in feed conversion (FCR) between the groups fed diet supplemented with or without betaine. Betaine supplementation 2g/kg diet to NED increased the total cost, total return, net profit, economic efficiency and performance index%, moreover improved the pancreatic and thyroid function and return the lipids indices of LED-fed birds to the normal level as in the NED-fed birds. No mortalities occur between the different groups. Therefore, betaine can be included in normal energy diets of broiler chickens by level of 2g/kg diet for improving the growth performance, economic value and welfare during the summer season.

Keywords: Betaine; Broiler chickens; Growth performance, Tonic Immobility, Fear level, Blood chemistry.

Introduction

In poultry production, broilers are generally characterized by rapid growth and high feed efficiency. High ambient temperature led to increased mortality, decreased feed intake and body weight gain and higher FCR [1,2], immunosuppression [3] and behavioral changes [4,5]. Amelioration of heat stress depressing effects may be achieved by understanding the biochemical changes of birds [4]. Fear is one component of stress in poultry [6], which can be measured by the duration of tonic immobility (TI). Tonic immobility is considered one of the most commonly fear tests for poultry and may be used as criteria for evaluating well being and stress levels [7]. Therefore, Nutritionists are searching for possible methods to reduce the negative effects of high ambient temperature in poultry [8,9]. The most significant nutritional approach is to change dietary protein or levels of energy by feed limitation in the summer period, addition of fat in the diet [10] or to supplement the diets with various additives such as vitamin C, ammonium chloride and potassium chloride [11,12] and sodium bicarbonate [13]. One of these nutritional methods for alleviating stress in the broiler is using betaine as a feed

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supplement in the poultry diets [14]. Betaine is a general name for trimethylglycine, a substrate for betaine-homocysteine methyl transferase (BHMT) in the liver and kidney. It is often found in high levels in vegetation exposed to scarcity, as a result of the osmoregulatory properties of betaine [1,15]. Earlier studies reported significant roles of betaine as improving growth performance, breast meat yield, fat metabolism and immunity [1,16-19] and decreasing the negative impact of heat stress by reducing fearfulness [6]. Betaine present in high levels in sugar beets in molasses aqueous form and are a by-product of sugar beet processing. As well, wheat and wheat bran contain significant amounts of betaine [20,21].

Betaine is considered as a lipotropic factor and has growth-promoting effects. Also, it has a role in protecting cells from changes in the osmolarity and maintains normal metabolic actions [20]. Though birds do not have a particular requirement for betaine, the osmolytic property of betaine could be useful to birds subjected to heat stress and may improve nutrient digestibility [22]. Betaine is concerned in energy and protein metabolism due to its role as a methyl group donor. Betaine supplementation reduced the fear response in broiler chickens that increased by heat stress, resulting in decreased duration of TI. Gudev *et al.* [23] suggested that tonic immobility does not seem to be a reliable marker of fear because of its complex nature and phenomenology. However, little studies have been carried out to evaluate betaine as a feed supplement to reduce negative effects of heat stress.

The aim of this work was to study the impact of supplementing graded levels of betaine to normal and low energy diets of broiler chickens during natural summer season on the growth performance, TI, economic value and some blood biochemical parameters.

Materials and Methods

Birds, housing, diets and experimental design

This study carried out at the Experimental Research Animal Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University, Egypt. Three hundred one day old chicks (Ross 308 broiler) were purchased from a commercial chick producer. Chicks were subjected to a 3 day adaptation period before the start of the experiment with average body weight of 58.54±0.07 gm. They were randomly assigned to 6 groups with 5 replicates for each (10 chicks/replicate). Factorial design (2x3) consisting of two energy levels (normal energy diet (NED) and low-energy diet (LED)). Each energy diet was supplemented with 0, 1 and 2 g betaine/kg diet (air dry basis) for 35 days (Betaine (Vistabet®) is a natural betaine source extracted from sugar beet molasses for use in poultry, swine, ruminant and aqua diets (AB Vista Headquarters, Woodstock Court, Blenheim Road, Marlborough Business Park Wiltshire SN8 4AN Marlborough). Feed and fresh drinking water were provided ad libitum to all birds during the entire experimental period. The average rate of temperature during the experimental period was recorded as follow, 35±2oC during the starter period, 36±2oC during the grower period and 37±1 oC during the finisher period. Chicks were reared under the same management, sanitary and ecological conditions all over the experimental period. Usual health and vaccination practices were done against New Castle (at 4th and 14th day) and Gumboro diseases (at 7th and 22 day). Daily observation was carried out on chicks for any health disorders. No mortalities occur among different groups. The proximate chemical composition of the basal diets presented in Table (1), which were formulated to follow Aviagen [24].

Table 1: Proximate and chemical composition of the normal and low energy diets "NED & LED" diets (%) based on Ross manual Guide [24]:

	Starter period (4-10 day)		**Grower period (11-23 day)**		**Finisher period (24-35 day)**	
	NED	**LED**	**NED**	**LED**	**NED**	**LED**
Yellow corn	55.6	54.34	59.43	59.62	62.43	62.21
Soybean meal, 48%	31.9	38.48	27.9	31.4	23.03	27.7
Corn gluten, 60%	5.98	1.5	5.57	3	6.57	3.27
Soybean oil	2	1.5	3	2	4	3
Calcium carbonate	1.3	1.3	1.2	1.18	1.05	1.07
Calcium dibasic phosphate	1.5	1.4	1.3	1.3	1.3	1.25
Common salt	0.15	0.15	0.15	0.15	0.15	0.15
Premix*	0.3	0.3	0.3	0.3	0.3	0.3
DL- Methionine, 98%	0.23	0.23	0.2	0.2	0.18	0.2
Lysine,Hcl, 78%	0.47	0.27	0.42	0.32	0.46	0.32
Choline	0.07	0.07	0.07	0.07	0.07	0.07
Threonine	0.1	0.1	0.1	0.1	0.1	0.1
Phytase	0.01	0.01	0.01	0.01	0.01	0.01
Antimycotoxin	0.1	0.1	0.1	0.1	0.1	0.1
NaCo3	0.25	0.25	0.25	0.25	0.25	0.25
Chemical composition (%)**						
ME (kcal/kg diet)	3008.93	2907.69	3104.47	3008.62	3210.19	3101.07
Crude protein	23.43	23.51	21.52	21.52	20.09	20.13
Calcium	0.97	0.96	0.87	0.87	0.81	0.81
Available Phosphrus	0.48	0.47	0.43	0.43	0.41	0.41
Lysine	1.44	1.439	1.29	1.29	1.19	1.19
Methionine	0.56	0.558	0.51	0.51	0.48	0.48
Threonine	0.97	0.97	0.88	0.88	0.81	0.81

*Premix per kg of diet: Vitamin A, 1 500 IU; Vitamin D3, 200 IU; Vitamin E, 10 mg; Vitamin K3, 0.5 mg; Thiamine, 1.8 mg; Riboflavin, 3.6 mg; Pantothenic acid, 10 mg; Folicacid, 0.55 mg; Pyridoxine, 3.5 mg; Niacin, 35 mg; Cobalamin, 0.01 mg; Biotin, 0.15 mg; Fe, 80 mg; Cu, 8 mg; Mn, 60 mg; Zn, 40 mg; I, 0.35 mg; Se, 0.15 mg.
** According to Aviagen [24]

Growth performance

The birds were individually weighed at the 4th day of age to obtain the average initial body weight then the body weight was recorded at 10, 23, 35 day to calculate the average body weight of the birds in each group. The body weight gain was calculated as $W_2 – W_1$, where W_2 is the final body weight at the intended period and W_1 is the initial body weight at the same period. Feed intake of each replicate was recorded as the difference between weight of the feed offered and residues left and then divided by the number of birds in each replicate to find out the average feed intake per bird. Feed conversion ratio (FCR) was estimated [25]. The relative growth rate (RGR) was calculated every week [26].

Tonic immobility

Tonic immobility was tested on 6 birds on day 34 from each group [27]. Tonic immobility was forced by putting the bird on his/her back with the head hanging in a U-shaped wooden cradle [28] and the bird was gently restrained for 10s. The observer sat at a distance of about 1 m from the bird without making unnecessary noise and movements. A stopwatch was launched to register latencies until the bird adjusts his/herself after finishing restraint by hands. If the position righting happened in less than 10s, means that the induction of tonic immobility not occurred and the restraint step was repeated. If tonic immobility was not induced after 3 attempts, the duration of tonic immobility was considered to be zero seconds. Conversely, if the bird failed to right his/herself after 10 min, the test was terminated and a maximum score of 600 s was recorded for TI duration [29].

Economic importance

Collective efficiency measures were calculated [30,31]. It includes: total return, total costs, variable costs and net profit. Also, the performance index (PI) was calculated [32].

Samples and clinico-biochemical analysis

At the end of the experiment, blood samples were randomly collected from five birds/ treatment after slaughter. The blood was drawn without anticoagulant into a clean dry centrifuge tube, left to clot at room temperature then centrifuged at 3000 rpm for 5 min for serum separation to perform the clinico-biochemical tests (lipid profile, pancreatic and thyroid function tests). Total cholesterol [33], triglycerides [34], HDL-C [35] and glucose [36] were estimated with colorimetric diagnostic kits of spectrum-bioscience (Egyptian Company for Biotechnology, Cairo, Egypt). The Iranian formula of LDL = TC / 1.19 + TG / 1.9 – HDL / 1.1 – 38 was used for LDL-C calculation. Meanwhile, chicken ELISA kits (My Biosource Co. of CAT. NO. MBS269454, MBS265796, MBS2500435, MBS701713, and MBS703565) were used for determining triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH), insulin and insulin-like growth factor 1 (IGF-1), respectively.

Statistical analysis

The data was analyzed by using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA) and expressed as the mean ± standard deviation (SD). The variation was assessed by two-way (ANOVA) and the differences between experimental groups were calculated by Duncan's multiple-range test [37]. Statistical significance of the results calculated at ($P < 0.05$).

Results

Growth performance

The results showed that there were no significant differences ($P >0.05$) between birds fed normal or low energy diet on the BW, BWG, FI, FCR and the overall performance (Tables 2 and 3). During the starter period, supplementation of betaine (1 and 2 g/kg diet) to the NED increased significantly ($P < 0.05$) the BW, BWG, FI and increased the efficiency of feed utilization. During the grower period, supplementation of betaine by 2g/kg diet to NED increased the BW and FI significantly ($P < 0.05$). Betaine supplementation to LED didn't cause any significant differences ($P > 0.05$) in growth performance of birds during the starter and grower period. During the finisher period, betaine supplementation (2g/kg diet) to the NED increased significantly ($P<0.05$) the BW, BWG. Betaine supplementation (1 and 2g/kg diet) to the NED decreased significantly ($P < 0.05$) FCR. Supplementing the LED by betaine (2g/kg diet) only significantly ($P < 0.05$) increased BWG and FI. The results of the overall performance showed that betaine supplementation by 2g/kg diet to both NED and LED increased significantly ($P < 0.05$) BW, BWG, FI and RGR, while no significant difference in FCR between groups supplemented with or without betaine.

Table 2: The effect of betaine supplementation to normal and low energy diet (NED & LED) on body weight, body weight gain, feed intake and feed conversion ratio of the broiler chicken.

		Starter period (4-10th day)					Grower period (11-23 day)				Finisher period (24-35 day)			
		Int. BW (g)	BW(g)	BWG(g)	FI(g)	FCR	BW(g)	BWG(g)	FI(g)	FCR	BW(g)	BWG(g)	FI(g)	FCR
Diet														
NED		58.48±0.18	209.63±21.92	150.93±22.32	213.11±19.35	1.42±0.09	723.70±33.14	514.06±36.23	888.93±55.88	1.73±0.17	1697.79±46.27	974.09±39.16	1734.47±32.29	1.79±0.15
LED		58.61±0.42	212.03±9.40	153.42±9.33	223.89±13.55	1.46±0.10	737.77±23.10	525.74±19.77	924.54±48.25	1.76±0.12	1694.02±22.41	956.25±88.63	1812.77±46.64	1.90±0.08
Betaine level (g/kg diet)														
0		58.44±0.16	195.70±16.32^{b}	136.92±16.81^{b}	201.33±16.22^{b}	1.47±0.08	723.89±15.97^{b}	528.18±16.33^{a}	874.75±55.56^{b}	1.66±0.13	1598.31±76.04^{b}	874.42±63.32^{b}	1683.65±10.04^{b}	1.92±0.08
1		58.55±0.36	215.13±11.70^{a}	156.58±11.95^{a}	224.30±9.62^{a}	1.43±0.11	711.94±27.40^{b}	496.80±37.06^{b}	896.49±25.14^{ab}	1.81±0.14	1655.27±55.92^{b}	943.33±34.78^{b}	1734.16±31.09^{b}	1.84±0.13
2		58.63±0.41	221.66±08.65^{a}	163.03±08.55^{a}	229.86±9.65^{a}	1.41±0.12	756.38±22.69^{a}	534.72±14.94^{a}	948.97±51.47^{a}	1.77±0.13	1834.13±76.03^{a}	1077.75±68.52^{a}	1903.05±83.36^{a}	1.77±0.13
Interaction														
	0	58.56±0.16	181.13±1.79^{c}	121.91±2.08^{c}	188.23±7.27^{c}	1.54±0.06^{a}	718.89±9.47^{bc}	537.75±8.94^{a}	835.33±27.61^{b}	1.55±0.02^{c}	1586.22±8.34^{c}	867.33±1.59^{c}	1691.74±85.42^{bc}	1.95±0.09^{a}
NED	1	58.49±0.28	223.33±5.07^{a}	164.83±5.23^{a}	227.50±4.40^{ab}	1.38±0.03^{ab}	690.55±15.90^{c}	467.22±13.57^{b}	898.83±38.72^{ab}	1.92±0.04^{a}	1626.55±34.95^{c}	936.00±22.99^{bc}	1640.55±27.95^{c}	1.75±0.12^{bc}
	2	58.38±0.09	224.44±8.09^{a}	166.05±8.11^{a}	223.61±4.58^{ab}	1.34±0.04^{b}	761.66±14.43^{a}	537.22±7.18^{a}	932.64±53.94^{a}	1.73±0.11^{abc}	1880.61±88.74^{a}	1118.94±74.32^{a}	1871.11±48.57^{ab}	1.67±0.08^{c}
	0	58.33±0.01	210.27±5.09^{ab}	151.94±5.09^{ab}	214.44±9.47^{b}	1.41±0.01^{ab}	728.89±21.74^{ab}	518.61±17.66^{a}	914.16±47.90^{ab}	1.76±0.09^{ab}	1610.41±18.09^{ab}	881.52±99.35^{c}	1675.55±50.92^{c}	1.90±0.08^{ab}
LED	1	58.61±0.48	206.94±10.74^{b}	148.33±11.21^{b}	221.11±13.46^{ab}	1.49±0.14^{ab}	733.33±15.8^{ab}	526.39±25.00^{a}	894.15±8.04^{ab}	1.70±0.09^{bc}	1684.00±64.21^{ab}	950.66±48.31^{bc}	1827.77±17.34^{abc}	1.92±0.08^{ab}
	2	58.89±00.49	218.88±9.94^{ab}	160.00±9.46^{ab}	236.11±9.73^{a}	1.48±0.14^{ab}	751.11±31.54^{ab}	532.22±22.087a	965.30±53.97a	1.81±0.17^{ab}	1787.66±9.88^{ab}	1036.55±33.50^{ab}	1935.00±19.33^{a}	1.87±0.10^{ab}

Initial body weight at 4th day; BW: Body weight; BWG: body weight gain ;FI: feed intake and FCR: feed conversion ratio .
Means within the same column carrying different superscripts were significantly different at (P<0.05

Table 3: The effect of betaine supplementation to normal and low energy diet (NED & LED) on the overall performance and tonic immobility of broilers chicken.

		BW(g)	TBWG(g)	TFI(g)	FCR	RGR	Tonic immobility time	Tonic immobility attempt
Diet								
NED		1697.79±46.27	1639.31±46.35	2836.52±43.22	1.73±0.08	186.60±1.06	95.70±36.52	2.55 ±0.82
LED		1694.02±22.41	1635.41±12.19	2961.20±26.60	1.81±0.07	186.58±0.77	95.77±35.80	2.00±0.76
Betaine level (g/kg diet)								
0		1598.31±76.04[b]	1539.87±76.05[b]	2759.74±53.37[b]	1.79±0.05	185.86±0.62[b]	115.64±29.88[a]	2.07±0.73[a]
1		1655.27±55.92[b]	1596.72±55.78[b]	2854.96±40.54[b]	1.78±0.07	186.32±0.41[b]	85.91±29.13[b]	2.33±0.98[a]
2		1834.13±76.03[a]	1775.50±76.23[a]	3081.89±21.77[a]	1.73±0.11	187.59±0.52[a]	82.33±39.70[b]	2.50±0.79[a]
Interaction								
NED	0	1586.22±8.34[c]	1527.66±8.23[C]	2715.31±99.73[c]	1.77±0.06	185.75±0.05[c]	117.12±35.91[a]	2.25±0.70[a]
	1	1626.55±34.95[c]	1568.06±35.02[c]	2766.88±61.50[c]	1.76±0.07	186.11±0.30[c]	70.00±13.35[a]	3.00±0.63[a]
	2	1880.61±88.74[a]	1822.22±88.79[a]	3027.36±6.94[ab]	1.66±0.07	187.93±0.54[a]	92.83±39.37[a]	2.50±1.04[a]
LED	0	1610.41±18.09[ab]	1552.08±18.09[c]	2804.16±27.21[bc]	1.80±0.06	185.97±0.96[c]	113.67±22.53[a]	1.83±0.75[a]
	1	1684.00±64.21[ab]	1625.39±63.94[bc]	2943.04±5.25[abc]	1.81±0.07	186.53±0.45[bc]	101.83±32.87[a]	1.66±0.81[a]
	2	1787.66±9.88[ab]	1728.77±9.85[ab]	3136.42±67.65[a]	1.81±0.10	187.24±0.11[ab]	71.83±40.66[a]	2.50±.054[a]

BW: body weight; TBWG: total body weight gain; TFI: total feed intake; FCR: feed conversion ratio and RGR: relative growth rate.
Means within the same column carrying different superscripts are significantly different at ($P<0.05$).

Tonic immobility

The results revealed that the duration of tonic immobility significantly (p=0.027) decreased with supplementation of betaine to the broiler's diet, while the energy level affected on the number of attempts done by the bird (Table 3).

Economic importance

The results showed that there was no significant effect (P >0.05) of NED and LED on the economic value (Table 4). Betaine supplementation (2g/kg diet) to NED resulted in significant increase (P < 0.05) in the final BW, feed cost, total cost, net profit, total return, economic efficiency and performance index. Betaine supplementation (2g/kg diet) to LED resulted in significant increase (P < 0.05) in the final BW, feed cost, total cost, and net profit. No significant difference (P>0.05) in feed cost/kg gain between all groups.

Biochemical results

Birds fed on diet with low energy showed significant (P< 0.05) decrease of serum TC, TG, LDL-C, T2, T4, TSH, insulin, IGF1 and glucose levels with no significant changes in serum HDL-C concentration when compared with birds fed normal energy diet (Table 5). Comparing with the NED birds, betaine supplementation showed no significant effect (P >0.05) on aforementioned parameters. Meanwhile, betaine supplementation to low energy diets compared to LED resulted in significant (P < 0.05) improvement in the thyroid, pancreatic function and lipid profile, which were completely return to the NED-fed birds by the concentration of 2g/kg diet for 35 days.

Discussion

Many problems are taxing poultry industry due to heat stress which results in high mortalities, low performance of the broiler chicks represented in decreased feed intake and poor feed conversion ratio [1,2] and immunosuppression [3]. Many nutritional approaches were done to reduce the negative effects of high temperature. One of these approaches for alleviating stress in broiler is betaine as a feed supplement in diets. The results of the present study showed that there were no significant differences in the growth performance between NED and LED groups without betaine supplementation during natural summer season. This was reflected on the economic value of the diets that was looked not different between these groups. These results improved by supplementation of betaine (2g/kg diet) to both NED and LED, where there was significant increase in BW, BWG, FI and RGR but FCR was not affected. Therefore, betaine supplementation improved the feed intake and increased the BWG, which decreased in NED and LED without betaine supplementation due to heat stress and this was reflected on the economic value of the diets.

There were significant increases of total return, net profit, economic efficiency and performance index in NED supplemented with 2g betaine/kg diet, while betaine supplementation to LED only led to an improvement of the total return. The results of the feed cost/kg gain not significantly different between groups that meant that the improvement in feed intake is the reason for improved growth performance and economic value. This improvement in BW and BWG due to betaine supplementation may be attributed to the osmolytic property of betaine that supports intestinal cell growth and enhances cell activity, resulting in improving nutrient digestibility [22].

Similarly, the positive effect of betaine supplementation during heat stress on body weight gain of broiler chickens was observed in other studies [38]. Akhavan-Salamat and Ghasemi [18] reported that betaine would improve the growth performance of the broiler chickens by reducing the stress through increasing the levels of total short-chain fatty acid (SCFA) and increasing the blood electrolyte [39,40].

Hassan *et al.* [41] and Sayed, and Downing [42] showed that the ability of betaine as methyl group donors could improve weight gain and feed efficiency. Also, it can improve the growth and feed efficiency of the broiler chickens by protecting the intestinal epithelia from the osmotic disorders [43,44].

Table 4: The effect of betaine supplementation to normal and low energy diet (NED & LED) on economic efficiency.

		FBW(Kg)	Feed cost (LE)	Total cost (LE)	Total return (LE)	Net profit (LE)	Economic efficiency	Feed cost/kg gain	Performance index (%)
Diet									
NED		1.69±0.14	18.45±1.12	25.95±1.12	33.95±2.92	8.00±2.06	0.43±0.09	11.28±0.53	98.32±12.76
LED		1.69±0.10	18.57±1.23	26.07±1.23	33.88±2.04	7.80±1.27	0.42±0.06	11.36±0.44	93.68±7.46
Betaine level (g/kg diet)									
0		1.59±0.07^{b}	17.62±0.91^{b}	25.12±0.91^{b}	31.96±1.52^{b}	6.84±0.96^{b}	0.38±0.05^{b}	11.44±0.37	89.25±5.35^{b}
1		1.65±0.05^{b}	18.22±0.70^{b}	25.72±0.70^{b}	33.10±1.11^{b}	7.37±0.99^{b}	0.40±0.05^{ab}	11.42±0.43	92.70±5.04^{b}
2		1.83±0.07^{a}	19.68±0.66^{a}	27.18±0.66^{a}	36.68±1.52^{a}	9.50±1.67^{a}	0.48±0.09^{a}	11.10±0.59	106.05±11.22^{a}
Interaction									
NED	**0**	1.58±0.01^{c}	17.66±0.64^{b}	25.16±0.64^{b}	31.72±0.16^{c}	6.56±0.51^{b}	0.37±0.04^{b}	11.56±0.37	89.30±2.57^{b}
	1	1.62±0.03^{c}	18.01±1.04^{b}	25.50±1.04^{b}	32.53±0.69^{c}	7.03±0.66^{b}	0.39±0.05^{b}	11.47±0.50	92.30±3.43^{b}
	2	1.88±0.08^{a}	19.69±0.04^{a}	27.19±0.04^{a}	37.61±1.77^{a}	10.41±1.74^{a}	0.52±0.08^{a}	10.82±0.49	113.36±10.85^{a}
LED	**0**	1.61±0.11^{c}	17.58±1.29^{b}	25.08±1.29^{b}	32.20±2.36^{c}	7.11±1.35^{b}	0.40±0.06a^{b}	11.33±0.41	89.21±8.07^{b}
	1	1.68±0.06b^{c}	18.45±0.03^{ab}	25.95±0.03^{ab}	33.68±1.28^{bc}	7.72±1.29^{b}	0.41±0.07^{ab}	11.36±0.46	93.09±7.17^{b}
	2	1.78±0.01a^{b}	19.67±1.05^{a}	27.17±1.05^{a}	35.75±0.19^{ab}	8.58±1.18a^{b}	0.43±0.08^{ab}	11.38±0.64	98.75±6.10^{b}

FBW: final body weight

Means within the same column carrying different superscripts are significantly different at ($P<0.05$)

Table 5: The effect of betaine supplementation to normal and low energy diet (NED & LED) on selective thyroid, pancreatic function tests and lipid profile.

		TC (mg/dL)	TG (mg/dL)	HDL-C(mg/dL)	LDL-C(mg/dL)	T3 (nmoL/L)	T4 (nmoL/L)	TSH(pg/mL)	Insulin(μIU/mL)	IGF1 (pg/mL)	Glucose(mg/dL)
Diet											
NED		184.55±3.57^{a}	155.33±6.57^{a}	60.22±5.23	144.09±5.14^{a}	18.33±2.34^{a}	291.55±11.37^{a}	95.44±3.35^{a}	26.73±0.42^{a}	129.11±5.34^{a}	117.77±9.14^{a}
LED		171.77±9.70^{b}	137.22±11.09^{b}	59.77±8.67	124.23±16.21^{b}	11.33±4.30^{b}	250.33±23.38^{b}	71.22±12.74^{b}	15.45±5.08^{b}	105.55±14.77^{b}	95.11±13.40^{b}
Betaine level (g/kg diet)											
0		173.50±8.13	140.00±16.40	60.00±4.93	126.93±20.46	12.00±6.48	256.33±37.42	75.83±21.36	18.06±9.52	108.00±22.74	100.33±19.95
1		177.83±13.18	144.83±11.05	60.33±8.52	132.82±13.70	15.33±3.72	273.00±22.05	83.33±12.94	21.71±5.80	118.33±11.72	105.67±15.21
2		183.16±4.66	154.00±6.98	59.66±8.18	142.73±7.66	17.16±3.18	283.50±16.53	90.83±6.85	23.50±3.65	125.66±7.11	113.33±12.30
Interaction											
NED	**0**	185.00±5.00^{a}	154.33±4.50^{a}	60.00±2.00	144.14±5.19^{a}	17.66±2.51^{a}	288.66±9.60^{a}	95.00±5.00^{a}	26.73±0.50^{a}	127.66±9.07^{a}	118.33±4.16^{a}
	1	185.00±2.64^{a}	153.33±9.07^{a}	60.66±6.50	143.01±3.68^{a}	18.33±1.15^{a}	291.33±13.61^{a}	95.00±2.00^{a}	26.73±0.64^{a}	128.66±1.52^{a}	118.33±9.01^{a}
	2	183.66±4.16^{a}	158.33±7.02^{a}	60.00±7.93	145.12±7.87^{a}	19.00±3.60^{a}	294.66±14.57^{a}	96.33±3.78^{a}	26.73±0.20^{a}	131.00±4.58^{a}	116.66±15.27^{a}
LED	**0**	162.00±3.60^{c}	125.66±6.02^{c}	60.00±7.54	109.73±11.47^{b}	6.33±1.52^{c}	224.00±16.52^{c}	56.66±3.78^{d}	9.40±1.03^{d}	88.33±7.09^{c}	82.33±2.51^{b}
	1	170.66±2.08^{b}	136.33±2.51^{b}	60.00±11.78	122.62±12.00^{b}	12.33±2.51^{b}	254.66±4.72^{b}	71.66±2.51^{c}	16.70±2.91^{c}	108.00±4.58^{b}	93.00±4.00^{b}
	2	182.66±6.02^{a}	149.66±4.04^{a}	59.33±10.21	140.33±8.22^{a}	15.33±1.52^{ab}	272.33±9.86^{ab}	85.33±3.51^{b}	20.26±1.41^{b}	120.33±4.50^{a}	110.00±10.58^{a}

TC: total cholesterol; TG: triglycerides; HDL-C: High density lipoprotein; LDL-C: Low density lipoprotein; T3: Triiodothyronine; T4: Thyroxine; TSH: Thyroid stimulating hormone and IGF-1: insulin-like growth factor 1.

Means within the same column carrying different superscripts were significantly different at ($P<0.05$)

Concerning the poultry welfare, results showed that the broilers fed on a diet the supplemented with betaine recorded the shorter duration of TI than broilers fed on diet without betaine indicating an improvement of chicken welfare. This may be attributed to the osmolytic effect of betaine and its positive effects on TI and reduced the stress [45]. Zulkifli *et al.* [46] classified the broiler chicken into low-fear and high-fear responders according to the duration of TI. Also, heat stressed broiler chicken recorded high intensity level of fear responses that evidenced with long TI [47]. The improved growth performance and increased adaptability to stress noted by broilers showed the shorter duration of tonic immobility [48]. These results are similar to that recorded by others [6] who demonstrated that TI duration decreased in broiler administered betaine and ascorbic acid comparing with that did not administer betaine. Therefore, its addition to birds diets may ameliorate the adverse impact of heat stress especially during hot-dry season. Energy level had no significant influence on TI duration, while the attempts done increased by birds fed on NED. The current study concluded that the betaine has an obvious role in stress tolerance of heat stressed broiler chicken that supported by the others [18].

Thyroxine (T_4) and triiodothyronine (T_3) play an important role in energy metabolism. Several dietary factors have a function in optimizing thyroid function. Though, either nutrient deficiencies or excess can activate or aggravate symptoms of hypothyroidism or hyperthyrodism [49]. Our results suggested that LED resulted in marked reduction in serum concentration of T_3, T_4 and TSH. Therefore, low energy can reduce the circulating T_3 by reducing the activity of iodothyronine deiodinases, which responsible for the conversion of T_4 to T_3 [50]. Energy limitation through changing dietary carbohydrates has concerned more interest in the prevention and treatment of metabolic disease. Long-period of energy limitation leads to a change of glucose homeostasis in humans [51] and rats [52-54] resulting in reduced glycemia and insulinemia. Energy limitation reduces serum IGF-1 level by 40% [55]. Our results indicate that betaine improved the altered pancreatic and thyroid function due to low calories diet as it considered as a modified amino acid.

Conclusion

Betaine can be included in the normal energy diets of broiler chickens by the level of 2g/kg diet for improving the growth performance, welfare and the economics during summer season.

Conflict of interest

The authors declare they have no conflict of interest.

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الملخص العربي

تأثير اضافة البياتين علي أداء النمو, منشط الجمود وبعض كيمياء الدم في بداري التسمين المتغذيه علي علائق منضبطة ومنخفضة الطاقة اثناء الاجهاد الصيفي الطبيعي

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أجريت هذه الدراسة لإختبار تأثير إضافة مستويات متدرجة من البياتين (فيستابيت) علي علائق بداري التسمين منضبطة ومنخفضة الطاقة أثناء الإجهاد الصيفي الطبيعي علي أداء النمو, منشط الجمود, القيمه الإقتصادية, بعض القياسات الكيميائية للدم. إستخدم في هذه الدراسة ٣٠٠ كتكوت روس عمر يوم وتم تقسيمهم عشوائيا علي ستة مجموعات. المعاملات الغذائية تتكون من عليقتين ضابطتين إحداهما صممت لتحتوي علي مستوي منضبط من الطاقة والأخري صممت لتحتوي علي مستوي منخفض من الطاقة. تم إضافة ثلاثة مستويات متدرجة من البياتين (صفر, ١, ٢ جم/كجم عليقة) علي كلا العليقتين الضابطتين. أجريت هذه الدراسة في فصل الصيف لمدة ٣٥ يوم. أوضحت النتائج عدم وجود إختلافات معنوية علي الأداء ما بين الدجاج المتغذي علي العلائق المنضبطة أو المنخفضة الطاقة. إضافة البياتين بمعدل (٢جم/كجم عليقة) لكل من العليقة المنضبطة والمنخفضة الطاقة أدي إلي زيادة معنوية في الوزن ومعدل النمو وكمية إستهلاك العليقة ومنشط الجمود بينما لا يوجود إختلافات معنوية في معدل التحويل بين العلائق الضابطة والعلائق المحتوية علي البياتين. إضافة البياتين بمعدل (٢جم/كجم عليقة) للعليقة منضبطة الطاقة أدي إلي زيادة معنوية في التكلفة الكلية, العائد الكلي, صافي الربح وكذلك الكفاءة الإقتصادية وأدي إلي تحسين وظائف الغدة الدرقية والبنكرياس وتعديل مستوي الدهون الذي إنخفض في الطيور المتغذية علي العلائق منخفضة الطاقة وعودتها الي المستوي الطبيعي كما في الطيور المتغذية علي العلائق منضبطة الطاقة. الخلاصة, يمكن إضافة البياتين بمعدل (٢جم/كجم عليقة) إلي علائق بداري التسمين منضبطة الطاقة لتحسين أداء النمو ورفاهية الدجاج أثناء فصل الصيف وكذلك لتحسين القيمة الإقتصادية للعلائق.