Pakistan J. Zool., vol. 49(5), pp 1857-1862, 2017. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2017.49.5.1857.1862

Effect of Betaine Supplementation on the Performance and Immune Response of Heat Stressed Broilers

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Article Information
Received 24 February 2017
Revised 24 March 2017
Accepted 31 March 2017
Available online 20 September 2017

Authors' Contribution
NC and RUK designed the study. HM conducted the study. SN edited the paper, SK and MSQ supported overall.

Key words
Broiler, Heat stress, Betain, Performance, Immunity.

ABSTRACT

This study was planned to investigate the effects of betaine supplementation on growth performance and immunity in broilers under natural summer stress. A total of one hundred and twenty day-old broiler chicks were divided into four different treatment groups such as Bet-0, Bet-1.0, Bet-1.5, Bet-2.0 having betaine supplementation at the rate of 0, 1, 1.5 and 2 g/kg feed, respectively. Significantly ($P<0.05$) higher weekly and total feed intake and weight gain were recorded in group Bet-2.0. Higher level of betaine supplementation significantly ($P< 0.05$) improved feed conversion ratio (FCR). Betaine supplementation significantly ($P< 0.05$) improved dressing percentage. The treatment groups had significantly ($P<0.05$) lower heterophil number and significantly ($P<0.05$) higher lymphocytes number than the control group. The heterophil/lymphocytes ratio and antibody titer was significantly ($P<0.05$) lower in the treated groups. Based on these results, it is concluded that supplementation of diets with betaine improved broiler feed intake, weight gain, FCR and increased immunity were under heat stress condition.

INTRODUCTION

In broiler production, recently a number of feed additives have been used to improve growth, feed efficiency, immune status and antioxidant capacity (Abudabos *et al*., 2016; Khan *et al*., 2016; Alzawqari *et al*., 2016; Shahid *et al*., 2015; Chand *et al*., 2014). Various techniques are also practised to reduce heat stress in poultry (Chand *et al*., 2016). Such methods include the use of electric fans, cooling pad system and sprinkling of water through foggers (Khan *et al*., 2014). As most of these methods cannot be practised due to high expenses, other strategies such as nutritional therapies including the use of balancing nutrient contents, addition of vitamin C, sodium bicarbonate, potassium carbonate and aspirin in drinking water can be followed. One of these nutritional strategies for reducing stress in the broiler is the use of betaine as a feed additive in the poultry diet (Zimmermann *et al*., 1996).

Betaine is a hydrocarbon consisting of hydrogen and carbon. It contains amide group attached with three (CH_3) methyl groups bound through the amino group of glycine amino acids. Betaine is made naturally as well as syntheticaly from a number of plants and animals (Boch *et al*., 1994). Betaine is found naturally in higher quantities in beet roots (Wang *et al*., 2004). Betaine in reaction with the homocysteine has methionine saving effect, where it donates methyl group instead of methionine (Paniz *et al*., 2005). Betaine is an osmolyte and assists in cellular water homeostasis (Klasing *et al*., 2002). Betaine supplementation in feed improves growth performance and feed intake under heat stressed condition (Hassan *et al*., 2005). The positive effect of betaine is due to the fact that it reduces the body temperature in chickens (Klasing *et al*., 2002).

The objective of this study was to evaluate the effects of betaine supplementation on the performance and immune response of summer stressed broilers.

MATERIALS AND METHODS

Bird's husbandry and experimental layout

After an adaptation period of one week, a total of 120 chicks were divided into 4 different treatments and designated as Bet-0, Bet-1.0, Bet-1.5, Bet-2.0 having three replicates of 10 birds each. Group Bet-0 was kept as a control and fed commercially available feed without supplementation, while groups Bet-1.0, Bet-1.5 and Bet-2.0 were provided with betaine (Nutricost, USA) at the

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0030-9923/2017/0005-1857 $ 9.00/0

rate of 1, 1.5 and 2g/kg feed, respectively. Humidity level was recorded with the help of hygrometer on daily basis. Minimum and maximum temperatures and humidity inside the shed were measured on daily basis (Table I). Birds were vaccinated against Newcastle Disease (ND). The experiment was lasted for 42 days including one week of adaptation period. Birds were fed standard commercial isocalrious and isonitrogenous feed throughout the experiment.

Table I.- Maximum and minimum temperature and humidity recorded during the experimental period.

Time	Temperature (ºC)		Humidity (%)	
	Min.	Max.	Min.	Max.
4:00 AM	25.4	26.8	66	79
8:00 AM	27.0	31.6	69	87
2:00 PM	31.0	35.0	53	79
6:00 PM	29.6	35.8	52	69
10:00 PM	27.4	33.6	66	80

Measurement of performance traits

Feed intake of each replicate was calculated by subtracting the left over feed from the provided on daily basis. Weekly body weight gain was calculated by subtracting weight at day 1 from body weight at day 7 of every week for a total of six weeks. Overall body weight gain was calculated from weekly body weight gain. Feed conversion ratio (FCR) of broilers was calculated on weekly basis. After six weeks of age, two birds per replicate were randomly selected, weighted and slaughtered. All visceral organs including head, neck, feet and shanks were removed and carcass was weighed again for determination of dressing percentage. The abdominal fat pads (AFP) was weighed after removing fat around the bursa of Fabricius and cloaca and was shown as g/100g body weight.

Antibody titre against ND

At the end of experimental period, two birds from each replicate were randomly selected for blood collection. From the brachial vein of the birds, 1.5ml of blood samples was collected in test tubes and centrifuged at 2000 rpm for 10 minutes to separate the serum. To determine antibody titer against ND, the Hemagglutination inhibition (HI) test was performed (Chand *et al*., 2014).

Heterophils and lymphocytes

At the end of experimental period, two birds from each replicate were randomly selected for blood collection. For heterophils and lymphocytes count, EDTA containing tubes were used for collection of blood samples. Blood smears were stained with May-Grunwald and Giemsa stains. A total of 100 cells each were counted for Heterophils and lymphocytes (Gross and Siegel, 1983) and then calculated Heterophil/lymphocyte ratio.

Statistical analysis

Data were statistically analyzed using Completely Randomized Design (CRD) while means were separated using Least Significance Test. Statistical package Statistix 8.1 was used to perform analysis. P values equal or less than 0.05 was considered significant.

RESULTS

Effect of betaine supplementation on weekly and total feed intake is given in Table II. Significantly higher weekly and total feed intake was recorded in Bet-2.0 compared to the control. Total feed intake of group Bet-2.0 was 8.7 % higher than the control. The effect of betaine supplementation on weekly and total weight gain is given in Table III. Among the treatment groups, Bet-2.0 birds had significantly higher weight gain followed by Bet-1.5, while the weight gain of Bet-1.0 was not significantly different from the control group during week 2 and week 5. Total weight gain increased significantly ($P<0.05$) with increasing level of betaine supplementation and significantly higher weight gain was recorded in the group Bet-2.0. Total weight gain of group Bet 2.0 has 12.5 % higher than the control group.

Table II.- Effect of betaine supplementation on mean feed intake (g) of heat stressed broiler chicks.

Group	Week 1 (Mean ±SE)	Week 2 (Mean±SE)	Week 3 (Mean ±SE)	Week 4 (Mean±SE)	Week 5 (Mean±SE)	Total (Mean±SE)
Control	420.53±8.41	609.60±1.57^{b}	752.63±3.40^{c}	843.53±4.17^{c}	701.43±1.76^{b}	3327.7±8.57^{d}
Bet-1.0	423.50±4.03	628.0±5.70^{ab}	786.63±5.73^{b}	863.13±7.63^{bc}	719.33±5.90^{b}	3420.6±9.76^{c}
Bet-1.5	422.53±7.43	645.97±22.22^{ab}	801.00±15.4^{b}	874.80±6.03^{b}	736.53±21.77^{ab}	3488.8±22.0^{b}
Bet-2.0	421.57±3.56	670.83±12.8^{a}	832.80±3.70^{a}	899.33±9.01^{a}	775.47±11.83^{a}	3618.0±12.21^{a}
P- value	NS	0.0535	0.0012	0.0030	0.0180	0.0000

Means in the same column with different superscripts are significantly different ($P\leq 0.05$). Bet represents Betaine; 1-2g per kg feed. NS, non-significant.

Table III.- Effect of betaine supplementation on mean weight gain (g) of heat stressed broiler chicks.

Group	Week 1 (Mean±SE)	Week 2 (Mean±SE)	Week 3 (Mean ±SE)	Week 4 (Mean±SE)	Week 5 (Mean±SE)	Total (Mean±SE)
Control	268.00±2.08	624.83±1.92^{c}	347.07±4.76^{c}	303.33±3.53^{c}	236.70±3.19^{c}	1779.9±11.52^{d}
Bet-1.0	272.00±4.04	630.40±1.24^{c}	367.30±4.41^{b}	321.83±3.76^{b}	251.20±9.51^{bc}	1842.7±17.29^{c}
Bet-1.5	274.33±11.35	640.00±3.33^{b}	378.33±4.27^{b}	337.83±9.46^{b}	274.50±5.11^{b}	1905.0±11.19^{b}
Bet-2.0	278.67±4.37	653.67±2.49^{a}	400.50±7.91^{a}	360.17±1.17^{a}	310.93±12.42^{a}	2003.9±4.62^{a}
P- value	NS	0.0001	0.0009	0.0005	0.0012	0.0000

For abbreviations and statistical details, see Table II.

Table IV.- Effect of betaine supplementation on mean Heterophil and Lymphocyte Ratio and ND antibody titer of heat stressed broiler chicks.

Group	Heterophil (Mean ±SE)	Lymphocyte (Mean ±SE)	H/L ratio (Mean ±SE)	ND Antibody Titer (Mean ±SE)
Control	46.5±2.03	45.17±2.76^{b}	0.065±0.117^{a}	3.222±0.40^{b}
Bet-1.0	40.16 ±2.79	55.333±2.60^{ab}	0.7831 ±0.085^{b}	6.556±0.89^{a}
Bet1.5	39.16 ±2.99	54.83±3.25^{a}	0.7457 ±0.109^{b}	5.333±0.41^{a}
Bet2.0	37.5 ±1.75	52.67±1.05^{a}	0.6818 ±0.045^{b}	5.444±0.38^{a}
P-value	NS	0.0382	0.0416	0.0019

For abbreviations and statistical details, see Table II.

Table V.- Effect of betaine supplementation on mean FCR and Dressing Percentage and AFP of heat stressed broiler chicks.

Groups	FCR (Mean ±SE)	Dressing% (Mean ±SE)	AFP (Mean ±SE)
Control	1.8697 ±0.013^{a}	65.387±0.268^{c}	1.7495±0.098
Bet-1.0	1.8565±0.014^{a}	66.541±0.207^{bc}	1.7717±0.210
Bet-1.5	1.8315±0.011^{ab}	67.149±0.557^{b}	1.8253±0.093
Bet-2.0	1.8055±0.010^{b}	69.088±0.697^{a}	1.8923±0.018
P-Value	0.0274	0.0038	NS

For abbreviations and statistical details, see Table II.

Data on FCR is presented in Table V. Higher level (Bet 1.5 and 2.0) of betaine supplementation significantly (P<0.05) improved FCR while the low level (Bet-1.0) supplementation had no significant effect on the FCR as compared to the control group. FCR of group Bet- 2.0 was 3.43 % lower (better) than the control group. Data on dressing percentage and abdominal fat is presented in Table V. Betaine supplementation at higher level significantly (P<0.05) improved dressing percentage while the low level had no significant (P>0.05) effect on the dressing percentage as compared to the control group. Significantly (P<0.05) higher dressing percentage was recorded in group Bet-2.0 and was followed by group Bet-1.5. Betaine had no significant effect on the abdominal fat pad at any level of supplementation.

Data on the number of heterophils, lymphocytes and their ratio is presented in Table IV. The HLR was significantly (P<0.05) higher in the control group as compared to the treated groups. Statistically similar HLR was recorded for all the treated groups. Data on the antibody titer against ND is presented in Table IV. The treatment groups had significantly (P<0.05) higher antibody titer against ND as compared to the control group.

DISCUSSION

Results of the present research work showed that high ambient temperature significantly affected the growth performance and immune status of broilers while betaine supplementation improved these parameters. Under heat stress condition, there is reduction in feed intake which may be due to little energy requirement for heat preservation (Freeman, 1988). Awad *et al*. (2014) reported that feeding of betaine at the rate of 1.5 g/kg in the diet results in significantly higher feed intake as compared to control group. Similarly, Sakomura *et al*. (2013) also reported that betaine supplemented to broilers significantly increased feed intake as compared to the control group.

Weight gain was significantly affected by betaine supplementation throughout the experimental period except first week. Birds in group Bet-2.0 had significantly higher weight gain as compared to the control. The difference between the different groups might be due to increasing level of betaine supplementation. The increase

in body weight gain may be due to the potential of betaine to improve the digestibility of specific nutrients (Eklund *et al*., 2006a, b). Sakomura *et al*. (2013) stated that during heat stress, there is an osmotic disturbance in broiler chickens while betaine improves the structural and functional characteristics of intestinal epithalia which results in better absorption of the nutrients. Furthermore, betaine is involved in the metabolism of protein and energy (Eklund *et al*., 2005). Attia *et al*. (2005) and Hassan *et al*. (2005) reported improved weight gain in poultry as a result of betaine supplementation. On the other hand, our findings did not agree with Zulkifli *et al*. (2004) and Esteve-Garica and Mack (2000) who stated that supplemental betaine in diet has no significant effect on body weight gain.

Our results showed that betaine supplementation significantly improved FCR at the rate of 1.5 to 2.0 g/kg feed. Findings of the present study are in line to the results of Attia *et al*. (2009) who stated that adding betaine at the rate of 1 kg/ton in poultry ration could partially alleviate chronic heat stress in poultry as compared to the negative treatment. Similarly, El-Husseiny *et al*. (2007) revealed that addition of betaine at the levels of 0.75 g/kg in diet significantly improved FCR as compared to the control group.

Our results indicated that betaine had no significant effect on the abdominal fat pad at any level of supplementation. Our results are in agreement with Sun *et al*. (2008) and Zulkifli *et al*. (2004). Contrary to the findings of the present study, Wang *et al*. (2004) and Zhan *et al*. (2006) reported that betaine supplementation reduced the abdominal fat pad because it has a role in lipid metabolism in the body. Our results are in contrast with Attia *et al*. (2005) who stated that there is an increase in abdominal fat pad by the supplementation of betaine.

Our results showed that betaine supplementation at higher level *i.e.,* Bet-2.0 and Bet-1.5, significantly ($P<0.05$) improved dressing percentage. The increase in dressing percentage may be due to the osmotic effects of betaine, which increases water retention (Waldroup and Fritts, 2005). Our results are in line with El-Shinnawy (2015) who reported that supplementation of betaine significantly increased the dressing percentage in chicken. Esteve-Garcia and Mack (2000) observed significantly better dressing percentage at 42 days of age at the rate of 1000 mg/kg betaine.

The present study showed that the treatment groups had significantly lower heterophil number and significantly higher lymphocytes number than the control group. Mashaly *et al*. (2004) reported that reduction of lymphocyte during heat stress is due to the increase of inflammatory cytokines which stimulate the hypothalamic production of corticotrophin releasing hormone under heat stress. These findings are in agreement with Nofal *et al*. (2015) who showed that supplementation of betaine in diet significantly decreased heterophil percentage but lymphocyte percentage was significantly increased, whereas, H/L ratio was significantly reduced. Awad *et al*. (2014) reported that adding betaine in diets significantly increased the lymphocyte percentage where as Heterophil and H/L ratio was significantly decreased as compared to the control group. Gudev *et al*. (2011) reported that supplementing betaine at the level of 1.5g/kg in feed significantly increased the lymphocyte and significantly decreased heterophil percentage.

The present study showed that betaine supplementation had significant effect on the antibody titer against ND vaccine. Our current results are in agreement with Farooqi *et al*. (2005) who showed that during heat stress, the addition of betaine in diets improved the immunity of birds. Similarly, Zhan *et al*. (2001) stated that addition of betaine significantly improved the antibody titer against ND. Whereas, our results are in disagreement with Zulkifli *et al*. (2004) who showed that betaine supplementation had no significant effect on antibody titer against ND.

CONCLUSION

It is concluded that supplementation of diets with betaine improved broiler feed intake, weight gain and FCR and increased immunity during heat stress.

Statement of conflict of interest

Authors have declared no conflict of interest.

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