Dietary Supplementation of Betaine (Betafin®) and Response to High Temperature Stress in Male Broiler Chickens

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ABSTRACT : The effects of supplemental betaine (Betafin®) in the drinking water (50 g/kg) (WB) or feed (100 g/kg) (FB) were investigated on male broiler chickens (Cobb×Cobb) exposed to 4 h episodes of heat stress at 34±1°C on day (d) 35 and 36±1°C from d 36 to 41. Prior to (d 1 to 34) and following heat exposure (d 35 to 41), betaine supplementation had no significant effect on body weight, total feed intake and cumulative feed conversion ratios of broilers. The total water intake of WB chicks was lower compared to controls. Prior to heat exposure, there was no difference in percentage of mortality among the three dietary groups. Following the heat challenge period, although higher percentage of control chicks succumbed to the heat challenge as compared to those of WB, it was not significantly different. The WB and FB chicks were less hyperthermic than controls in response to the heat challenge. Irrespective of treatment groups, the heat treatment resulted in a marked elevation in heterophil/lymphocyte ratios (HLR). The WB birds, however, had smaller increase in HLR than those of controls during heat exposure. Antibody production against Newcastle disease vaccine on day 35 was not affected by betaine supplementation. On d 42, WB birds had higher antibody production than those of FB. It is concluded that the WB treatment, as measured by HLR, antibody production and mortality rate, has advantages over the FB group under heat stress conditions. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 2 : 244-249)***

Key Words : Betaine, Heat Stress, Broiler Chickens

INTRODUCTION

Betaine, or glycine betaine, is widely found in nature and is synthesized by a variety of plants and organisms (Boch et al., 1994). There is a growing interest in using betaine as a feed additive in the diets of poultry (Saunderson and MacKinlay, 1990; Zimmermann et al., 1996) and swine (Smith et al., 1994; Matthews et al., 1995). Previous studies suggested that betaine might have a methionine sparing effects in non-ruminant animals. Betaine has also been shown to protect cells from osmotic stress and allowed them to continue regular metabolic activities in conditions that would normally inactivate the cell (Kidd et al., 1997). Within plant tissues, betaine functions as a cellular osmotic protectant. This osmolyte property protects plant cells from stress due to freezing, drought or salinity.

During heat stress, the body cells of birds are subjected to osmotic stress. In such instances, water is pulled out of the cell because of a higher concentration of salts or solutes outside the cells. This loss of water can cause the cells to shrink in volume and if this water loss is not corrected, the cell will eventually die. Although poultry do not have a specific requirement for betaine, the osmolytic property of betaine could be beneficial to heat stressed birds. Currently, literature concerning the effects of supplemental betaine on broiler chickens under heat stress conditions is limited. Thus, the objective of the present study was to determine the influence of dietary supplementation of betaine (Betafin®) on performance, body temperature, heterophil/lymphocyte ratios and antibody response in broiler chickens subjected to high ambient temperatures.

MATERIALS AND METHODS

One hundred and fifty day-old male broiler chicks (Cobb×Cobb) were received from a commercial hatchery and placed in an environmentally controlled chamber (2.3× 9.1×3.8 m) with a photo-regimen of 24 h light. The chicks were wing banded, weighed individually and randomly assigned in groups of 5 to 30 battery cages with wire floors. Ambient temperature on day (d) 1 was set at 32±1°C and then gradually reduced until 24±1°C was reached by d 21. The chicks were administered (intraocularly) live Newcastle disease (ND) vaccine (Nobilis ND Clone 30, Intervet International, 58,300 AA Boxmeer, The Netherlands) on d 7 and 21. Feed and water were provided for *ad libitum* consumption. Birds were fed starter and finisher diets from d 1 to 20 and d 21 to 42, respectively that met the nutritional requirements of the broiler (NRC, 1984). The basal diet was a typical corn-soy diet (Table 1). Commencing from day 1, the birds were subjected to one of three dietary treatments with 10 cages per group. The three dietary groups were: (1) basal diet and untreated drinking water (control); (2) basal diet and drinking water treated with 50 g/kg betaine (WB); (3) basal diet supplemented

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Table 1. Nutrient composition of broiler starter (d 1 to 20) and finisher (d 21 to 42) diets

Ingredients (%)	Starter	Finisher
Corn	53.89	60.30
Soybean meal	36.19	31.86
Fishmeal	3.00	3.00
Palm oil	3.73	2.44
Choline chloride (60%)	0.25	0.20
Vit-min. mix[1]	0.10	0.10
Salt	0.20	0.10
Antioxidant (santoquin)	0.01	0.01
DL-methionine	0.18	0.033
Limestone	1.30	1.30
Dicalcium phosphate	1.15	1.15
Total	100	100
Calculated composition (%)		
AME, kcal/kg	3,000	3,000
Crude protein	21.30	20.00
Crude fat	6.31	5.22
Crude fibre	3.80	3.65
Calcium	1.02	0.90
Available phosphorous	0.45	0.35

[1] Supplied per kg diet: Fe, 100 mg; Zn, 100 mg; Mn, 110 mg; Cu, 20 mg; I, 2mg; Co, 0.6 mg; Se, 0.2 mg; antioxidant (santoquin), 0.6 mg; folic acid, 0.33 mg; thiamin, 0.83 mg; pyridoxine, 1.33 mg; biotin 2%, 0.03 mg; riboflavin, 2 mg; phylloquinone, 1.33 mg; cyanocobalamin, 0.03 mg; pantothenate, 3.75 mg; niacin, 23.3 mg; retinol, 2 mg; cholecalciferol, 0.025 mg; dl-α-tocopherol, 23 mg.

with 100 g/kg betaine and untreated drinking water (FB). The dosages of betaine supplemented in feed and drinking water were as recommended by the manufacturer (Finfeeds, P.O. Box 777, Marlborough, Wiltshire, SN8 1XN, UK). Plastic bottle drinkers with a capacity of four litres were used (one drinker per cage). For estimation of evaporative water loss, three similar drinkers were placed at various locations in the house which were inaccessible to the birds. Body weight, feed intake, cumulative feed conversion (feed:gain) and water intake were determined for each cage at 7, 14, 21, 28, 35 and 42 d of age. Mortality was recorded daily.

Prior to (d 35) and following 7 d of heat challenge, (d 42), 10 birds per group were randomly selected, killed by cervical dislocation and the abdominal fat pads (AFP) (including fat surrounding bursa of Fabricius and cloaca) were removed and weighed. Weights of AFP were expressed relative to body weight (g/100 g body weight).

On d 35 all chicks (at the same time) were subjected to 34±1°C and 75% relative humidity for 4 h in the environmentally controlled chamber to evaluate their ability to withstand high ambient temperatures. The increase from 24±1°C to this temperature occurred over an approximately 1 h period. From d 36 to 41, the heat challenge was increased to 36±1°C for 4 h/day.

Prior to heat challenge (d 35), 10 birds per dietary group were randomly chosen and bled (1.5 ml) for heterophil (H) and lymphpocyte (L) counts and ND antibody titres. Blood samples for for H and L counts were collected in tubes containing EDTA as an anticoagulant. Blood smears were stained within 2 to 3 h of preparation using May-Grunwald and Giemsa stains and H and L were counted to a total of 60 cells (Gross and Siegel, 1983). Heterophil/lymphocyte ratio (HLR) was calculated by dividing the number of heterophils by that of lymphocytes. Blood samples for ND antibody titres were serum separated and stored at -20°C. Serum samples for ND antibodies were analysed using ELISA kit (IDEXX Laboratory, Inc., Westbrook, ME 4029). Similar procedures were repeated following 4 d (d 38) and 7 d (d 41) of heat treatment.

On d 35, 38 and 41, immediately following heat challenge, 10 birds per dietary group were randomly chosen for recording of rectal body temperature using an electronic thermometer. The probe was inserted about 3 cm into the rectum for about 30 seconds.

Data were analyzed using General Linear Models procedure of SAS® software (SAS Institute, 1982). Dietary treatment, stage of heat challenge and their interactions were considered as the main effects for the analyses of body temperature, HLR, relative AFP weight and ND antibody titre data. Antibody titre and AFP weight data were transformed to common logarithm and arc sine square roots, respectively, prior to statistical analysis. Duncan's multiple range test was performed for multiple comparisons of means. Data on mortality rate were analyzed by chi-square test. The α value was 5%.

RESULTS

The performance data are presented in Table 2. Body weights of birds were not affected by dietary treatment throughout the period of study. Prior to heat challenge, feed consumption of control, WB and FB birds was not significantly different. However, during the heat treatment period (d 35 to 41), the FB and WB birds consumed significantly more feed than controls. Despite consuming significantly more feed from d 35 to 41, the FCR of FB and WB birds were similar to their control counterparts. There was no significant difference in the amount of water consumed by all the birds during the first three weeks of life. From d 21 to 27 and d 28 to 34, however, the control birds consumed more water than their WB counterparts. The total water intake (d 1 to 41) of control chicks was greater than those subjected to WB. The total water intake of FB chicks was similar to both WB and control groups. Prior to heat exposure, there was no difference in percentage of mortality among the three dietary groups. Irrespective of dietary group, the 7 d heat challenge resulted high mortality of birds. Although higher percentage of control chicks succumbed to the heat challenge as compared to those of

Table 2. Effect of betaine supplementation on body weight, feed intake, feed conversion ratio, water intake and mortality rate of broiler chickens subjected to heat challenge between 35 to 41 d of age (Mean±SEM)

	Treatment		
	Control	WB	FB
Body weight (g)			
d 0	41±0.4	41±0.6	40±0.3
d 7	143±3.4	147±2.0	145±2.7
d 14	439±10.3	428±6.4	432±7.4
d 21	644±13.2	646±9.0	621±30.5
d 35	1,079±24.0	1,084±13.5	1,115±15.4
d 38	1,629±23.7	1,599±24.6	1,642±21.0
d 42	2,006±26.2	1,982±27.7	2,005±29.4
Feed intake (g/bird)			
wk 1	153±7.1	157±4.2	144±5.8
wk 2	346±13.6	349±8.7	340±10.0
wk 3	486±10.7	487±13.3	489±7.6
wk 4	807±16.4	783±15.1	795±13.1
wk 5	1,044±20.9	979±23.9	1,022±18.6
wk 6	1,334±19.5^{a}	1,254±22.8^{b}	1,248±33.8^{b}
Total	4,169±72.5	4,009±69.6	4,038±59.1
FCR (feed/gain)			
wk 1	1.06±0.03	1.07±0.03	1.00±0.03
wk 2	1.14±0.06	1.19±0.04	1.12±0.03
wk 3	1.42±0.03	1.43±0.02	1.48±0.09
wk 4	1.59±0.04	1.56±0.02	1.52±0.02
wk 5	1.74±0.02	1.72±0.01	1.70±0.02
wk 6	2.09±0.06	2.02±0.03	2.02±0.04
Water intake (g/bird)			
wk 1	217±3.6	231±13.0	198±9.2
wk 2	475±13.9	472±15.8	428±10.4
wk 3	1,041±43.3	984±33.5	981±26.1
wk 4	1,255±42.0^{a}	1,102±38.0^{b}	1,139±26.9^{b}
wk 5	2,544±83.8^{a}	2,158±74.5^{b}	2,475±94.1^{a}
wk 6	2,756±92.7	2,611±114.4	2,901±122.2
Total	8,287±214.0^{a}	7,557±217.7^{b}	8,167±231.5^{ab}
Mortality (%)			
0-34 d	2	2	2
35-42 d	26.5	16.3	22.4

^{a,b}Means within a row with no common letters differ at p≤0.05. WB=basal diet and drinking water treated with 50 g/kg betaine. FB=basal diet supplemented with 100 g/kg betaine and untreated drinking water.

WB, it was not significantly different.

The betaine treatment×stage of heat treatment interactions for relative AFP weight, body temperature, HLR and ND antibody titre data were significant (Table 3). The effect of diet on AFP weight was only noted following 7 d of heat treatment. On d 41, except for the FB group, birds deposited more fat with age. While diet had negligible effect on body temperature on d 35 and 38 (1 and 4 d of heat exposure, respectively), the WB and FB birds were less hyperthermic than controls at 41 d of age (7 d of heat exposure). Irrespective of dietary groups, the heat exposure resulted in a significant elevation in HLR. Significant effect of betaine treatment on HLR was only noted on d 38 (4 d of heat exposure) with WB birds exhibited lower values than controls. The HLR of FB chicks were similar to both FB and control groups. Heterophil/lymphocyte ratio was not significantly affected by diet on HLR on d 35 and 42 (1 and 7 d of heat exposure). While diet had no significant effect on ND antibody titres on d 35 (prior to heat exposure) and 38 (4 d of heat exposure), subjecting birds to WB resulted higher antibody production than those of FB on d 42 (7 d of heat exposure). The mean ND antibody titres of control birds on d 42 were similar to both FB and WB groups.

DISCUSSION

Collectively, the physiological data presented here suggested that betaine supplementation could help to

Table 3. Relative abdominal fat pad weight, rectal body temperatures, heterophil/lymphocyte ratios, ND antibody titres of broilers subjected to heat challenge between 35 to 41 d of age when diet×stage of heat treatment interaction was significant (Mean±SEM)

Parameter	Treatment		
	Control	WB	FB
Relative abdominal fat pad weight			
d 35	0.74±0.09^{y}	0.91±0.07^{y}	0.83±0.09
d 42	1.09±0.01^{abx}	1.28±0.06^{ax}	0.96±0.15^{b}
Rectal body temperature (°C)			
d 35	42.79±0.18^{y}	42.36±0.11^{z}	42.85±0.16^{y}
d 38	44.82±0.37^{x}	44.18±0.21^{y}	44.39±0.43^{x}
d 41	45.61±0.28^{ax}	44.75±0.21^{bx}	44.76±0.31^{bx}
Heterophil/lymphocyte ratio			
d 35	0.31±0.33^{y}	0.36±0.05^{y}	0.34±0.02^{y}
d 38	1.18±0.24^{ax}	0.54±0.03^{bx}	0.94±0.11^{abx}
d 42	0.78±0.06^{x}	0.68±0.06^{x}	0.87±0.10^{x}
ND antibody titres			
d 35	1,258±224.4	1,511±527.7	1,137±181.2
d 38	957±134.6	864±395.7	527.7±128.3
d 42	1,127±115.8^{ab}	1,518±160.4^{a}	950±185.0^{b}

^{a,b} Means within a row with no common letters differ at p≤0.05. ^{xz} Means within a column with no common letters differ at p≤0.05.
WB=basal diet and drinking water treated with 50 g/kg betaine. FB=basal diet supplemented with 100 g/kg betaine and untreated drinking water.

alleviate the stress attributed to high temperatures. Heat-elicited elevation in HLR and body temperature was all reduced by supplementing chicks with betaine. Heterophil/lymphocyte ratios appear to be a reliable measure of thermal stress in poultry (Zulkifli et al., 1994a,b; 1999, 2000; 2002). One of the earliest studies to report on the effects of high temperature as a stressor on blood leucocyte was that by Chancellor and Glick (1960). They reported an initial decrease in H and an increase of L after exposure for 15-30 min. However, 2 h later the cellular numbers were reversed. Our results are in accordance with the findings of Zulkifli et al. (1999) that HLR correlated well with body temperature. The WB birds had lower body temperature and HLR than their control counterparts following heat exposure.

Although there was a marked numerical difference in the mortality rate between WB (16.3%) and control (26.5%) chicks, statistical analyses revealed no significant difference. It is interesting to note that although the control chicks were more hyperthermic and "stressed" (as measured by HLR) than those of WB, the mortality rate of both groups did not differ significantly. Pathological state is an extreme consequence of biological response and occurs when prolonged and intense physiological reactions are involved (Sapolsky, 1992). According to Moberg (1985), most stressful encounters in animals can be coped sufficiently without adverse effect on survivability.

In the present study, irrespective of treatment, all birds had similar body weight, total feed intake and cumulative FCR prior to and following heat exposure. It is interesting to note although the FB and WB birds consumed significantly less feed than controls during the heat challenge period, all the three groups had similar body weight on d 42. Reduced feed intake under heat stress is due, in part, to lower energy requirements because allocation of energy for heat conservation is minimal (Freeman, 1988). The lower feed intake and similar body weight of FB and WB birds as compared to controls, however, did not improve FCR. Thus, there was little evidence to suggest that betaine supplementation in feed or drinking water could significantly improve the performance of broiler chickens under heat stress conditions.

In the present study, although there was no difference in the amount of water consumed during the heat exposure period, the total water intake of WB birds was lower than those of controls. Despite consuming less water, the WB chicks exhibited similar growth performance to their control counterparts. These results confirmed earlier findings that betaine supplementation may improve osmoregulation in poultry (Kidd et al., 1997). Excessive drinking of water might result in wet droppings, damp litter, higher amount of atmospheric ammonia and susceptibility to hockburn and foot necrosis (Appleby et al., 1992). Under the hot and humid tropical conditions, this may lead to obnoxious odour and the development of larger number of flies. Hence, wet droppings and consequent damp litter can be reduced by betaine supplementation in the drinking water.

Betaine has been shown to interact with lipid metabolism by stimulating the oxidative catabolism of fatty acids via its role in carnithine synthesis, and thus can be used as a mean to increase lean and decrease fat in poultry carcass (Saunderson and MacKinlay, 1990). On the contrary, the present findings together with those of Schutte et al. (1997) suggest that betaine has no significant influence on

abdominal fat deposition in broiler chickens. The mean relative AFP weights of control birds were not significantly different from those of FB and WB birds throughout the period of study. Thus, it can be concluded that the effect of betaine on fat deposition in broilers is inconsistent. The increase in AFP weight with age is expected. It is well documented that older birds have a higher fat content than younger birds (Leenstra, 1986). The number of fat cells in both layer- and broiler-type chickens increases until about 14 weeks of age (Pfaff and Austic, 1976; March and Hansen, 1977). The heat treatment from d 35 to 41 may also contribute to the increase in AFP weight. Rearing temperature is considered as one the most prominent environmental factors in determining fat deposition (Leenstra, 1986). Fischer (1984; as cited by Leenstra, 1986) noted a liner increase of 0.19% total body fat per degree increase in temperature between 10 and 30°C.

Converging evidence suggests that extreme environmental conditions influence the health and well being of animals. These stressors directly alter host immune function. In the present study, irrespective of dietary group, the ND antibody titres remained consistent throughout the duration of heat exposure. Following 7 d of heat exposure although the WB treatment failed to enhance ND antibody titres as compared to controls, the former had higher response than those of FB. Hence, it appears that the effect of betaine on antibody production in heat-stressed broiler chickens is inconclusive.

Collectively, our results suggest that, for best results, betaine has to be supplemented in the drinking water. Based on HLR, mortality rate and ND antibody titres, WB birds were better able to withstand high ambient temperatures than their FB counterparts. There appears to be no obvious explanation for the apparent difference in effectiveness to reduce heat stress problems between FB and WB and only a speculative one can be offered at this stage. It is well established that as ambient temperature rises feed intake declines but water intake increases. Thus, there is a possibility that the intake of betaine by the FB birds is insufficient as compared to their WB counterparts to ameliorate response to high temperature. In view of this, the optimum concentration of betaine supplementation in broiler feed during period of heat stress merits further investigation.

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