



RESEARCH ARTICLE

Ameliorating Effect of Different Anti-Stressors on Growth Performance and Immunophysiological Responses in Heat Stressed Broilers Chickens

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ABSTRACT

This study was conducted to ascertain the effects of betaine, chia seed, potassium chloride, and vitamin C on growth performance, immune response and serum biochemistry of broilers chickens reared during hot (30-37°C) and humid (52-80%) climate. A total of 320 birds were divided into five treatment groups (A-E) with four replicates, having 16 birds in each. Group A served as control. Birds in groups B, C, D and E were respectively fed diets supplemented with betaine (2g/Kg), chia seed (5g/Kg), potassium chloride (2g/Kg) and vitamin C (600mg/Kg). Daily feed intake and weekly body weights were recorded. Blood samples were collected at 7th, 17th, 36th and 42nd day for estimation of Newcastle disease virus titer and at 6th week of age for serum cholesterol and glucose estimation. Results showed highest ($P<0.05$) feed intake and antibody titer against ND in birds supplemented with chia seed. Betaine supplementation resulted in highest ($P<0.05$) body weight gain and better FCR. Supplementation of vitamin C in diet resulted in lower ($P<0.05$) serum cholesterol and glucose levels. These findings showed that use of appropriate feed supplements helped to reduce the deterioration in performance of heat stressed broilers. Among different anti-stressors used in present study betaine, chia seed and vitamin C offered better performance in competing negative effects of heat stress in broiler chickens.

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INTRODUCTION

Homeostasis is the mechanism that maintains the internal temperature of bird and keeps all physiological functions normal. Birds have ability to maintain their body temperature under normal conditions but when the internal heat production and heat gain from the environment are greater, then the rate of heat dissipation decreases, and body temperature increases. A bird is said to be in heat stressed when their temperature go above the upper critical level of the thermo neutral zone (Khan *et al.*, 2011). Heat stress is one of the major stressors in poultry that induces many physiological alterations which includes an increase in plasma concentrations of corticosterone hormone, protein and glucose levels (Whitehead and Keller, 2003).

Several methods have been proposed for reducing heat stress in poultry including nutritional manipulations. Supplementing diets with different feed additives such as vitamin C, betaine and use of electrolytes showed beneficial responses in heat stressed birds (Whitehead and Keller, 2003; Hijati *et al.*, 2015). Betaine is a multi-nutritional agent that may help chickens to resist heat stress. Broilers reared under heat stressed conditions showed an improvement in growth, feed conversion ratio and immune response when fed a diet supplemented with betaine (Wang *et al.*, 2004). In broilers, supplemental ascorbic acid (Sahin and Kucuk, 2001) resulted into increased performance in the birds reared under heat stress conditions. Supplementation of ascorbic acid in combination with vitamin E showed improved body weight gain in birds exposed to heat stress (Ciftci *et al.*, 2005).

Chia seed can also be used as an anti-stressor, because it is potentially a great source of antioxidants, the immense antioxidant potential can be utilized for better health (Uribe *et al.*, 2011). In stress condition, the amount of free radicals within the body increases, chia seed has the ability to neutralize these radicals and can protect the body during stress (Nadeem *et al.*, 2014). Potassium chloride (KCl) has been testified to reduce mortality, elevate growth rate and immune response in heat stressed birds (Borges *et al.*, 2004). Therefore, the present study was conducted with the aim of analyzing the effects of different anti-stressors including- betaine (2g/Kg), chia seed (5g/Kg), potassium chloride (2g/Kg), and vitamin C (600mg/Kg) on growth performance, immune response and serum chemistry of heat stressed broilers.

MATERIALS AND METHODS

A total of 320 commercial day-old male Cobb broiler chicks, purchased from a local hatchery were divided into five different treatment groups (A-E) according to completely randomized design. Group A served as control, birds in groups B, C, D and E were fed diets supplemented with betaine (2g/Kg), chia seed (5g/Kg), Potassium Chloride (KCl) (2g/Kg), and Vitamin C (600mg/Kg) respectively. Each treatment was replicated 4 times having 16 birds in each. Betaine (BetaWin) and Potassium Chloride were purchased from Ghazi Brothers Pvt. Ltd. vitamin C was supplemented in the form of C Vita 100 was purchased from ZGI International Limited, while powder of natural chia seed was purchased from local grocery shop. Feed composition of starter, grower and finisher diets is given in Table 1. All the diets were provided as mash. The present study was conducted at Poultry Research and Training (PRTC) Centre, Ravi Campus Pattoki, University of Veterinary and Animal Sciences, Lahore, to compare the efficacy of different anti-stressors on growth performance antibody response against Newcastle Disease (ND) and serum biochemistry of broilers kept during hot-humid season (July-August). Stocking density was 0.65ft per bird; rice husk was used as bedding material. Birds were vaccinated according to prevailing schedule of the area. A lighting schedule of 24 hours was followed throughout the entire experiment. Feed and water were offered *ad-libitum* throughout the experimental period. Natural environmental conditions were used throughout the experimental period as the temperature and humidity are high in July-August in Pakistan. Environmentally controlled conditions were not maintained in the shed to reduce temperature and/ or control humidity during research period. Temperature and humidity (Table 2) were recorded daily, twice a day (morning and afternoon). Daily feed intake and weekly body weight were measured to calculate the feed conversion ratio (FCR). The record of the birds died during the experimental period was maintained to calculate mortality (%). Blood samples from two birds per replicate were collected at 7th, 17th, 36th and 42nd day for estimation of antibody titer against Newcastle Disease Virus. NDV titer was determined following the procedure described by Rubbani *et al.* (2001). In addition to this serum cholesterol and glucose levels were also determined from blood samples collected on day 42.

Serum samples were extracted by centrifugation and stored at -20°C for measuring glucose and cholesterol using Glucose Liquid color (GOD-PAP-Method; Human cat # 10260) and Cholesterol Liquid color (CHOD-POP-Method; cat # 10017) respectively.

Statistical analysis: The data obtained were analyzed statistically using a completely randomized design with the statistical package Statistics (copyright 1985-2005; Analytical Software, USA). Values were considered significant at $P < 0.05$. In the case of significant differences, Duncan's multiple range tests was used to compare differences among treatment means.

RESULTS

Growth performance: Results of the present study showed significant ($P < 0.05$) differences among different anti-stressors regarding overall feed intake, body weight gain, FCR and mortality percentage (Table 3). Birds fed chia seed had higher feed intake ($P < 0.05$) compared to control group. Supplementation of betaine resulted in highest body weight ($P < 0.05$). Improvement in weight gain was also reflected in better (lower) FCR values.

Table 1: Nutrient composition of starter, grower and finisher diets

| Nutrients | Starter | Grower | Finisher |
|---------------------------------------|---------|--------|----------|
| ME (Kcal/kg) | 2700 | 2850 | 3100 |
| CP (%) | 21.00 | 20.0 | 18.11 |
| Fat (%) | 3.00 | 4.11 | 3.79 |
| Fiber (%) | 4.65 | 4.31 | 4.14 |
| Calcium (%) | 0.88 | 0.82 | 0.78 |
| Phos. Avail. (%) | 0.44 | 0.4 | 0.4 |
| Lysine dig. (%) | 1.17 | 1.05 | 0.99 |
| Meth dig. (%) | 0.50 | 0.49 | 0.46 |
| M+C dig. (%) | 0.80 | 0.77 | 0.72 |
| Argin dig (%) | 1.20 | 1.1 | 1.02 |
| Threo dig. (%) | 0.70 | 0.66 | 0.63 |
| Tryp dig. (%) | 0.20 | 0.18 | 0.16 |
| Isoleu dig. (%) | 0.70 | 0.68 | 0.62 |
| Valine dig. (%) | 0.83 | 0.76 | 0.68 |
| Ingredient used in experimental diets | | | |
| Maize | 35.13 | 54.85 | 58.70 |
| Rice polish | 0.00 | 5.00 | 6.00 |
| Wheat Bran | 1.00 | 3.00 | 4.00 |
| Canola Meal | 15.00 | 6.05 | 1.67 |
| Rapeseed Meal | 4.00 | 4.00 | 4.00 |
| Soybean Meal | 15.65 | 16.00 | 17.50 |
| Corn Gluten Meal | 1.20 | 1.60 | 1.60 |
| Poultry Byproduct meal | 0.00 | 2.00 | 1.00 |
| Fish Meal | 2.00 | 2.50 | 0.00 |
| Marble Chips | 0.80 | 0.55 | 0.73 |
| DCP | 0.80 | 0.53 | 1.12 |
| Lysine sulphate | 0.57 | 0.48 | 0.53 |
| DL Methionine | 0.09 | 0.18 | 0.19 |
| Threonine | 0.06 | 0.05 | 0.08 |
| Molasses | 0.63 | 2.50 | 2.25 |
| Premix | 0.24 | 0.43 | 0.26 |
| Salt | 0.18 | 0.23 | 0.32 |
| Phyzyme | 0.05 | 0.05 | 0.05 |
| Rice Broken | 22.60 | 0.00 | 0.00 |
| Total | 100.00 | 100.00 | 100.00 |

Table 2: Record of Temperature and humidity:

| Week | Ambient Temperature (Range) (Min-Max °C) | Humidity (Range) (Min-Max %) |
|------|--|------------------------------|
| 1. | 32.1-33 | 52-70 |
| 2. | 32-40 | 54.5-74 |
| 3. | 30-41 | 54.5-74 |
| 4. | 29-38 | 74-82.5 |
| 5. | 29-40 | 72-80.5 |
| 6. | 30-36 | 73.5-80 |

Table 3: Growth performance influenced by different Anti-stressors in broilers reared during hot-humid season

| Treatment | Means | | | |
|-----------|-----------------------------|----------------------------|-------------------------|---------------|
| | Feed intake (g) | Body weight (g) | FCR | Mortality (%) |
| Control | 3085.89±81.0 ^b | 1696.65±66.0 ^b | 1.82±0.04 ^a | 2.08±2.08 |
| Betaine | 3167.41±24.20 ^{ab} | 1870.43±79.7 ^a | 1.70±0.06 ^b | 0±0 |
| Chia seed | 3248.56±66.0 ^a | 1837.30±67.1 ^{ab} | 1.77±0.03 ^{ab} | 0±0 |
| KCl | 3060.83±52.9 ^b | 1740.33±59.1 ^{ab} | 1.76±0.06 ^{ab} | 2.08±2.08 |
| Vit.C | 3177.35±34.3 ^{ab} | 1819.46±35.9 ^{ab} | 1.74±0.03 ^{ab} | 0±0 |

*Different alphabets on means show significant results (P<0.05).

Table 4: Anti-body titer and serum chemistry influenced by different Anti-stressors in broilers reared during hot-humid season

| Treatment | Means | | | | Cholesterol Level | Glucose level |
|-----------|---------------------|----------------------|------------------------|-------------------------|---------------------------|---------------------------|
| | Day 7 th | Day 17 th | Day 36 th | Day 42 nd | | |
| Control | 5.00±0 | 4.25±0.25 | 2.25±0.62 ^b | 2.62±0.40 ^c | 152.37±0.75 ^a | 248.26±0.60 ^a |
| Betaine | 6.50±0.50 | 4.75±0.25 | 3.75±0.25 ^a | 3.30±0.40 ^{bc} | 144.12±3.20 ^{ab} | 226.43±0.49 ^b |
| Chia seed | 5.50±1.50 | 4.00±0.40 | 4.00±0.40 ^a | 3.62±0.28 ^a | 140.85±4.40 ^b | 225.48±0.42 ^{bc} |
| KCl | 5.00±1.00 | 4.50±0.28 | 3.75±0.25 ^a | 3.37±0.25 ^{ab} | 145.37±2.77 ^{ab} | 224.38±0.53 ^c |
| Vit.C | 5.50±0.50 | 4.50±0.28 | 3.75±0.25 ^a | 3.50±0.28 ^a | 139.75±2.92 ^b | 216.76±0.57 ^d |

*Different alphabets on means show significant results (P<0.05).

Mortality: Mortality percentage remained non-significant throughout the experiment. Highest mortality percentage was observed in control group and KCl supplemented birds (2.08±2.08). All other dietary treatments showed least (0±0) mortality rates (Table 3).

Antibody titers for Newcastle (ND): The results of the study revealed that hemagglutination inhibition (HI) titers against ND showed non-significant differences (P>0.05) when compared on days 7 and 17 but, on 35th and 42nd day the birds fed on diet supplemented with chia seed showed significantly (P<0.05) higher anti-NDV antibody titers as compared to control group (Table 4).

Serum biochemistry: Results for serum biochemistry revealed significant (P<0.05) differences among all treatments for blood cholesterol and glucose level (Table-4). All dietary supplements improved the physiological parameters of broilers during heat stress. Lowest (P<0.05) cholesterol and glucose levels were observed in the serum samples of the birds supplemented with vitamin C and chia seed compared to control group that were reared in heat stress condition and were not supplemented with any dietary treatment.

DISCUSSION

Growth performance: From the findings of the present study it is clear that high ambient temperature accompanied with high humidity levels (high heat index) adversely affect the overall performance of broiler. According to results the high temperature along with excessive humidity reduced the feed intake which resulted into impaired growth. Poor performance was exhibited by the birds owing to higher ambient temperature, as the birds would have been subjected to metabolic disorder and struggling to manage homeostasis leading to low feed with higher water intake along with dissipation of extra energy. This might be due to the fact that body temperature of the bird increases with an increase in temperature and humidity that consequently results in reduced feed consumption, growth rate and feed efficiency (Khan *et al.*, 2011).

Highest feed intake in chia seed group may be associated with the presence of appropriate amount of anti-oxidants in it that combat against the production of free radicals in the body during the heat stress (Nadeem *et al.*, 2014). A wide range of vitamins (A, C, E) and

mineral contents like sodium, potassium, chloride are also present in chia seed, that might contribute in the reduction of heat stress on birds and results in an increased feed intake. The birds supplemented with betaine showed best results in improving growth parameters as compared to other supplements used to reduce heat stress in the present study. The positive effect of betaine may be attributed to the functions it performs as an osmolyte that reduces the dehydration condition in the body of bird during the heat stress (Kettunen *et al.*, 2002). It has the ability to influence the movement of water in intestinal mucosa, in hyperosmotic medium, tend to reduce the reflux of water between duodenum and jejunum and aids in the growth of broilers (Kettunen, 2002). Betaine also increases the natural production of some amino acids in the body which can help to reduce the depression that is helpful in stress condition. An increase in feed efficiency may be related with the effective digestibility of nutrients in digestive tract because betaine can act as betaine hydrochloride to increase the concentration of hydrochloric acid in the stomach, which helps in the breakdown of feed and effective utilization of nutrients increase the growth of body (Marc *et al.*, 2013). Results for lowest mortality rates may be attributed to the least stress on the birds supplemented with betaine. The evidences regarding the use of betaine in heat stress condition proves that it is an important nutrient due to its multiple functions.

Increased body weight of broilers along with improvement in FCR and lowest mortality rates when betaine is supplemented during heat stress in the present study has been also reported by others (Farina *et al.*, 2012). Chen and Chiang (2002) stated that dietary supplementation of betaine under high temperature improved the weight gain of broilers. Another study reported that birds fed on diets supplemented with betaine showed better feed efficiency (Waldroup *et al.*, 2006). In addition to this, Farina *et al.* (2012) observed lowest mortality rates in birds that were reared in heat stress and supplemented with betaine.

Antibody titers against Newcastle Disease virus: Immune response of broilers improved significantly (P<0.05) when fed at a diet having chia seed. These results might be associated with the presence of antioxidants; as it has been reported that the immense antioxidant level in Chia seed could be beneficial for better health (Uribe *et al.*, 2011). Chia seed is the power

house for omega-3 fatty acids (that include n-3 polyunsaturated fatty acid (PUFA) and α -linolenic acid, ALA) (Vuksan *et al.*, 2007) and the Omega-3 affects the antibody production in chickens while, n-3 PUFA have higher ability to increase immune response compared with ALA (Kamran *et al.*, 2009). Fernandez *et al.* (2006) reported the therapeutic properties of chia seed including the reduction of serum cholesterol level that prevents stress and also contributes in the improvement of immune system which was also proved in this study.

Similar to the present findings Friedman and Sklan, (1995) reported higher antibody production in broilers treated with high levels of ALA. In contrast to this Kamran *et al.* (2009) does not observe any significant difference for antibody production in the birds supplemented with ALA enriched diets and control birds.

Serum chemistry: In present study, vitamin C supplementation resulted in lower glucose and cholesterol concentration compared to control group. Similar findings have been reported by Samar *et al.* (2014) that addition of vitamin C in diet increases the protein concentration in blood serum, while cholesterol and glucose concentrations decrease. In contrast, Mosleh *et al.* (2018) stated that serum parameters were not influenced by the addition of vitamin C when used in heat stress condition. Lower levels of serum cholesterol and glucose in birds fed diets supplemented with vitamin C during heat stress might be attributed to fact that vitamin C reduces the breakdown of body lipids in heat stress condition and lowers the blood cholesterol levels (Gursu *et al.*, 2004). During heat stress feed intake of the bird is being decreased, thus lipolysis phenomenon compensates this deficiency of energy by converting cholesterol into glucose (Rashidi *et al.*, 2010). Borges *et al.* (2007) stated that production of glucose is directly proportional to the release of glucocorticoid hormone which is being produced under the heat stress conditions and increase the production of glucose in body by initiating the process of gluconeogenesis. However, vitamin C reduces the release of glucocorticoids in such conditions (Sahin and Kucuk, 2003) and hence decreased the serum glucose level as observed in the present study.

Conclusions: From the current study, it can be concluded that heat stress adversely affects the growth performance as well as the physiology of birds, and these adverse effects can be amended by the use of anti-stressors. Supplementation of betaine in diet proved comparatively more efficient than other anti-stressors used in present study, in terms of growth parameters while, immune response of broilers could be improved by the use of chia seed. Vitamin C played an important role in improving physiology of bird against heat-induced free radical damage. Further studies are needed to evaluate possible synergistic effect of these supplements in heat stress birds.

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Authors contribution: TA and SM conceived and designed the study. TA, SM, AB and ASJ performed experiment, participated in all sample collection and

pathological studies. GS contributed to the analysis of data. JH and FH helped in sample collection. AM and RY collaborated in the sample collection and paper writing. All authors approved the final manuscript.

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