INTRODUCTION

Metabolism of glucose in birds is considerably different from mammals while blood glucose concentration is much higher in birds and insulin levels are low (Brooks et al., 2016). Birds as compared to mammals are considered to be less sensitive to insulin (Scanes, 2009) and effect of chromium to enhance insulin sensitivity in mammals is well documented (Vincent, 2001). Heat or cold stress increase circulating concentrations of corticosterone in broilers and it is well documented that corticosterone reduces insulin sensitivity in broilers (Zhao et al., 2009). Poultry is reared in open side houses in most of the tropical countries like India which results in huge temperature variation in shed (Rajkumar et al., 2011) causing stress which results in increased demand of antioxidant supplementation. Since cooling of poultry houses (environment control) is very expensive thus methods are focused on nutritional modifications (Attia et al., 2015) like search of new feed additives along with their different combinations to increase the performance of birds naturally. Stress increases mineral and vitamin mobilization from tissues and their production is decreased (Smith and Kay, 2009). Thus, it becomes necessary to provide additional antioxidants to poultry during stress and other critical periods of life to improve performance. Antioxidants are the substances which trap free radicals and prevent them from doing any damage (Mayer et al., 2010). Antioxidants are used to reduce the harmful effects of excess reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Mustafa et al., 2003). There is a possibility the combined use of chromium and antioxidants can be effective in improving poultry performance.
excretion (Siegel, 1995), thus may exacerbate a marginal vitamin and mineral deficiency or an increased mineral and vitamin requirement. It has been reported that the negative effects of environmental stress could be prevented by the use of some minerals and vitamins supplements such as vitamin C and chromium (Sahin and Sahinm, 2001; Sahin and Kucuk, 2001). Chromium is postulated to function as an antioxidant (Farag et al., 2017, Kholy et al., 2017) and its deficiency can disrupt carbohydrate, protein metabolism along with impaired growth rate (Pagan et al., 1995). Though birds do not require any dietary vitamin source as it can synthesize vitamin (Pardue and Thaxton, 1986) reported that particular environmental stressors could alter ascorbic acid utilization or synthesis in poultry. It is well documented that under stress conditions such as low or high environmental temperatures, humidity and high productive rate ascorbic acid synthesis is inadequate (McDowell, 1989). Poultry cannot synthesize vitamin E thus vitamin E requirements must be met from dietary sources (Chan and Decker, 1994) in case of increased demand in stress. Vitamin E is a biological chain-breaking antioxidant that protects cells and tissue from lipoperoxidative damage induced by free radicals (McDowell, 1989). Sahin et al. (2002) reported that broilers supplemented with dietary chromium and vitamin E significantly alleviated the heat stress related decrease in performance suggesting that additional supplementation into diets may be necessary under stress conditions in growing birds.

As per available literature scanty work has been done on combination of chromium yeast alone and in combination with ascorbic acid and vitamin E. In this view, the present experiment was designed to study the effect of dietary supplementation of chromium yeast alone and in combination with antioxidants on feed conversion, growth and nitrogen retention in broiler chickens during the winter season.

**MATERIALS AND METHODS**

**Experimental Design**

One hundred eighty day old Cobb-400 broiler chicks of comparable body weight were equally distributed into four treatment groups viz. T₀, T₁, T₂ and T₃. Each treatment group contained forty five chicks with three replicates of fifteen birds in each. The diet (Table 1) was maize-soybean-based broiler mash was formulated as per BIS (Bureau of Indian Standards, 1992) specifications to meet the nutrient requirements of broilers. The standard starter mash (0 - 3 weeks) and finisher mash (4 - 6) were fed. Chicks were fed corn-soya based broiler mash (basal diet) maintained as control (T₀), the basal diet supplemented with chromium at 0.5 mg Cr/kg diet from chromium yeast (T₁), the basal diet + 0.5 mg Cr/kg diet from chromium yeast + 250 mg of ascorbic acid/kg of diet (T₂) and the basal diet + 0.5 mg Cr/kg diet from chromium yeast + 250 mg vitamin E/kg of diet (T₃).

| Ingredients | Starter Mash | Finisher Mash |
|-------------|--------------|---------------|
| Maize       | 56.2         | 62.5          |
| Soybean meal| 37.6         | 31.3          |
| Vegetable oil| 2.0         | 2.0           |
| Dicalcium Phosphate | 2.6 | 2.6         |
| Calcite     | 1.0          | 1.0           |
| Trace mineral mixture * | 0.2 | 0.2          |
| Salt        | 0.4          | 0.4           |

**Other supplement (g/100 kg)**

| Ingredients | Starter Mash | Finisher Mash |
|-------------|--------------|---------------|
| Vitamin premix** | 25 | 25          |
| Methionine  | 155          | 34            |
| Lysine      | 63           | 17            |
| Choline Chloride | 60 | 60          |
| Maduramicin | 50           | 50            |
| Toxin binder| 100          | 100           |

**Nutrient analysis of broiler starter and finisher mash (% DM)**

| Nutrients | Starter mash | Finisher mash |
|-----------|--------------|---------------|
| Dry matter| 93.78        | 93.02         |
| Crude protein | 22.84 | 20.61        |
| Ether extract | 4.14  | 4.56         |
| Crude fiber | 3.73  | 3.44         |
| Nitrogen free extract | 63.12 | 64.98      |
| Total ash | 6.17         | 6.41          |
| Calculated ME (kcal/kg) | 2888 | 2942        |

*1 Kg contains Mn 90 g; Zn 80 g; Fe 90 g; Cu 15 g; I 2.0 g and Se 300 mg.

**500 gm contains Vit.A 12; 50 MIU; Vit.D₃ 2.80 MIU; Vit.E 30.00 g; Vit.K 2.00 g; Vit.B₁ 2.00 g and Vit.B₂ 2.00 g.

**Data Collection**

The maximum and minimum in house air temperature and relative humidity were recorded daily during the 42 day experimental period (5th February to 17th March) by thermo-hygrometer and temperature humidity index (THI) was calculated. Record of weekly feed offered and weekly feed leftover from different treatment groups was maintained. The data obtained was used for the calculation of weekly feed consumption of broilers in each treatment groups during particular week. Body weight of individual chick was recorded using digital weighing balance at day one and thereafter at weekly interval till six weeks of age and weekly body weight gain was calculated. Feed conversion ratio (FCR) was calculated from weekly feed consumption and body weight gain. At the end of fifth week, three birds (one from each replicate) from each treatment group were randomly selected for metabolic trial which

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**Table 1: Composition of broiler mash (%)**

**Table 2: Nutrient analysis of broiler starter and finisher mash (% DM)**

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Table 2: Mean weekly in house air temperature, relative humidity and temperature humidity index

| Weeks | Mean in house air temperature (°C) | Avg. house air humidity (%) | Temperature humidity index |
|-------|-----------------------------------|-----------------------------|---------------------------|
|       | Minimum                           | Maximum                     |                           |
| 1     | 12.9±0.49                         | 32.12ab±0.81                | 35.50ab ±1.44             | 20.98ab ±0.36 |
| 2     | 13.92ab±0.7                        | 28.32±0.51                 | 21.24±0.32                | 19.94±0.25   |
| 3     | 15.67±1.08                        | 28.71±0.28                 | 22.17±0.61                | 20.84±0.56   |
| 4     | 11.88±0.75                        | 34.00±1.77                 | 22.25±1.0                 | 21.05±0.95   |
| 5     | 11.52±0.69                        | 36.82±0.64                 | 24.1b±0.63                | 22.26±0.59   |
| 6     | 11.87±0.51                        | 37.77±0.78                 | 24.84±0.61                | 22.48±0.51   |

Means bearing different superscripts (a,b,c) in column differ significantly (P<0.01).

Table 3: Effects of treatments on feed consumption (g) of broilers

| Weeks | T_0  | T_1  | T_2  | T_3  |
|-------|------|------|------|------|
| 1*    | 122.88±0.41 | 123.64±0.32 | 126.13b±1.02 | 123.62a±0.70 |
| 2#    | 298.33±1.35 | 302.91±4.65 | 305.31±4.08 | 300.12±1.16 |
| 3#    | 673.85±3.53 | 672.70±7.89 | 672.77±6.11 | 682.13±2.07 |
| 4#    | 908.77±11.21| 831.39±15.15| 863.30±23.23| 842.79±8.26 |
| 5#    | 1035.33±0.99| 1033.95±25.89| 1058.95±4.95| 1046.79±15.32|
| 6**   | 1114.81±2.89| 1125.75±4.61| 1138.48±8.37| 1086.39±6.04|

Mean bearing different superscripts (a,b) in row differ significantly (P<0.05)*,(P<0.01)** and # Non significant.

T_0 = Control; T_1 = basal diet supplemented with 0.5 mg Cr/kg diet; T_2 = basal diet supplemented with 0.5 mg Cr /kg diet + 250 mg of ascorbic acid /kg of diet; T_3 = basal diet supplemented with 0.5 mg Cr /kg diet + 250 mg vitamin E /kg of diet

Table 4: Effects of treatments on feed conversion ratio of broilers

| Weeks | T_0  | T_1  | T_2  | T_3  |
|-------|------|------|------|------|
| 1     | 1.89±0.03 | 1.79±0.02 | 1.84±0.01 | 1.86±0.03 |
| 2     | 1.68±0.02 | 1.66±0.029 | 1.66±0.01 | 1.66±0.03 |
| 3     | 2.17±0.05 | 2.18±0.01 | 2.11±0.05 | 2.22±0.04 |
| 4     | 2.30±0.21 | 1.92±0.01 | 2.04±0.10 | 2.02±0.01 |
| 5     | 2.13±0.04 | 1.85±0.14 | 1.81±0.02 | 1.89±0.11 |
| 6     | 2.16±0.02 | 2.13±0.02 | 2.06±0.08 | 2.15±0.17 |
| Average | 2.05±0.25 | 1.92±0.26 | 1.92±0.25 | 1.97±0.24 |

#Non significant difference was recorded for weeks 1 to 6.

T_s = Control; T_1 = basal diet supplemented with 0.5 mg Cr/kg diet; T_2 = basal diet supplemented with 0.5 mg Cr /kg diet + 250 mg of ascorbic acid /kg of diet; T_3 = basal diet supplemented with 0.5 mg Cr /kg diet + 250 mg vitamin E /kg of diet

RESULTS AND DISCUSSION

The climatic data revealed that there were huge variations in temperature within a day (Table 2). The difference between minimum and maximum temperature in a day from morning to evening was more than double during the experiment. The maximum average in house air temperature of 37.77°C was recorded during 6th week and minimum of 11.52°C in 5th week. The data of in house relative humidity was conducted for 5 consecutive days for determining the nitrogen retention collected at 24 hourly basis. During metabolic trial, daily record of feed offered, feed left over and faeces voided was maintained and used for nitrogen analysis as per AOAC (1990).

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The data generated throughout the experimental period was analyzed statistically by applying the Completely Randomized Design to study the effect of treatment on various parameters (Snedecor and Cochran, 1994). The climatic data regarding in-house air temperature and humidity was analyzed using RBD to study the weekly variations (Snedecor and Cochran,1994).
Table 5: Effects of treatments on body weight gain (g) of broilers.

| Weeks | Groups |
|-------|--------|
|       | T₀ | T₁ | T₂ | T₃ |
| 1     | 64.89±1.34 | 68.48±0.93 | 68.15±0.55 | 66.46±1.50 |
| 2     | 176.89±3.53 | 181.54±2.40 | 183.73±3.18 | 180.17±4.25 |
| 3     | 310.71±6.79 | 308.11±2.11 | 318.31±11.22 | 307.35±7.32 |
| 4     | 394.22±3.23 | 432.11±6.14 | 424.44±15.57 | 416.97±3.82 |
| 5     | 484.78±9.42 | 566.44±17.67 | 584.80±5.21 | 556.24±31.95 |
| 6     | 509.17±11.18 | 502.82±7.95 | 552.54±25.58 | 475.89±27.25 |

#Non significant difference was recorded for weeks 1 to 6.

T₀ = Control; T₁ = basal diet supplemented with 0.5 mg Cr/kg diet; T₂ = basal diet supplemented with 0.5 mg Cr/kg diet + 250 mg of ascorbic acid /kg of diet; T₃ = basal diet supplemented with 0.5 mg Cr/kg diet + 250 mg vitamin E /kg of diet

Table 6: Effects of treatments on total nitrogen intake, excretion and retention of broilers.

| Group | Nitrogen intake** (g) | Nitrogen excretion (g) | Nitrogen retention* (g) | %Nitrogen retention* |
|-------|----------------------|-----------------------|------------------------|---------------------|
| T₀    | 4.16±0.09            | 1.52±0.11             | 2.64±0.24              | 63.46±1.76         |
| T₁    | 4.91±0.12            | 1.50±0.23             | 3.4±0.23               | 69.24±1.40         |
| T₂    | 5.01±0.08            | 1.41±0.09             | 3.6±0.04               | 71.85±1.38         |
| T₃    | 4.23±0.24            | 1.35±0.20             | 2.87±0.14              | 67.84±1.51         |

Mean bearing different superscripts (a,b,c) in column different significantly (P<0.05)*, (P<0.01)** &# Non significant T₀ = Control; T₁ = basal diet supplemented with 0.5 mg Cr/kg diet; T₂ = basal diet supplemented with 0.5 mg Cr/kg diet + 250 mg of ascorbic acid /kg of diet; T₃ = basal diet supplemented with 0.5 mg Cr/kg diet + 250 mg vitamin E /kg of diet.

depicts gradual increase from 35.50% at first week to 44.21% at 3rd week thereafter gradual reduction in relative humidity was recorded and it was 29.01% at 6th week and THI ranged from 19.94 (2nd week) to 22.48 (6th week). As THI exceeds 20.4°C, broilers show reduced performance and increased variability in performance metrics (Purswell et al., 2012). These variations in the environment are indicator of imposing environmental stress on birds. Environmental temperature varies in many regions of the world during winter months and such conditions cause adverse effects on intake, nutrient digestibility and feed efficiency in poultry (Sahin, 2001). Environmental stress causes deficiency of chromium and vitamin C due to increased chromium mobilization from tissues and its excretion, also depresses ascorbic acid synthesis for poultry (NRC, 1997), thus justifying the use of chromium and antioxidants.

Feed consumption in general did not exhibited much significant difference among all treatment groups with exception of 1st and 6th week wherein T₂ group supplemented with chromium and ascorbic acid reflected significantly (P<0.05) higher feed consumption in 1st week (Table 3). In 6th week T₂ (chromium + vitamin E) supplemented groups recorded the lowest (P<0.01) feed intake due which the values became statistically significant but rest groups had no significant difference between them. The results are in agreement with Anandhi et al. (2006); Sukosombat and Kanchanatawee (2005); Noori et al. (2012) except in the 1st week that exhibit a significant increase in T₂ group, and in 6th week that exhibit a significant decreased in T₃ group for feed consumption.

FCR among the treatments were recorded as 2.05, 1.92, 1.92 and 1.97 for T₀, T₁, T₂ and T₃ groups respectively with non-significant difference (Table 4). However supplemented groups had lower FCR than the control group and T₁ and T₂ recorded same FCR. Toghyani et al. (2006); Chaudhari et al. (2006); Anandhi et al. (2006) and Sukosombat and Kanchanatawee (2005) however, observed the non-significant effect on weekly feed conversion ratio in the chromium supplemented broilers and their observations are in tune with present findings. The findings of present experiment were found contrary to Samanta et al. (2008) who observed the significantly improved feed conversion ratio in broilers at 2nd, 3rd and 5th week, however, Kaoud (2010) observed increased feed conversion ratio only at 3rd week in broilers.

The mean weekly live body weights at 6th week were 1981.85, 2100.50, 2160.53 and 2044.97 g in T₀, T₁, T₂ and T₃, respectively (Table 5). An extra weight of 178.68 g/bird was recorded in group T₂ followed by 118.65 g/bird in T₁ and 63.12g/bird in T₃ as compared to control group in 6th week. However, all the supplemented groups recordd numerically higher weights than control but difference between groups was statistically not significant. There were no significant differences in mean weekly gain in body weight. However numerically the average weight gain of
all the supplemented groups was higher than control and chromium and ascorbic acid combination recorded maximum gain than other groups. Similar findings of non-significant gain in body weight in broilers have been reported by supplementation of organic chromium (Liarn, 1993; Anandhi et al., 2006).

The results of metabolic trial indicated average nitrogen retention values of 63.46, 69.24, 71.85 and 67.84% in T₀, T₁, T₂ and T₃ group, respectively (Table 6). The observation revealed significantly higher nitrogen retention in all supplemented groups than control group. The higher nitrogen retention was recorded in T₂ group followed by T₁, T₃ and T₀ (control) group. The results are suggestive of the fact that the dietary chromium yeast supplemented groups exhibited superior nitrogen retention than control group and it was maximum (71.85%) in chromium yeast plus ascorbic acid supplemented (T₂) group. Amatya et al. (2004) also reported significantly (P<0.05) increased crude protein metabolizability in chromium supplementation in broilers from chromium yeast in comparison to control. These findings are in agreement with observations in the present study.

CONCLUSION

Results indicated that supplementation of chromium alone or in combination with ascorbic acid/vitamin E have little influences on feed consumption, body weight gain and feed conversion ratio, however increased the nitrogen retention in broilers. According to numerical values the best performer group was chromium yeast plus ascorbic acid suggesting some synergistic action between them and must be evaluated further.

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CONFLICT OF INTEREST

The authors have no conflict of interest with each other or any organization.

AUTHORS CONTRIBUTION

This manuscript is the part of MVSc thesis work of the first author.

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