Growth rate, feed intake and antioxidant enzyme activity in Sahiwal calves supplemented with chromium propionate during winter season

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Abstract In order to observe the effect of chromium propionate supplementation on growth rate, feed intake and antioxidants activity during winter season, twelve Sahiwal calves were selected and further divided equally i.e. control and treatment group. Both groups were fed normal diet except treatment which was fed chromium propionate @0.5mg/kg dry matter intake/day additionally. Blood samples was collected on day 0, 15, 30, 45 and 60 and analysed for catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR). As a result of this study, Body weight gain was significantly (P<0.05) higher in treatment than control group. Correspondingly, dry matter intake was higher in treatment group (P>0.05). The Superoxide dismutase activity was significantly (P<0.05) higher in control group. In the present study, chromium supplementation increases the antioxidants activity and reduces oxidative stress in Sahiwal cows. Thus, Supplementation of chromium propionate could be used as one of the major thermal stress ameliorative measure.

Keywords: Antioxidant enzymes, body weight, chromium propionate, feed intake, Sahiwal calves

Introduction

Successful livestock production requires applying strategies that optimizes the use of the environment and available nutrient sources in order to capitalize on the livestock's production potential. Cold exposure is one of the stresses that results in a variety of negative effects on productivity of livestock through modified digestive, metabolic, and endocrine functions (Anderson, 1987; Amatya, 2004). Oxygen which is essential for all aerobic organisms has been termed the "oxygen paradox". Excessive production of free radicals and concomitant damage at cellular and tissue levels are controlled by cellular antioxidants defense systems. Antioxidants prevent or remove oxidative damage to target molecules (Halliwell and Gutteridge, 2007). The preventive body antioxidative defense systems can be accomplished by enzymatic (SOD, GSHPx and Catalase) and non-enzymatic mechanisms (Vitamin E and Selenium). Excessive production of free radicals and ROS, and/or a decrease in body antioxidant defense, lead to damage of biological macromolecules and disruption of normal metabolism and physiology (Trevisan et al. 2001). When ROS are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress results. Therefore, an imbalance between increased production of ROS and reduced availability of antioxidant defenses near the time of parturition increases oxidative stress and may contribute to periparturient disorders in dairy cows (Waller, 2000; Gitto et al. 2002). Unfortunately, oxidative stress as it is not a classical disease, does not exhibit a specific clinical picture. The determination of products of peroxidative damage to macromolecules, and antioxidant substances like glutathione and enzymes (SOD, GSHPx and Catalase) are useful markers for the oxidative stress and antioxidant status respectively.

Chromium (Cr) is well established as an essential trace element for man and animals (NRC, 1997) especially during stress conditions such as thermal, physical, biological etc. Cr supplementation protects against stress-induced losses of several trace elements (Schrauzer et al. 1986). The organic form of Cr is utilized more effectively than the inorganic form (Page et al. 1993). However, as per our knowledge, literature on the oxidative stress and antioxidant status during winter season though replete but in Indian perspective there are meager reports has been figured out so far. No systematic information is available in the literature regarding the ameliorative effect of Cr during cold stress in growing Sahiwal calves. Therefore, the present investigation was planned to
explore the effect of Cr supplementation on growth performance, feed intake and antioxidant status in Sahiwal calves.

**Materials and Methods**

**Experiment Design**

The experiment was conducted in the Livestock Research Centre of National Dairy Research Institute (NDRI), Karnal, Haryana. It is situated on an altitude of 250 metres above mean sea level, latitude and longitude position being 29° 42"N and 79° 54"E respectively. Minimum ambient temperature is recorded near 0°C in winter, and maximum temperature goes up to 45°C in summer with annual rainfall of 700 mm. For the present study, twelve Sahiwal female calves were selected from the herd of NDRI, Karnal. These animals were further divided equally into 2 groups (6 calves each) i.e. control and experimental (Initial body weight of control and treatment group were 87.10 ± 5.50 and 87.18 ± 8.50 kg). Experiment was approved by the institutional animal ethics committee (IAEC) constituted as per the article no.13 of the CPCSEA rules, laid down by Govt. of India.

**Feeding and management of animals**

The experimental animals were kept in separate pens throughout the study. Deworming of all the animals were done before the start of the experiment. Animals were let loose every week for exercise.

All the female Sahiwal calves were fed as per NRC (2001) requirement. The concentrate mixture (Table 1) was offered in the morning (7:00 am) whereas, the chaffed green fodder was offered at 11:00 am. Throughout the experiment, depending upon the availability of green fodder (green maize, jowar, berseem etc.) was supplied to the experimental animals.

The experiments on both groups of animals were conducted for 45 days during winter season. Experimental animals were supplemented @ 0.5mg chromium propionate/kg of dry matter intake over and above the feeding of control group. Chromium propionate was supplemented to experimental animals by dissolving in distilled water and directly putting in their mouth. The feeds offered to the animals and residue left were recorded fortnightly interval to find out the total dry matter intake (DMI) of the animals. Fortnightly body weight of experimental animals was also recorded to observe changes in growth rate due to dietary treatment.

**Blood collection**

Peripheral blood samples were collected at 0700 hours in heparinized vacutainer tubes (Becton Drive, Franklin Lakes, NJ, USA) by jugular vein puncture, posing minimum stress to Sahiwal calves, on days 0, 15, 30, 45 and 60 in winter and summer season. The samples were brought to the laboratory in chilled iceboxes soon after collection and centrifuged at 1200 g at 4 °C for 20 min to separate the plasma for the analysis of catalyse (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) (Wheeler et al., 1990).

**Laboratory analysis**

Feed intake after weighing feed refusals was recorded and dry matter (DM) intake was calculated daily. Feed and fodder offered to animals were analysed for DM, crude protein (CP), ether extract (EE), crude fibre (CF) and total ash (AOAC, 1990). Detergent method was used for the estimation of neutral detergent fibre (NDF) and acid detergent fibre (ADF) in feed and fodder offered to calves during experimental period (Van Soest et al. 1991).

Catalase was determined in plasma by using Catalase Assay kit (Cayman Chemical Company, USA; Catalog No.707002) according to manufacturer's protocol (Johansson and Borg, 1988). The range of the assay was 2-35 nmol/min/ml. The intra and the inter assay coefficient of variation assay coefficient of variation were 3.8% and 9.9%, respectively.

Superoxide Dismutase was determined in plasma by using Superoxide Dismutase Assay kit (Cayman Chemical Company, USA; Catalog No.706002) according to manufacturer's protocol (Marklund, 1980). The range of the kit was 0.025-0.25 units/ml SOD. The intra and the inter assay coefficient of variation assay coefficient of variation were 3.2% and 3.7%, respectively.

Glutathione Peroxidase (GPx) was determined in plasma by using Glutathione Peroxidase Assay kit (Cayman Chemical Company, USA; Catalog No.703102) according to manufacturer's protocol (Ursine et al., 1985). The range of the kit was 50-344 nmol/min/ml GPx. The intra and the inter assay coefficient of variation assay coefficient of variation were 5.7% and 7.2%, respectively.

Glutathione Reductase (GR) was determined in plasma by using Glutathione Reductase Assay kit (Cayman Chemical Company, USA; Catalog No.703202) according to manufacturer's protocol (Inoue et al., 1987). The range of the kit was 20-255 nmol/min/ml GR. The intra and the inter assay coefficient of variation assay coefficient of variation were 3.7% and 9.3%, respectively.

**Statistical analysis**

Data of present study were normally distributed as checked by Shapiro-Wilk test in SAS system. Data were analyzed by...
Results and Discussion

The average daily weight gain (ADG)

The fortnightly average body weight of calves is presented in Table 2. Body weight ranged from 65.6 kg to 100.1 kg in the control group and 64.1 to 109.3 kg in treatment group. On an average, the body weight at the end of four fortnights was 108.28±5.87 and 113.48±11.20 kg in control and treatment group, respectively. But average daily weight gain (ADG) in control group was 350 g in control group while in treatment group it was 440 g/day in winter season. Body weight gain was found to be significantly (P<0.05) higher in treatment group compared to control group (Figure 1). Chromium supplementation increased more body weight of experimental calves at 1st, 2nd, 3rd and 4th fortnights by 2.46%, 2.59%, 2.94% and 4.80% respectively when compared to control group (fed basal diet without Chromium propionate). Body weight gain are in agreement with those obtained by Barajas et al. (2005) who noticed that Chromium methionine supplementation tended (p = 0.06) to increase 2.5% ending calves weight (251.38 vs. 257.75 kg), Barajas et al. (2008) further reported that chromium supplementation increased (p<0.01) ending bulls weight (466 vs. 500 kg) and average daily gain (1.28 vs. 1.47 kg/d). The increase of body gain in these calves could be attributed to the increase in Dry Matter Intake (DMI). This expatiation agrees with those obtained by Kraidees et al. (2009). However, the results of the present study are not in general agreement to other workers in lambs (DePew et al. 1996; Forbes et al. 1998) and calves (Kegley et al. 1997; 2000; Swanson et al. 2000), who did not find any appreciable higher body weight gain in Cr supplemental group compared to control group

Superoxide Dismutase (SOD)

The mean± SE of plasma SOD activity of control group was 25.70 ± 0.44 μM at the starts of experiment and it increased to 27.02 ± 0.27 μM (Table 2). Whereas, in treatment group the

Table 1 Feed ingredients and chemical composition used in the experimental ration

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Concentrate</th>
<th>Wheat straw</th>
<th>Berseem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>28</td>
<td>91.10±0.12</td>
<td>12.10±0.18</td>
</tr>
<tr>
<td>Ground nut cake</td>
<td>10</td>
<td>91.80±0.50</td>
<td>91.25±0.48</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>15</td>
<td>3.07±0.10</td>
<td>17.08±0.12</td>
</tr>
<tr>
<td>Mustard cake</td>
<td>13</td>
<td>0.76±0.03</td>
<td>3.01±0.05</td>
</tr>
<tr>
<td>Rice polish (deoiled)</td>
<td>11</td>
<td>79.19±0.04</td>
<td>41.10±0.08</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>13</td>
<td>54.16±0.45</td>
<td>21.23±0.40</td>
</tr>
<tr>
<td>Bajra</td>
<td>5</td>
<td>46.88±0.26</td>
<td>57.70±0.24</td>
</tr>
<tr>
<td>Salt</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* By calculation
SOD activity was 25.72 ± 0.94 μM the starts of experiment and it decreased to 25.48±0.89 μM. The overall mean (± SEM) of plasma SOD activity was found significantly (P< 0.05) lower in treatment group as compared to control group (Figure 4).

The role of intracellular SOD is to scavenge the superoxide (oO-2) that is produced by a number of reaction mechanisms, including several enzyme systems, as a part of normal cellular functions (Valentine et al., 2005).

The oxidation or autooxidation of hemoglobin (Hb-Fe2+) into the erythrocytes results in the continuous formation of oO-2 (Hebbel and Easton, 1989). There are three distinct types of SOD classified on the basis of the metal cofactor: 1) Copper/zinc (Cu/Zn - SOD), 2) Manganese (Mn-SOD) and 3) Iron (Fe-SOD) isozymes (Bannister et al. 1987). In this study, higher SOD activity found in control group was probably a response to higher oO-2 generation. This may indicate that control group was more stressed as compare to treatment group. An increase in superoxide dismutase activity (P<0.001) and in serum malondialdehyde concentration (P<0.001) were observed in the mild temperature humidity index (MTHI) and high temperature humidity index (HTHI).

**Table 2** Effect of chromium propionate supplementation on DMI (kg/day), Body wt. (kg), Average body weight gain (kg), CAT (nmol/min/ml), SOD (μM), GPx (nmol/min/ml) and GR activities (nmol/min/ml) in control and treatment group of Sahiwal calves in Winter Season

<table>
<thead>
<tr>
<th>Fortnight</th>
<th>Dry Matter Intake (kg/day)</th>
<th>Body weight (kg)</th>
<th>Average body weight gain</th>
<th>Catalase activity (nmol/min/ml)</th>
<th>SOD activity (μM)</th>
<th>Glutathione peroxidase (nmol/min/ml)</th>
<th>Glutathione reductase (nmol/min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td>0</td>
<td>3.06 ±0.14</td>
<td>3.09 ±0.19</td>
<td>87.10 ±5.50</td>
<td>87.18 ±8.50</td>
<td>7.91 ±0.44</td>
<td>25.70 ±0.60</td>
<td>22.1 ±0.70</td>
</tr>
<tr>
<td>1</td>
<td>3.07 ±0.11</td>
<td>3.15 ±0.18</td>
<td>94.70 ±5.55</td>
<td>97.03 ±8.50</td>
<td>12.76 ±0.44</td>
<td>25.95 ±0.94</td>
<td>24.20 ±0.70</td>
</tr>
<tr>
<td>2</td>
<td>3.14 ±0.11</td>
<td>3.39 ±0.17</td>
<td>99.36 ±5.76</td>
<td>101.94 ±8.75</td>
<td>15.22 ±0.70</td>
<td>25.01 ±1.36</td>
<td>24.4 ±1.95</td>
</tr>
<tr>
<td>3</td>
<td>3.20 ±0.13</td>
<td>3.52 ±0.20</td>
<td>104.69 ±5.87</td>
<td>107.77 ±10.42</td>
<td>15.54 ±1.49</td>
<td>26.71 ±2.38</td>
<td>26.22 ±1.95</td>
</tr>
<tr>
<td>Overall</td>
<td>3.22 ±0.13</td>
<td>3.37 ±0.20</td>
<td>98.82 ±5.73</td>
<td>101.48 ±8.42</td>
<td>13.33 ±0.84</td>
<td>26.08 ±1.36</td>
<td>25.21 ±0.55</td>
</tr>
<tr>
<td>Mean</td>
<td>±0.06 ±0.08</td>
<td>±2.74 ±0.43</td>
<td>±4.37 ±0.01</td>
<td>±0.02 ±0.02</td>
<td>±0.84 ±0.02</td>
<td>±0.29 ±0.03</td>
<td>±0.35 ±0.02</td>
</tr>
</tbody>
</table>

Table Data: The values are the MEAN±SE of 6 observations on six animals. The different superscript in row indicate the significant difference (P<0.05)

**Figure 1.** Average body weight (kg) of control and treatment group of Sahiwal calves during winter Season

**Figure 2.** Dry Matter Intake (kg/Day) of control and treatment group of Sahiwal calves during winter Season

SOD activity was 25.72 ± 0.94 μM the starts of experiment and it decreased to 25.48±0.89 μM. The overall mean (± SEM) of plasma SOD activity was found significantly (P< 0.05) lower in treatment group as compared to control group (Figure 4).
periods compared with the low temperature humidity index (LTHI). Cows supplemented with Cr had lower (P = 0.009) serum concentrations of cholesterol but greater (P < 0.001, respectively) serum levels of heat shock protein (Hsp72) and IL-10 compared with those without Cr supplementation in the HTHI period. (Zhang et al. 2014). As such direct relationship of organic Chromium propionate with SOD activity has not been reported so far. The exact mechanism by which that action is brought about is also not yet known. As there is less SOD activity in treatment group compares to control, we may conclude that Cr supplementation has some role to play in stress condition.

Catalase (CAT)

The mean± SE of plasma CAT activity of control group was 7.91±1.44 nmol/min/ml at the starts of experiment and it increased to 15.24±1.58 nmol/min/ml. Whereas, in treatment group the CAT activity was 8.83±0.49 nmol/min/ml the starts of experiment and it increased to 14.29±2.05 nmol/min/ml (Table 2). The overall mean (± SEM) of plasma CAT activity did not differ significantly (P< 0.05) among both groups (Figure 3).

Catalase is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen. The enzyme is found in all aerobic eukaryotes and is important in the removal of hydrogen peroxide generated in peroxisomes (microbodies) by oxidases involved in β-oxidation of fatty acids and purine catabolism. In peroxisomes catalase takes care of the cytocylic and mitochondrial peroxides formed during urate oxidation (Alberts et al., 2002). Since SOD activity increases H₂O₂ production, protection from reactive oxygen would only be conferred by a coordinated increase of catalase and glutathione peroxidase activities thus a positive and significant correlation exist between catalase activity and SOD activity (Clemens and Waller, 1987; Frei, 1994; Kehrer and Smith, 1994; Sharma et al. 2011; Aggarwal et al. 2012). In support of this conjecture, catalase activity was found to be increased in control group in present study and also lower catalase activity in treatment as compared to control group indicating less oxidative stress in treatment as compared to control group. Organic Chromium propionate interaction with CAT activity has not been reported so far. The exact mechanism by which that action is brought about is also not yet known.

Glutathione peroxides (GPx)

The mean± SE of plasma GPx activity of control group was 2.21±0.60 nmol/min/ml at the starts of experiment and it increased to 3.38±0.70 nmol/min/ml (Table 2). Whereas, in treatment group the GPx activity was 2.25±0.70 nmol/min/ml the starts of experiment and it increased to 3.15±0.55 nmol/min/ml. The overall mean (± SEM) of plasma GPx activity did not differ significantly (P> 0.05) among both groups (Figure 5).

Glutathione peroxidase is selenium dependent enzyme and it has also antioxidant property. It converts hydrogen peroxide to water. Plasma glutathione peroxidase is considered as an indicator of oxidative stress (Tüzün et al. 2002; Sharma et al. 2011). In present study GPx-P activity was found to be increased in control group indicating more oxidative stress during severe winter. Since, less enzymatic activity was found in treatment group , this may be due to less cold stress on these animals compared to control one. Increases in Glutathione peroxidise activity have been noted in dairy cattle during times of physiological and metabolic stress and it was suggested to be a cytoprotective response resulting from altered oxidative status (Bernabucci et al. 2005).

Glutathione reductase (GR)

The mean± SE of plasma GR activity of control group was 34.51±7.39 nmol/min/ml at the starts of experiment and it increased to 75.07±2.34 nmol/min/ml (Table 2). Whereas, in treatment group the GR activity was 38.52±1.36 nmol/min/ml the starts of experiment and it increased to 70.18 ± 4.19 nmol/ml.
min/ml. The overall mean (± SEM) of plasma GR activity did not differ significantly (P>0.05) among both groups (Figure 6).

Glutathione reductase (EC 1.8.1.7) catalyzes the reduction of glutathione disulfide (GSSG) to the sulphydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell (Mannervik, 1987; Meister, 1988; Deponte, 2013). There is no such reported evidence of relationship between Cr propionate and GR activity. There is a need to continue to explore the relationship between Cr propionate supplementation and GR activity. By decrease level of GR activity in treatment group as compare to control one. We may suggest that Cr supplementation played role in alleviation of stress in winter.

Conclusions

In conclusion, based on the results of the present study, it can be stated that the supplementation of dietary chromium helped in improving the DMI, body weight gain and reduction in oxidative stress of Sahiwal calves during winter season. Therefore, the supplementation of Chromium propionate could be used as one of the major ameliorative agent to cope up with the adverse effect of lower ambient temperature during cold stress for sustaining animal productivity.

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