



Amelioration of adverse effects of heat stress using custom design shelter system and supplementing chromium propionate to growing Karan Fries calves during summer

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ABSTRACT

Climate change has become a threat to the sustainability of livestock systems throughout the world. Present study was carried out to evaluate the ameliorative effect of custom design shelter system and chromium propionate supplementation in crossbred calves, for which 15 growing Karan Fries crossbred calves were further divided equally into three groups based on their body weight and age. Groups 1, 2 and 3 animals were kept in an open shed (control), under custom designed shed and under custom designed shed supplemented with chromium propionate @ 0.5 mg/kg DMI through chromium propionate, respectively. These animals were fed on the fodder and concentrate (60:40) *ad lib*. All calves were kept under similar feeding and management schedule for 8 days under preliminary period to adapt on the diet followed by 90 days experimental period. Blood samples were collected on day 0, 45 and 90 for the analysis of biochemical parameters (cortisol, catalase, IgA and IgM) and expression of growth related gene (NFIL3). Results showed that the custom designed shed reduced the T_{max} (32.94°C) by 3.24°C compared to the open shed (36.18°C) and improved the dry matter intake (DMI), average daily gain (ADG) and decreased water intake of growing calves of group 3 and 2 over group 1. Results further indicated lower stress (cortisol, catalase) levels and better immunity (IgA and IgM) in group 3 compared to group 2 and 1. The gene related to growth (NFIL3) showed higher expression in group 3 compared to the group 2 and 1. The improvement in body weight gain with better immunity and lower stress level in group 3 and 2 indicated beneficial effect of custom designed shelter system and chromium propionate supplementation to Karan Fries growing calves. Therefore, micro environment modification by shed along with chromium supplementation can be suggested as an important management practices during heat stress for crossbred calves.

Key words: Chromium propionate, Custom designed shed, Gene expression, Heat stress, Immunity

Livestock production system is largely affected by climate change resulting in an imbalance between metabolic heat production inside the animal body and its dissipation to the surroundings results to heat stress. Livestock productivity requires applying strategies that optimizes the use of the environment and available nutrient sources in order to capitalize on the livestock's production potential. Heat exposure is one of the stress that results in a variety of negative effects on productivity of livestock through modified digestive, metabolic, and endocrine functions (Amatya *et al.* 2004). Chromium (Cr) occurs widely in nature, it is a transition element, which occurs in a number

of oxidation states. Burton (1995a) has found that the most stable form is the trivalent state, which is the one involved in glucose tolerance factor (GTF). Chromium is established as an essential trace element for man and animals, especially during stress conditions such as thermal, physical, biological etc (NRC, 1997). Cr supplementation protects against stress-induced losses of several trace elements (Schrauzer *et al.* 1986). Interest in adding Cr to livestock feeds has evolved in the last 2 years, in part due to information from human nutrition and in part due to results of studies where organic Cr complexes added to animal diets have resulted in beneficial effects on growth, reproduction, and immune response (Burton *et al.* 1996). The focal point is the function of Cr in glucose utilization and its consequent impact on utilization of dietary sugars particularly in rapidly growing animals or in those under physiological stress whether due to disease challenge or the demands of high production or reproduction. Several studies in cattle have demonstrated a favourable response to chromium supplementation,

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especially if the animals are under some physiologic stress (Al-Saiadya *et al.* 2004). These positive studies examined the effects of supplementation of chromium during the periparturient period in dairy cattle. The positive findings included improved humoral and possibly cell mediated immune response, improved energy status (reduced liver triglyceride accumulation), DMI etc (Burton *et al.* 1996). However, as per our knowledge, literature on the oxidative stress and antioxidant status during summer season is available but in Indian perspective meagre reports have appeared so far. No systematic information is available in the literature regarding the ameliorative effect of Cr during heat stress in growing Karan Fries calves. Therefore, the present investigation was planned to explore the mitigation of heat stress using custom design shed and supplementation of chromium to growing Karan Fries calves.

MATERIALS AND METHODS

Experimental design: The experiment was conducted at the Climate Resilient Livestock Research Centre (CRLRC), at NDRI, Karnal, Haryana. It is situated on the latitude and longitude position being 29°42"N and 79°54"E respectively. Minimum temperature is recorded near 0°C in winter and maximum temperature goes up to 44°C in summer season with annual rainfall of 750 mm. In the present study, 15 Karan Fries female calves (1–2 years) were selected from the herd of NDRI, Karnal, and divided into 3 groups (5 calves each) i.e. group 1 (control), group 2 (shed) and group 3 (shed with chromium propionate).

Feeding and management of animals: The group 2 and 3 animals were kept in custom designed animal shed and group 1 animals were kept under open sheds. All experimental calves were fed as per NRC (2001). The concentrate mixture and chaffed green fodder (berseem/oats and maize) was offered at 10.00 AM. The experiment was conducted for 90 days during hot dry (April to June) season. Group 3 animals were supplemented with 0.5 mg chromium/kg DM intake through chromium propionate over and above the feeding of group 1 and group 2. The feeds offered to the animals and residue left were recorded at weekly interval for consecutive two days to find out the total DMI of the animals. Fortnightly body weights of experimental animals were also recorded to observe changes in average daily weight gain due to dietary treatment.

Physiological response: The physiological responses i.e. respiration rate, pulse rate, rectal temperature and skin temperature were recorded by flank movement, palpation of coccygeal artery, using digital thermometer and infrared thermometer respectively throughout the study.

Blood collection: Blood samples were collected on days

0, 45 and 90 before feeding in morning using vacutainers (Becton Drive, Franklin Lakes, NJ, USA) from jugular vein posing minimum stress to experimental animals during summer. The blood samples were brought to the laboratory immediately after collection and centrifuged at 3,000 rpm for 30 min to separate the plasma for the analysis of biochemical parameter (Catalase, Cortisol, IgA and IgM) and PBMC was separated for RNA isolation and cDNA preparation for gene expression study.

Chemical analysis of feeds and fodders: Feed offered and feed residue were recorded to calculate feed DM intake presented. Feed and fodder offered to animals were analysed for DM, crude protein (CP), ether extract (EE), and total ash (AOAC 1990). Detergent method was used for the estimation of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) (Van Soest *et al.* 1991) in feed and fodder offered to calves during experimental period. The data on chemical composition of feed and fodders are shown in Table 2.

Biochemical estimation: According to manufacturer's protocol, Catalase, Cortisol, IgA and IgM was determined in plasma using MyBioSource ELISA kit (MyBioSource, Inc., USA) with Catalog No. MBS701325, MBS039175, MBS564143 and MBS564147, respectively.

RNA extraction and cDNA synthesis: Total RNA was isolated from the blood drawn from animals of 3 groups using RNeasy Mini Kit (Qiagen India Pvt. Ltd.) according to the manufacturer's protocol. Possible genomic DNA contamination in RNA preparation was removed by using the RNase-Free DNase treatment (Qiagen India Pvt. Ltd). Purity of the RNA was checked in a Bio spec nanodrop (Shimadzu, Japan) with the ratio between OD at 260 nm to 280 nm above 2. For each sample, about 200 ng/µl of total RNA was used for cDNA synthesis using Revert Aid First strand cDNA synthesis kit (Fermentas, USA) by reverse transcription (RT) PCR according to the manufacturer's protocol. The RT reaction was carried out at 65°C for 5 min, 42°C for 60 min, 70°C for 5 min in a thermal cycler (Bio-Rad, USA).

Quantitative PCR (qRT-PCR): Primers for the present study were designed using primer 3.0 software (Table 1). The qRT-PCR reaction was carried out using Applied Biosystems 7500 Real-Time PCR system. Each reaction was performed in a final volume of 15 µl containing 1 µl diluted cDNA as template, 0.5 µl 200 nM of each specific primer, 7.5 µl of 2 × SYBR Green Master mix (Thermo Scientific, USA) and 5.5 µl DNase-/RNase-free water. The first segment of the amplification cycle comprised of a denaturation programme of 5 min at 95°C, followed by the second segment that comprised three-step programme cycle:

Table 1. Primer sequence forward and reverse, temperature and product size of gene

Gene name	Primer sequence (F)	Primer sequence (R)	Annealing temperature (°C)	Product size (bp)
NFIL3	CAACGTGGACAAGATGATGG	ACCTCTTGGCAGCTTCGTTA	58	126

Table 2. Chemical composition of feed and fodders used in the experimental ration

	Concentrate	Fodders		
		Oat	Berseem	Maize
DM (%)	89.27±0.87	19.28±0.36	14.27±0.22	19.14±0.29
OM (%)	92.32±0.92	88.56±0.96	87.44±0.91	89.82±0.90
CP (%)	21.65±0.43	12.19±0.14	18.42±0.32	9.33±0.09
EE (%)	4.67±0.09	3.14±0.05	2.89±0.03	1.84±0.03
NDF (%)	36.28±0.26	52.87±0.51	45.62±0.61	55.64±0.72
ADF (%)	17.49±0.17	30.14±0.42	28.81±0.33	36.85±0.42
Hemicellulose (%)	18.79±0.19	22.73±0.19	16.81±0.28	18.79±0.23
ADL (%)	4.87±0.08	4.52±0.04	4.27±0.04	6.91±0.11
Ash (%)	7.68±0.11	11.44±0.10	12.56±0.14	10.18±0.18

primer annealing, extension and quantification with 40 cycles of 95°C for 18 sec, 60°C for 20 sec and 72°C for 20 sec. A melting curve was produced after completion of the PCR programme to assess the specificity of the amplified PCR product.

Statistical analysis: The data on biochemical parameters and gene expression were analyzed using the GraphPad Prism (version 5.01). Experimental results were presented as mean±SEM (standard error mean). The data on dry matter intake, average daily gain, water intake and physiological parameters were analyzed statistically using one way ANOVA technique as per Snedecor and Cochran (1994) and means were separated for significance by using Tukey Kramer range tests.

RESULTS AND DISCUSSION

Dry matter intake, average daily gain and water intake: The initial body weight of experimental animals ranged from 91.8 to 228.1 kg, with an overall average body weight of 164.56±10.79 kg/group. The average daily gain (g/day) was higher in group 3 (731) followed by group 2 (639) and 1 (590). DM intake was 4.91, 5.40 and 5.58 kg/day in group 1, 2 and 3, respectively. Average daily gain of chromium supplemented group during present study were in agreement with Barajas *et al.* (2005), who also reported the higher body weight in calves supplemented with chromium. The increase of body weight gain in these calves could be attributed to the increase in dry matter intake (Kraidees *et al.* 2009). Soltan (2009) also reported that chromium supplemented diet had lower body weight loss and improved dry matter intake with consequent reduction in the energy balance deficits during the first period after calving when compared with the control group. In the present investigation, the water intake was lower in group 3

Table 3. Body weight, dry matter intake, Water intake and Average daily gain in Karan Fries calves during hot dry season

Parameters	Group 1	Group 2	Group 3	P-value
Initial body weight (kg)	164.86±19.07	165.50±19.19	163.32±22.13	0.996
Final body weight (kg)	205.49±39.16	222.97±20.07	229.09±23.50	0.836
Average daily gain (g/d)	590±0.14	639±0.09	731±0.21	0.786
Dry matter intake (kg/d)	4.91±0.35	5.40±0.33	5.58±0.35	0.254
Water intake (l/d)	13.26±0.32	12.10±0.49	11.57±0.44	0.058

followed by group 2 and 1 but the results were nonsignificant. Water intake decreased because less heat stress was occurring in the group 3 due to Cr supplementation, probably due to decreased stress level (catalase and cortisol), increased immunity level (IgA and IgM) and comparatively low temperature in custom designed shed compared to open shed. Results of water intake during present study were in agreement with previous reports which indicated that the animals housed in shed show change in feed and water intake (Sharma *et al.* 2016, Beatty *et al.* 2006). Studies also reported the increase in feed intake after supplementation of chromium during lactation in Holstein cattle (Al-Saiady *et al.* 2004).

Physiological parameters: Tmax (32.94°C) was lowered by 3.24°C in custom designed shed than open shed (36.18°C). The rectal temperature (°F), heart rate (number) and surface body temperature (°C) at dorsal, ventral and ear were significantly (P<0.05) lower in group 3 as compared to groups 2 and 1. The respiration rate was also lower numerically in group 3 as compared to other two groups (Table 4). The findings of present study on physiological variables are consistent with previous finding

Table 4. Rectal temperature, respiration rate, heart rate and temperature of dorsal, ventral and ear in Karan Fries calves during hot dry season.

Parameters	Group I	Group II	Group III	P-value
Rectal temperature (°F)	103.24±0.11 ^b	101.77±0.04 ^a	101.68±0.03 ^a	0.000
Respiration rate per minute	46.01±1.67	46.62±2.12	45.41±2.06	0.909
Heart rate per minute	83.21±1.33 ^b	77.73±1.98 ^a	76.47±1.96 ^a	0.026
Dorsal temperature (°C)	39.12±0.22 ^b	36.41±0.38 ^a	36.26±0.38 ^a	0.000
Ventral temperature (°C)	38.02±0.27 ^b	36.21±0.31 ^a	36.01±0.28 ^a	0.000
Ear temperature (°C)	37.40±0.21 ^b	35.64±0.48 ^a	35.45±0.47 ^a	0.002

Means bearing different superscripts in the row differ significantly (P<0.05).

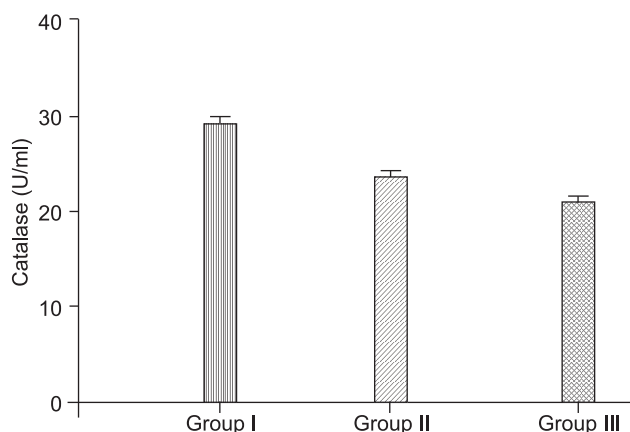


Fig. 1. Catalase level in different groups of Karan Fries calves during hot dry season.

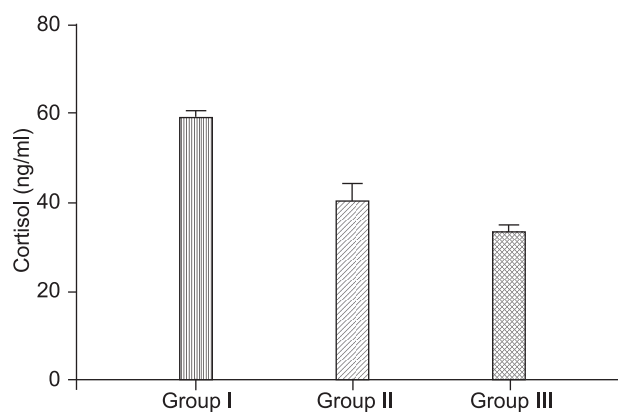


Fig. 2. Cortisol level in different groups of Karan Fries calves during hot dry season.

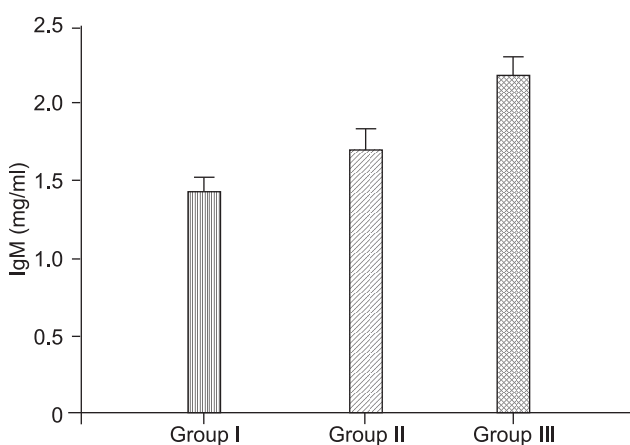


Fig. 3. IgM in level in different groups of Karan Fries calves during hot dry season.

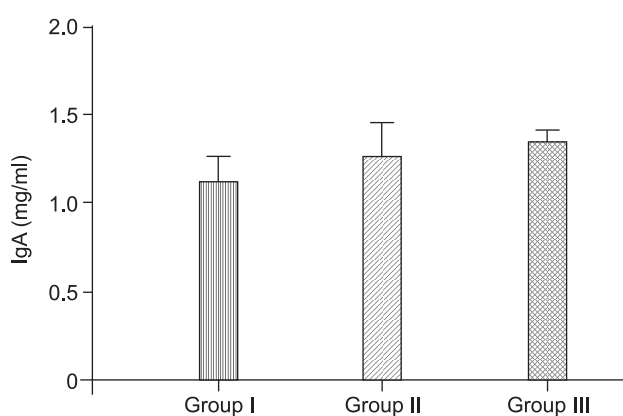


Fig. 4. IgA level in different groups of Karan Fries calves during hot dry season.

which indicated the no effect of Cr supplemented to transported stresses calves on physiological parameters (AnQiang *et al.* 2009).

Stress biomarkers: The levels of stress biomarkers (catalase and cortisol) were lower ($P < 0.05$) in group 3 as compared to group 2 and 1 (Figs 1, 2), indicating beneficial effect of Cr supplementation, to sensitive Karan Fries breed during heat stress. Low plasma cortisol levels in group 3 observed during the present study was within the normal physiological range. Results were in agreement with previous reports which indicated lower level of serum cortisol after Cr supplementation in periparturient buffaloes (Kumar *et al.* 2013 and Deka 2013). Kafilzadeh *et al.* (2012) also reported that Cr improved some aspects of the immune response by reducing cortisol secretion and increasing neutrophil count. Catalase level was also significantly ($P < 0.05$) lower in groups 3 and 2 compared to group 1. Catalase is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen. The enzyme 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

cytoytic and mitochondrial peroxides formed during urate oxidation (Alberts *et al.* 2002).

Immunity status: The level of immunity (IgA and IgM) was significantly ($P < 0.05$) improved in group 3 as compared to group 1 and 2 (Figs 3, 4). Cr supplementation to Karan Fries calves seems to improve the immunity system under stress conditions of hot dry season by reducing the free radical production and improving feed intakes. Spears (2000) reported that imbalances of dietary nutrients can alter enzymes activity thereby impairing immune function. Functionality of numerous structural proteins, enzymes and cellular proteins depends on nutrients, including minerals, being ingested in appropriate amounts (Nocek *et al.* 2006 and NRC 2001). The result of present study showed improvement in immunity and stress levels in groups 3 and 2 indicating ameliorative effect of custom designed shed and chromium fed to Karan Fries growing calves over control group. Our results were in agreement with previous reports which indicated that supplemental Cr can alter specific immune responses of stressed cattle (Burton *et al.* 1996, Burton 1995b and Millard *et al.* 1994).

Gene expression: The growth related gene (NFIL3) showed higher expression in group 3 followed by groups 2

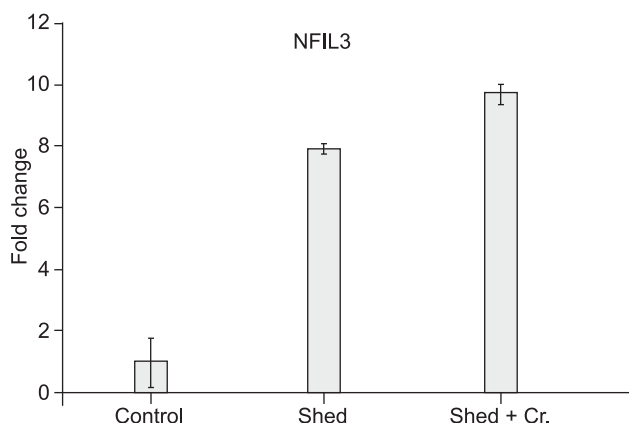


Fig. 5. Expression of in different groups of NFIL3 gene Karan Fries calves during hot dry season.

and 1 (Fig. 5). NFIL3 has been shown to have many functions in the immune system. It was essential to intrathymic natural killer cell development and it regulates cytokine production in multiple T cell subsets and has a role in macrophage function (Hidalgo *et al.* 2012, Kashiwada *et al.* 2011 and Motomura *et al.* 2011). In the present study, higher expression of NFIL3 gene indicating higher immune tolerance in Karan Fries calves kept in custom design shed with Cr supplementation (group 3) followed by groups 2 and 1 during summer.

The improvement in body weight gain, dry matter intake and water intake with better immunity and lower levels of stress biomarker in Karan Fries growing calves indicating ameliorative effect kept of custom designed shed alone and additional in group 3 i.e. custom design shed with Cr supplementation during heat stress conditions. Therefore, supplementation of chromium propionate along with protection of animals using suitable shelter may be the answer for improving growth rate, lowering the free radical production and reducing the stress biomarker levels.

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