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Inorganic zinc supplementation modulates heat shock and immune response in heat stressed peripheral blood mononuclear cells of periparturient dairy cows

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ABSTRACT

Thermal stress in India is one of the major constraints affecting dairy cattle productivity. Every attempt should be made to ameliorate the heat and calving related stress in high producing dairy cows for higher economic returns. In the current study, inorganic zinc was tried to alleviate the adverse effects of thermal stress in periparturient cows. Twelve cows, six each of Sahiwal and Karan Fries (KF) in their second parity with confirmed pregnancy were chosen for the experiment. The blood samples were collected periparturiently on three occasions viz. −21, 0 and +21 days relative to calving. The *in vitro* study was conducted after isolating peripheral blood mononuclear cells (PBMC) from whole blood. The cultured PBMC were subjected to three different levels of exposures viz. 37°C as control, 42°C to induce thermal stress and 42°C + zinc to ameliorate the adverse effects of high temperature. Heat shock lead to a significant ($P<0.05$) rise in the level of heat shock proteins (HSP). HSP was more on the day of calving as well. KF showed more HSP concentration than Sahiwal breed indicating the heat bearing capacity of later. Zinc treatment to thermally stressed PBMC caused a fall in the HSP concentration in both the breeds during periparturient period. Moreover, heat stress increased significantly ($P<0.05$) the Interleukin 6 (IL-6) concentration which declined upon zinc supplementation to PBMC. IL-6 levels decreased periparturiently. Heat and calving related stress caused a fall in the IL-12 levels which increased significantly ($P<0.05$) with zinc supplementation. These findings suggest that zinc supplementation attenuates the HSP response and augments immunity in PBMC of periparturient dairy cows. The study could help to alleviate the heat stress and potentiate immunity by providing mineral supplements in periparturient dairy cattle habituating tropics.

1. Introduction

Climate change is one of the major threats to the livestock industry in India, where the temperature in different seasons varies tremendously from 10°C to 44°C. High ambient temperature has adverse effects on biochemical functions of high yielding dairy cows. The stress of periparturient period is aggravated more due to thermal stress. The management of cows during the transition period especially in summer season is important to protect them from various diseases. Zinc is one of the most important trace elements in the body owing to its role in cell proliferation, genomic stability and antioxidant defense [1]. In human beings, it has been seen that zinc and chaperones are indispensable for immune function [2]. Zinc deficiency compromises both innate immunity and the T-cell compartment and increases susceptibility to infections [3,4]. High ambient temperature impairs animal health, milk yield and reproduction [5,6], resulting in estimated economic loss of 897 million dollars in the USA, in spite of intensive cooling every year [7]. To alleviate thermal stress from high producing animals, genetic modification is time taking, so technical modification of environmental conditions (i.e. sprinklers, fans, shading, etc.) and optimized feeding are crucial elements in the short-term management of the anticipated climatic challenges for dairy farmers to avoid severe production losses and maintain animal health [8]. Trace minerals participate in a wide range of body functions such as reproduction [9,10], oxidative metabolism [11],

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enzyme synthesis [9,12] and regimen of energy metabolism in dairy cows [11] and are indispensable for the growth and development of animals. During the transition period, high producing cows kept under thermal stress condition are faced with depressed feed intake, negative energy balance (NEB) [13], oxidative stress [14] and compromised immune function [15]. During the transition period of dairy cows, the increase in oxygen demand with increased metabolic requirements result in augmented production of oxygen derived free radicals [16]. Trace mineral requirement of dairy cows under all physiological and management conditions are not certain and thus determining the optimum levels and sources under each specific situation present challenges at the farm level [11]. Various supplements and additives, zinc being one amongst them are used in dairy animal rations during the periparturient period to maintain the productive and reproductive performance of animals [17]. Zinc treatment to heat stressed lymphocytes can alleviate the adverse effects of heat stress and improve the immune status [18]. Dietary supplements such as vitamins, trace elements and minerals are exclusively utilized to alleviate the unfavorable effects of thermal stress [19]. Khorsandi et al. [20] reported that supplementing heat stressed transition cows with ruminal bolus lead to a higher milk yield which suggests that it mitigates the adverse effects of thermal stress. Zinc is an activator of many enzymes and therefore influences pH, immune competence and basic cellular functions [21,22]. An adequate daily intake of zinc is necessary to achieve a steady state for proper immune function, because there is no specialized zinc storage system in the body. Zinc deficiency contributes to the cellular immunosenescence [23]. Some reports describe that zinc supplementation to human peripheral blood mononuclear cells leads to a more mRNA production and release of the monokines IL-6, IL-1β and TNF-α [24]. In an *in vitro* culture of peripheral blood mononuclear cells, Zn deficiency does not affect oxidative burst and phagocytosis of monocytes but only affects the production of TNF-α and IL-6 [25]. Zn is the only naturally occurring lymphocytic mitogen [26,27]. The nutritional strategies of high producing dairy cows need more focus to meet their overall demand for milk production, health and reproductive potential [28]. Though the crossbred population in India has considerably increased the production levels, they are also found to be more prone to heat and nutritional stress [29]. Antioxidant nutrient supplementation especially vitamins C, A and E, zinc and chromium can be used to attenuate the negative effects of environmental stress [30].

Based on the literature cited, we experimented with the assumption that inorganic zinc supplementation abates the adverse effects of heat stress and potentiates the immunity in periparturient dairy cows.

2. Materials and methods

2.1. Chemicals and reagents

These were chiefly obtained from Sigma Chemical Co. (St. Louis, MO, USA), Hyclone Laboratories, Inc. (Logan, UT, USA), Thermo Scientific (Rockford, IL, USA), MP Biomedicals, Cusabio Biotech Co. Ltd, Abcam, Cell Signaling Technology, etc. unless otherwise indicated. Disposable cell culture grade plasticware and vacutainer tubes were purchased from BD-Plymouth (UK), Becton, Dickinson and Co. (Lincoln Park, NJ, USA), or Nunc, Roskilde (Denmark).

2.2. Experimental animals

The experiment was carried out on 12 animals (Six each from Karan Fries (*Bos indicus* × *Bos taurus*) aged about 4.5 years with an approximate body weight of 450 Kg and Sahiwal (*Bos indicus*) aged about 5 years with an approximate body weight of 390 Kg) selected from Livestock Research Center (LRC), ICAR-National Dairy Research Institute (NDRI), Karnal. The animals in their second parity were confirmed to be pregnant and had no history of reproductive disorders. All the cows were maintained under uniform management conditions. The study was approved by the Institutional Animal Ethics Committee (IAEC) of Indian Council of Agriculture Research (ICAR)-National Dairy Research Institute (NDRI) constituted as per the article 13 of the CPCSEA rules, laid down by the Government of India. The experimental animals were maintained and fed as per standard practices followed at LRC, NDRI, Karnal for transition animals. The ingredients of total mixed ration (TMR) are presented in Table 1.

2.3. Blood sampling

Fresh blood (6–7 ml) from each animal was drawn aseptically in Potassium-EDTA coated vacutainer tubes (BD-PlymouthPL6 7BP, UK) on days −21, 0 and +21 relative to expected date of calving. The samples were immediately taken to the laboratory under refrigeration conditions and processed for PBMC isolation within 1 h of collection.

2.4. PBMC isolation and determination of their viability

Density gradient centrifugation was used to separate PBMC from whole blood. The whole blood was diluted with 1X DPBS (Thermoscientific, Waltham, MA USA) and was layered gently over Histopaque-1077 (Sigma Aldrich, St. Louis, MO, USA). Manufacturer's instructions were followed for the isolation of PBMC. The isolated PBMC pellet was washed twice with 1X DPBS (Thermoscientific, Waltham, MA USA). To determine the viability of isolated PBMC, Trypan blue dye exclusion method [31] was used. The cell pellet obtained was diluted with serum-free RPMI-1640 medium (Hyclone Laboratories, Inc., Logan, UT, USA) and the cell number and viability was determined by Haemocytometer (Rienfeld, Germany) using 0.04% Trypan blue (Sigma Life Sciences, St. Louis, MO, USA). The cell viability in different experiments was found to be more than 85% within 3 h of processing.

2.5. Culture and exposure of peripheral blood mononuclear cells

The PBMC suspension was adjusted to 1×10^6 viable cells per ml of culture medium, RPMI-1640 (Hyclone Laboratories, Inc., Logan, UT, USA) containing 10% FBS (Hyclone®, Thermoscientific, Canada). The cells were seeded @ 1×10^6 viable cells per ml of medium in 75 cm^2 culture flasks (Nunclon™ delta Surface, Nunc, Roskilde, Denmark). 5 ml of medium was dispensed into the flasks. PHA-P (Sigma Aldrich, Saint Louis, Missouri USA) @ 5 μg/ml was used to provide maximum stimulation to bovine PBMC. The culture flasks (Nunclon™ delta Surface), one pretreated with zinc (0.01 mM) and two without zinc were incubated at 37°C in a humidified CO_2 incubator (5% CO_2 and 95% air) (Thermo Electron Corporation) for 48 h. Zinc (0.01 mM), was used in its salt form, Zinc sulphate heptahydrate (Sigma Aldrich, USA), suitable for cell culture after standardizing its concentration to provide optimal stimulation of PBMC culture. The culture was maintained free from contamination by using 1% Penicillin-Streptomycin solution (MP Biomedicals, Santa Ana, California, USA) and 2.5 mg/ml Amphotericin B (Sigma Aldrich, St. Louis, MO USA). After 48 h, the culture flasks were subjected to various levels of exposures viz.

a) 37°C (Flask without zinc pretreatment, kept as Control)
b) 42°C (Flask without zinc pretreatment, kept as Treatment I)
c) 42°C (Flask pretreated with zinc, kept as Treatment II)