

A REVIEW ON THE EFFECTS OF MICRONUTRIENTS IN HEAT STRESS ALLEVIATION IN DAIRY ANIMALS

ABSTRACT

Heat stress from global warming and climate change, which result in an ongoing rise in Earth's temperature, has a detrimental effect on the growth and health of dairy animals. The animal experiences heat stress when it cannot release enough heat to maintain homeothermy. The degree of heat stress dairy animals experience depends on the ambient temperature and humidity. In dairy animals, heat stress reduces feed intake, milk production, reproductive efficiency, and immune function. This article will concentrate on the micronutrients that lessen the damaging effects of heat stress on dairy animals. Micronutrients are vital substances that life requires in minute amounts. Major minerals, trace minerals, and vitamins are all included. Micronutrients support the maintenance of animal production, enhance nutrient utilization, effectively combat oxidative stress, and strengthen the weak immune system. Minerals are crucial for maintaining an animal's normal physiological processes. However, it's believed that animals' reactions to heat stress increase mineral loss through excretion. Therefore, adding minerals to the diet (such as DCAD, Zinc, Chromium, Selenium, etc.) may help to reduce the harmful effects of heat stress. Vitamins serve as cofactors for enzymes, act as catalysts in a number of metabolic pathways, and are crucial for an animal's normal growth and development. The addition of vitamins (such as Vitamin E, Niacin, etc.) to dairy animals' diets may also help to mitigate the harmful effects of heat stress.

INTRODUCTION

In the present scenario, heat stress is a major concern in rearing dairy cattle. Heat stress occurs when an animal's heat load is greater than its capacity to lose heat. Dairy animals feel hot sooner than humans do. Factors like high air temperature, humidity, solar

radiation and low air movement contribute to increased risk. A living body can only maintain its core temperature when heat production and heat loss are balanced. The thermoneutral zone is characterized as the range of surrounding temperatures where the body can keep up its core temperature exclusively by regulating dry heat loss, i.e., skin blood flow (Kingma *et al.*, 2014; Singh *et al.*, 2020a; Singh, 2021).

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Temperature humidity index (THI) is a simple method to measure and assess heat stress. It depends on humidity and ambient temperature. Present-day high-producing cattle begin to experience heat stress at a THI of 65-68. As a broad classification, the level of stress can be separated into light (68-71), moderate (72-79), severe (80- 89), very severe (>90), and deadly (>100). Signs of heat stress include reduced rumination, increased CO₂ output, increased respiration and panting, reduced feed intake, decrease in growth, reduced milk production, reduced carcass quality, decreased fertility, increased sweating, and finally, these all lead to an increase in mortality rate (Bin-Jumah *et al.*, 2019). Older dairy animals seem to be more severely affected compared to younger animals. Heat stress reduces dry matter intake and leads to as much as a 30% drop in milk yield in dairy animals.

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Strategies for the alleviation of heat stress include physical modification of the environment, genetic development of less sensitive breeds, and improved nutritional management schemes using dietary water, protein, fat, fibre, micronutrients, and feed additives (Conte *et al.*, 2018) (Fig.1). Dietary yeast, chemical additives, fermentates, betaine, dietary cation-anion difference, propionate supplementation, and micronutrients are the main components of anti-heat stress additives. These substances are essential for improving the metabolic status of cattle as well as their immune system and energy metabolism (Zou *et al.*, 2022). Micronutrients are essential elements needed by life in small quantities. ~~It~~ *They* includes major minerals, micro/trace minerals and vitamins. Micronutrients help to maintain the production of the animals, improve nutrient usage, effectively neutralize oxidant stress and strengthen the compromised immune system (Mir *et al.*, 2018). Based on different relevant studies, *the* list of micronutrients that are important for heat stress alleviation is given in Fig. 2.

During heat stress, there is an extra need for micronutrients. This is because the reduced feed intake and heat stress increase mineral excretion, whereas it decreases serum and liver concentrations of vitamins and minerals. Moreover, the excretion of vitamins and minerals and their mobilization from tissues are increased under stress conditions (Siegel.,

1995). Recent research has demonstrated that combining micronutrients (Zn, Se, Vitamin E, and Vitamin C) has improved animals' resistance to the harmful effects of HS. Animal performance under HS may be improved by the substances' capacity to prevent cell damage and enhance intestinal integrity and renal function (Ortega *et al.*, 2022). The metabolic and physiological processes of several species of animals can also be improved by adding vitamins and minerals (Singh, 2019; Singh *et al.*, 2020e). This may have an impact on the mineral digestibility and electrolyte balance of HS-affected animals. (Sivakumar *et al.*, 2010; Liu *et al.* 2018). Stress may aggravate a marginal vitamin and mineral deficiency or lead to increased vitamin and mineral requirements (Sahin *et al.*, 2005). National Research Council recommendation says that mild to severe heat stress in dairy cattle has been estimated to cause an increase in maintenance requirements of micronutrients by 7 to 25%.

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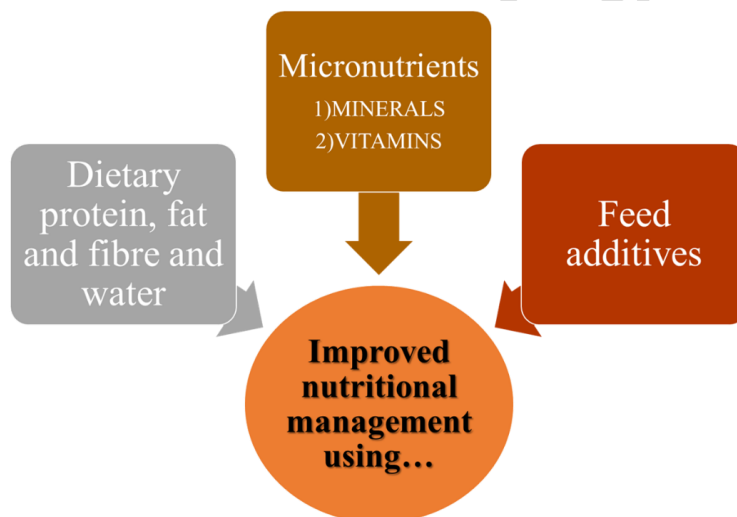


Fig.1 Improved nutritional management schemes during heat stress in animals

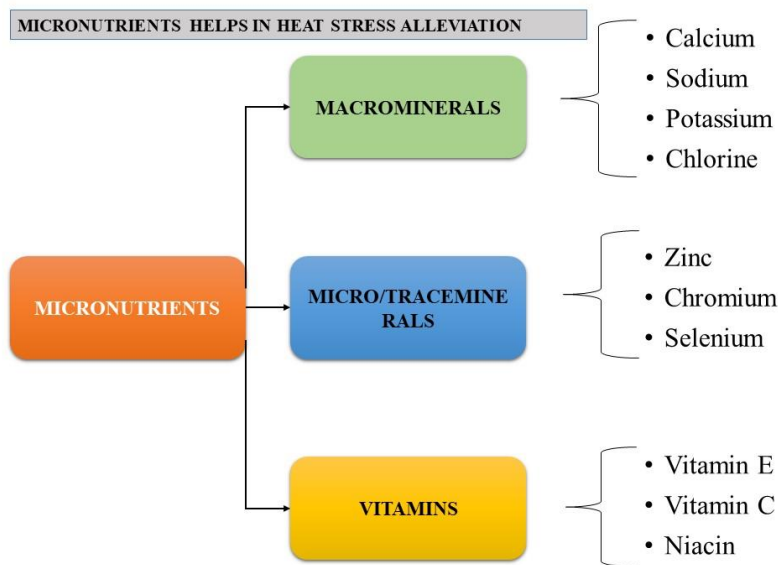


Fig. 2 Classification of important micronutrients helps in heat stress alleviation

MACROMINERALS

Heat stress-induced disturbances of acid-base status are related significantly to macromineral elements. The main change occurring during heat stress is an increase in the respiratory rate which causes carbon dioxide expulsion. That will lead to altered blood carbonic acid to bicarbonate ratio and the pH level increases. This condition is known as respiratory alkalosis. To maintain the pH, the bicarbonate will be expelled through urine. Rumen needs bicarbonate for buffering acids during rumen fermentation. Since the bicarbonate ~~are~~ is excreted through urine, the rumen will not get enough bicarbonate ion and this ultimately leads to subacute ruminal acidosis (Sanchez *et al.*, 1994). The demand for cations by the kidney is increased because the excretion of bicarbonate ions must be accompanied by the excretion of the cation. Potassium or sodium is a possibility; however, sodium is more likely to be excreted (El-Nouty *et al.*, 1980). Sodium and potassium help to increase dry matter intake, ~~increase in~~ milk yield, ~~increase in~~ milk fat percentage; and dietary Na and K are very important in the maintenance of water balance and acid-base physiology of heat-stressed dairy animals (Mallonee *et al.*, 1985).

The increased amount of dietary chlorine in feed is much more detrimental to dry matter intake during summer. A study shows that ingestion of CaCl_2 increases plasma

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chlorine concentration, which exceeds the bicarbonate buffering power capacity and produces metabolic acidosis, thereby appearing to suppress appetite in pigs (Yen *et al.*, 1981). When animals, particularly cattle, are given diets with negative cation to anion balance (i.e. more anions than cations) or calcium chloride causes an acidifying effect, it is known as hyperchloremic metabolic acidosis.

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Dietary cation-anion difference (DCAD), has been defined as milliequivalents of $(Na + K) - (Cl + S)$ per kilogram of dry matter and has a direct impact on blood acid-base metabolism (Block *et al.*, 1994). Dietary cation-anion difference calculated using Na^+ , K^+ , and Cl^- concentrations, has a significant effect on health status and productivity by influencing acid-base balance (Hu *et al.*, 2004). Serum total amino acid and essential amino acid concentrations and the ratio of essential amino acid to total amino acid were greater for high DCAD. These results suggest that increasing DCAD improves amino acid availability for protein synthesis, which would otherwise be taken for the maintenance of acid-base balance (Wildman *et al.*, 2007). A positive Dietary cation-anion difference diet of +350 mEq/kg dry matter improved the immunity status and nutrient intake by ameliorating heat stress (Suman *et al.*, 2018). Keeping the dietary cation-anion difference at a healthy lactating level remains a good strategy for reducing thermal stress during the warm summer months.

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Serum calcium is lowered in heat-stressed lactating dairy animals. This may be due to respiratory alkalosis. During respiratory alkalosis, the relative amount of bicarbonate ions in the blood will be increasing, along with which pH also increases. To neutralize it, the blood proteins release H^+ ions and they react with bicarbonate ions to form water and CO_2 . The free form of calcium present in the blood will get attracted to these negatively charged blood proteins and form calcium-bound proteins. Hence free calcium ions in the blood decrease during heat stress. So, there is a need for extra allowance of calcium for animals under heat stress (Odom, 1989; Sanchez *et al.*, 1994).

MICRO/TRACE MINERALS

Supplementation of micro/trace minerals () in heat-stressed dairy animals helps in improved improving performance and productivity, maintenance of proper immunity, udder health and reproduction, and reducing oxidative damage of tissues.

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Zinc

Zinc is an important micromineral, involved in productive performances like growth (Underwood *et al.*, 1999), immune system, and reproduction (Patel *et al.*, 2017; Abdel *et al.*, 2011), and involved in a wide range of metabolic activities. By using transcellular transport mechanisms, Zn is absorbed by the small intestine and activates antioxidant peptides and enzymes by promoting the expression of metallothionein, which is essential for shielding cells from ROS (Jarosz *et al.*, 2017; Maares *et al.*, 2020). Zinc plays a critical role in antioxidant defense as an integral part of superoxide dismutase (SOD), which is an essential enzyme. The deficiency of zinc has been reported to cause an increase in oxidative DNA damage and impair antioxidant functions (Underwood, 1999; Jing *et al.*, 2007). Zinc supplementation lessens the heat shock protein response and enhances immunity in heat-stressed peripheral blood mononuclear cells of periparturient dairy cows (Sheikh *et al.*, 2017). Inorganic salts like zinc sulfate ($ZnSO_4$), zinc oxide (ZnO), and zinc chloride ($ZnCl_2$) are the major sources of zinc in mineral supplements formulated for animal feeding (Batal *et al.*, 2001). Mammary integrity should be maintained in high-producing cattle during heat stress to prevent a decrease in milk production. Studies indicate that dietary organic Zn complex (40 ppm) can improve mammary epithelial integrity, and the mammary cells displayed more integrity (Weng *et al.*, 2018). High-yielding HF cows received a base diet containing 0.131 percent of diet DM of the zinc-methionine (Zn-Met) complex, which improved their oxidative status as evidenced by higher total antioxidant status and lowers malondialdehyde concentrations. Overall, the findings of this study demonstrated that feeding RPZM to animals during times of heat stress could maintain their ability to produce milk and the composition of their milk. Animals' improved performance after taking Zn-Met complex supplements may have improved oxidative and immune status (Kargar *et al.*, 2022). Supplementing multiparous lactating Holstein with 35 mg of Zn/kg of DM from Zn hydroxy chloride and 40 mg of Zn/kg of DM from Zn-Met complex had a significant impact on mammary cell turnover (Rivas *et al.*, 2021). These findings imply that cows receiving Zn supplements have increased milk secretion capacity and the ability to maintain milk yield under heat stress by safeguarding mammary epithelial cells and their capacity for secretion.

Chromium

Chromium is another favourable micronutrient for defying the adverse effects of heat stress in animals. It is a necessary mineral and is crucial for the metabolism of glucose as glucose tolerance factor (Lashkari *et al.*, 2018). It acts as an excellent antioxidant that prevents heat-stress-induced lipid peroxidation. Chromium improves cortisol hormone

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activity and nutrient metabolism. It promotes insulin action in responsive tissues, thereby increasing farm animal productivity (Bin-Jumah et al., 2020). Appropriate amounts of chromium-containing additives should be added to the ration for dairy cows during hot summers due to the low concentration of chromium in the ruminant feed ingredients. (Shan et al., 2020). 10 mg/day Cr given to dairy animals from 21 days prepartum to 35 days postpartum showed an increase in milk production which was associated with decreased lipolysis and increased glucose uptake (McNamara et al., 2005). Later studies also demonstrated that oral administration of various Cr concentrations increased dairy cows' DMI while not affecting milk yield (Vargas-Rodriguez et al., 2014; Leiva et al. 2017). Cr-methionine at 0.05 mg /kg increases feed efficiency and dry matter intake in cows (Arthington et al., 1997) and significantly reduced the respiration rate in dairy calves (Mousavi et al., 2019). A study showed that supplementation of inorganic Cr to the diet of buffalo calves reared under high ambient temperature improved heat tolerance, and immune status without affecting nutrient intake, and growth performance (Kumar et al., 2015). The estimated requirement of chromium of buffalo calves in summer conditions was calculated to be 0.044 mg/kg body mass and 10.37 ppm per day and the supplementation of Cr had a positive effect on its balance and plasma concentration without interacting with other trace minerals (Kumar et al., 2013). By lowering respiration rates and rectal temperatures, increasing dry matter intake and milk lactose content, and boosting antioxidant and immune function, chromium yeast (CY) supplementation at 0.36 mg Cr/kg DM improved the welfare of mid-lactation dairy cows (Shan et al., 2020). Additionally, it is said that Cr supplementation can guard against stress-related losses of Zn, Fe, Mn, etc (Sirirat et al., 2012).

Selenium

Selenium is an essential trace mineral that is an indispensable component of the antioxidant system (Oltremari et al., 2014). It has been demonstrated that dietary Se supplementation under heat stress supports reproductive physiology, production performance, and gastrointestinal health. The underlying mechanisms involve antioxidant status, immunocompetence, and regulation of nutrient digestibility influenced by gastrointestinal microorganisms (Zheng et al., 2021). Se, which is absorbed by active transport via a sodium pump in the duodenum and cecum, functions as a dietary antioxidant by forming selenoproteins and controlling the activity of endogenous enzymes (Mehdi et al., 2013; Kiełczykowska et al., 2018). Selenium decreases the adverse effects of heat stress on metabolism and redox balance, resulting in improved dairy animal health, immune function,

and milk quality (Sejian et al., 2012). Studies show that in heat-stressed animals, there is a significant reduction in plasma selenoprotein, diet supplementation with selenium can significantly raise plasma selenoprotein and selenium concentrations, which might be a potential mechanism to protect dairy animals from heat stress (Hill et al., 2012). Se controls the production of selenoproteins, which take part in several cellular defense responses and shield cells from stressors like heat shock and oxidative damage (Kumar et al., 2017). Supplementing Se can significantly lower cellular HSP production, reduce the need for HSP defense against high-temperature stress, and reduce oxidative stress. (Zou et al., 2019). The supplementation of organic selenium in the diet had a positive effect on the percentage of milk fat and it improved mammary gland health (Oltamari et al., 2014). Sun et al. (186) found that Holstein dairy cows kept stable under heat stress with the addition of 0.3 mg/kg DM organic Se to their diets in the middle of lactation, while the GPx activity in the serum of the cows in a similar inorganic Se group gradually decreased. Studies have shown that selenium supplementation can stop heat stress-induced apoptosis in cells (Tang et al., 2019).

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VITAMINS

Vitamins function as enzyme cofactors (coenzymes), participate in a variety of metabolic pathways as catalysts and are essential for the normal growth and development of animals. The addition of vitamin supplements to the diet of dairy animals might also contribute to the relief of the negative effects of heat stress (Min et al., 2019).

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Vitamin E

Vitamin E is an antioxidant that is essential for body functions like growth, immunity, tissue integrity, and reproduction (Dalle et al., 2011; Singh et al., 2020b) and to prevent oxidative stress. In membrane and plasma lipoproteins, vitamin E functions as a free radical-suppressing antioxidant (Reboul et al., 2017; Singh et al., 2020c). Elevated temperature and humidity in summer will lead to greater oxidative stress for animals, so feeding of additional vitamin E is required. Vitamin E includes a group of tocopherols and tocotrienols, among them, α -tocopherol has greater biological activity. Due to the potent antioxidant properties of tocopherols, the impact of α -tocopherol in the prevention of chronic diseases believed to be associated with oxidative stress has often been studied and beneficial effects have been observed (BrigeliusFlohé et al., 1999; Singh, 2019; Singh et al., 2020d; Singh et al., 2020e).sSupplementation of vitamin E along with selenium (500 IU Vitamin E + 0.3mg selenium per kg DM) alleviated the heat stress in dairy cows. Supplementation of chromium

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along with vitamin E and selenium (chromium propionate (0.5 mg) + vitamin E (500 IU) + selenium (0.3 mg)) in calves during heat stress decreased significantly ($P < 0.05$) the cortisol levels and rectal temperature (Sultana et al., 2022).

Vitamin C

According to Padilla et al. (2006), heat stress reduces the plasma vitamin C concentration in lactating cows; therefore, vitamin C supplements are required during the HS period. Reactive oxygen species (ROS) can damage cell membranes, DNA, proteins, and lipids during OS. Vitamin C is also crucial for the regeneration of other antioxidants, such as glutathione and alpha-tocopherol (vitamin E) (Padayatty et al., 2016). Dairy cattle are more susceptible to oxidative stress after calving and when under heat stress, and total ascorbic acid in plasma significantly drops during the peripartum period (Tanaka et al., 2011). According to experimental data with cattle, dairy cows need to have access to vitamin C to successfully adapt to stressful situations when there is heat stress (Ranjan et al., 2012). By lowering IL-6 levels and reducing ROS production, polyherbal vitamin C (20 g/d) can reduce inflammation. It also enhanced HF cow performance under heat stress.

Niacin

Niacin has been reported to be a vitamin that resists heat stress in cattle by increasing evaporative heat loss *in vivo* and cellular heat shock response by increasing gene expression of heat shock proteins during thermal stress *in vitro* (Zimbelman et al., 2013). Niacin (vitamin B3) supplementation increases resistance to heat stress by inducing greater cutaneous vasodilatation and blood flow and the increase in the rate of blood flow to the skin after niacin supplementation is associated with an increase in the evaporative heat loss from the skin surface and sweating rate (Di et al., 1997; Khan et al., 2012). The greater cutaneous vasodilatation after niacin supplementation is caused by prostaglandin D produced by epidermal Langerhans cells, which acted on vascular endothelial prostaglandin D2 receptors (Maciejewski et al., 2006; Cheng et al., 2006). When rumen-protected niacin was fed to Holstein cattle (12 g/day) during heat stress, the treatment group showed a small but detectable reduction in the rectal and vaginal temperature over the control group (Zimbelman et al., 2010). A study ~~shows~~ showed that 800 ppm niacin supplementation to lactating crossbred cows resulted in better heat stress alleviation (Khan et al., 2018). Another study conducted on Jinjiang cattle under heat-stress conditions showed that adding niacin reduced

the body temperature and respiratory rate ($P < 0.05$), and the reason is considered as niacin supplementation may alleviate heat stress by proliferating those bacteria belonging to the phylum *Succiniclaticum*, which may further contribute to the digestion of cellulose and the improvement of the metabolic function (Zou et al., 2022).

A combination of these micronutrients in adequate quantity will be a better alternative for reducing the detrimental effects of heat stress. Supplementation of trace elements (zinc, selenium, copper, cobalt, iodine, and manganese) and vitamins (Vitamin A, Vitamin D₃, and Vitamin E) above the National Research Council recommendation using a sustained-release source had positive effects on reproductive and lactation performance of dairy animals kept under thermal stress condition (Khorsandi et al., 2016).

CONCLUSION

The most adverse effects in heat-stressed dairy animals are pronounced reduction in feed intake and milk production. Concerning these issues, several nutritional strategies using micronutrients summarized in this article are capable of significantly increasing feed intake and milk production in heat-stressed dairy animals. Supplementation of micronutrients will also play an auxiliary function in heat-stressed dairy animals, such as the amelioration of defects in immune function, reproductive performance, heat dissipation, water intake, energy balance, and mammary health. So adequate amount of micronutrient supplementation in feed improves the growth, productivity, and overall well-being of the animal during heat stress.

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