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Immune Status of Cattle During Thermal Stress

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Abstract

Animal husbandry and agriculture are the climate sensitive sectors and majorly affected by climate change. This change in climate will be a greatest threat to about one third population of the world which depends on animal husbandry for their livelihood. Among all climate changes, thermal stress is the principal factors that enforce negative impacts on production and reproduction in cattle. Further, it also alters the immune functions of the animals and makes them susceptible to infections.Thermal stress either enhances or suppresses the immune functions in farm animals, which depends on time and intensity of exposure. The stress signal acts mainly through hypothalamo-pituitary-adrenal (HPA) axis to modulate the immune response. Thermal stress generally acts to shift the adaptive immune function from cell mediated to humoral immunity and thus weakens the animal immune action to microbes. Emerging and re-emerging nature of pathogens is another aspect of this climatic change for which livestock needs fine-tuned immune system to fight. Hence, the thermal stress-immune system interactions need to be studied thoroughly to introduce various management and nutritional strategies to alleviate the ill-effects of thermal stress in farm animals.

Keywords: Thermal stress; Immunity; Livestock; Pathogens; Production; Reproduction

Introduction

Livestock sector contributes about 53 percent of world agricultural GDP [1], and to the economy of farmers by means of milk, meat, hide, eggs, drought power, manure etc and hence its alive ATM for farmers. It also provides employment to 1.3 billion worldpopulation [2]. To ensure nutritional security to the current growing population, animal protein seems to be inevitable as it's cheap source of high quality protein with essential vitamins and micronutrients. To provide quality animal meat to the consumers, livestock should be healthy in terms of physiology, temperament and immunity. However, there are various biotic and abiotic stressors, which challenges the animal's comfort. Microbes such as bacteria, viruses, fungi and protozoa, as well as helminths and arthropod vectors, are the biotic stressors whereas temperature, solar radiation, photoperiod, humidity, geographical location, nutrition and socioeconomic signals are the major abioticstressors. Disease is an outcome of the interaction of host, pathogen and environment. Due to global warming, abrupt climatic conditions like storms, droughts, floods and extreme hot andcold temperatures are prevailing around theglobe. High temperatures, accompanied by highrelative humidity, favour the survival and multiplication of animal disease vector like ticks, fleas, tabanid flies, etc.; thus, therisk of spread of vector borne diseases also increases [3]. Abiotic stressors such as thermal stress and nutritional stress have a major impact on live stock productivity [4] and influences the animals' productive and reproductive traits in a major way. Thermal stress is one of the crucial factors affecting live stock productivity [5] and affects animal productive performances like milk yield, meat quality and reproductive performances like age at first calving, ovulation defects, embryo survival, etc. [6]. Thermal stress also weakens the animal's immune system and makes them more prone to diseases. For mitigating immune suppression by means of various nutritional, managemental and other interventions, depth understanding of the mechanism by which thermal stress affects immune function is mandatory.This chapter deals with the mechanism by which thermal stress affect immune response, the relationship between hypothalamo-pituitary-adrenal axis and immune axis and various interventions need to be taken to mitigate thermal stress.

Stress and immunity

Seyle in 1946 who gave the first definition about the animal response to stress asthe 'general adaptation syndrome'. Stress, the biological responseelicited when an animal perceives a threat to its homeostasis [7]. Stress mediated interactions with the immune system suppresses its normal functions and leads the animal to a disease-prone state [7]. When an animal exposed to a stressor, the central nervous system first begins to respond by sending signals to any of the biological systems viz. behavioural, autonomic nervous system, neuroendocrine system and immune system to alleviate or compensate the threat. The immune system reacts to stress by increased or decreased immune functions [8].

Thermal stress and immunity in calves

The disease process involves factors; namely, host, pathogen and environment. Environment modifies the host and pathogen interactions in a way that the pathogen overcomes the immune barrier of the host. In calf neonates, the first18 hours of post-natal life is very crucialas it determines the immune status of theanimal. During this period, the intestinal epithelium is permeable to colostral proteins, particularly immunoglobulinsand thus passive transfer of immunity occurs from dam to off spring. Exposing dams and neonates to thermal stress has majori mpacts on the calf's immunity. Exposing heifers to high temperature during late pregnancy and early postpartum period notonly reduces the concentrations of IgG, milk proteins and fatty acids in colostrums [9] but also lowers the intestinal absorption of immunoglobulins [10]. The reduced intestinal absorption and low quality of Igs in turn lead to calf mortality at high temperature [11].

Thermal stress and disease occurrence in adult cattle

Thermal stress directly or indirectly favours disease occurrence in animal host. Directly, high temperature favours the survival of organisms outside the host for longer time. This can be seen in the case of spores of Bacillus anthracis and Clostridium chauvoei [12], which survive for a long period under high temperature and made availableto infect the animals. Indirectly, chronic thermal stress causes immune suppression in animals and makes them susceptible to diseases.Moreover, global climatic change has created huge modifications on the macro and micro climate of both the host and the parasite and also altered certain trends of host-microbe interactions, namely: (a) shortened generation time of microbes which prefer high temperature leading to increased pathogen population and in turn increased risk of infection, and (b) population of vectors like ticks, flies, midges, fleasnormally requires high temperature, and hence they increase in number with increased feeding frequency. These lead to more chances for spread of pathogen to host. The sub-tropicalhot and humid conditions favour the tick population like Boophilus microplus, Haemophysalis bispinosa and Hyalomma anatolicum [13]. (iii) The extrinsic incubation period reducesdue to high temperature has been seen in case of Culicoides [3]. High ambient temperature combined with relative humidity predisposes dairy cows to clinical mastitis [14].The direct effect of heat compromises the udder immune system and also favours the housefly population [15].

Thermal stress and gastro-intestinal tract

Optimum production performance depends upon efficient feed conversion. The gastrointestinal tract health is crucial in cattle for proper digestion and in turn production performance. Besides, the microbial population in rumen and digestive tract is important for better digestion. However, the blood flow to internal organs gets compromised and peripheral blood flow increases to dissipate the internal bodyheat [16]. The decreased blood flow leads to ischemia and necrosis of intestinal epithelial cells [17], decreases tight junctions between enterocytes which favour entry of bacterial pathogens and its antigenic components, particularly lipopolysaccharides leading to endotoxemia [18]. The toxin in turn affects the normal liver metabolism, leading to steato hepatitis and decreased productive performance.

Transition period, thermal stress and immunity in dairy cows

The transition period is one of the most critical times in the life of the dairy cows' specifically on immune system. Reduced immune function associated with failure of neutrophils to move into tissues (uterus) being invaded by pathogens and reduced capability of neutrophils and other immune cells to effectively carry out phagocytosis and killing mechanisms allows the dairy cow to be highly sensitive to infectious disease. This can occur not only at the time of calving but throughout the lactation cycle, more adversely on pick lactation during summer. Cows with compromised immune systems are predisposed to mastitis, metritis and other types of infectious and metabolic diseases [19]. Calving during the month of hot summer is more stressful as compared to monsoon and winter respectively, hence breeding management to augment more calving in later seasons is advisable to reduce thermal stress on cow and calf. Management strategies to improve neutrophil function during stress will likely improve immune function and aid in disease resistance.

Milk production and composition during thermal stress

Thermal stress adversely affects milk production and its composition in dairy animals, especially animals of high genetic merit [20,21]. Berman (2005) estimated that effective environmental heat loads above 35°C activate the stress response systems in lactating dairy cows. In response dairy cows reduces feed in take which is directly associated with negative energy balance (NEB), in turn largely responsible for the decline in milk synthesis [20]. Moreover, maintenance requirements of energy also increased by 30% in thermal stressed dairy animal [21]. Therefore, energy in take would not be enough to cover the daily requirements for milk production. Hot and humid environment not only affects milk yield but also effects milk quality [22]. Reported that milk fat, solids-not-fat (SNF) and milk protein percentage decreased by 39.7, 18.9 and 16.9%, respectively.

Effects of thermal stress on reproductive performance of dairy cows

Thermal stress reduces the length and intensity of oestrus besides increased incidence of anoestrous and silent heat in farm animals [23,24]. It increases ACTH and cortisol secretion [24], and blocks oestradiol-induced sexual behaviour [25,26]. Reported that developed follicles suffers damage and become non-viable when the body temperature exceeds 40°C. Multifactorial mechanisms involved in reducing fertility of dairy animals depending on the magnitude of thermal stress. Thermal stress reduces oocyte development by affecting its growth and maturation [24]. It increases circulating prolactin level in animal's results to acyclicity and infertility [24,27]. Moreover, 80% of oestrus may be unnoticeable during summer [28] which further reduces fertility. A period of high-temperature results to increase secretion of endometrial PGF-2α, thereby threatening pregnancy maintenance leads to infertility [29]. Plasma follicle-stimulating hormone (FSH) surge increases and inhibin concentrations decreases during thermal stress leading to variation in follicular dynamics and depression of follicular dominance that could be associated with low fertility of cattle during the summer and autumn [26]. However, FSH secretion is elevated under thermal stress condition probably due to reduced inhibition of negative feedback from smaller follicles which ultimately affect the reproductive efficiency of dairy animals [30]. Conception rates were drop from about 40% to 60% in cooler months to 10-20% or lower in summer. Embryonic growth and survival also affected during thermal stress in dairy animals. Thermal stress causes embryonic death by interfering with protein synthesis [31], oxidative cell damage [32], reducing interferon- tau production for signalling pregnancy recognition [29] and expression of stress-related genes associated with apoptosis [33]. Low progesterone secretion during thermal stress limits endometrial function and embryo development [30,32].

Mechanism of affecting live stock immune systems by thermal stress

Different stressors including heat stress induces the endocrine system to increase glucocorticoids and catecholamines. These hormones modulate the cytokine release and there by regulates immune responses. Glucocorticoid also induced lympholysis [35]. Experiments in poultry revealed that decrease in relative weights of immuneorgans like spleen, cloacal bursa andthymus observed during thermal stress. Stress induced glucocorticoids act to inhibitthe pro-inflammatory cytokines requiredto initiate an innate immune response. Stress leads to activation of hypo-thalamo-pituitary adrenal axis and ultimately the release of glucocorticoids. Chronic rise in glucocorticoid levels is inhibitory to of cytokines.

Strategies to minimize thermal stress in cattle

The various strategies include housing management, cooling systems such as misting, fogging, pad cooling, sprinkling or dripping, modified nutrition strategy and genetic selection of animals for heat tolerant genes need to be explored for management of animals during thermal stress.

Physical modifications of environment

The most common approach to combat thermal stress is to alter the cow's environment through provision of house or shade (along with feed and drinking water), evaporative cooling system with water in the form of fog, mist or sprinkling with natural or forced air movement, and possibly cooling ponds [36]. Modification of micro environment to enhance heat dissipation mechanism to relieve thermal stress is one of the most important measures to be considered in hot environment.

Optimum nutrition to mitigate thermal stress

Balanced nutrition including energy, vitamins, minerals, antioxidants, prebiotics, probiotics, symbiotic is required to alleviate thermal stress. Vitamin A maintains the mucosal and epithelial barrier [37]. Hence, it is required for maintaining udder health in milch animals. Selenium and vitamin E plays an important role in immune functionin combination. Selenium supplementation decreased incidence of clinical mastitis and somatic cell count [38]. Selenium– vitamin E supplementation positively influences chemotaxis [39], phagocytic ability [40] and oxidizing property of neutrophils [39]. Copper is essential for the development of immune organs and antibody production [41]. Zinc is involved in various stages of keratinization [42]. Zinc supplementation in summer helps in maintaining udder health by strengthening keratin layer and preventing mastitis [43]. Iron helps in the development of immune organs like thymus, spleen, lymph node and cloacalbursa. Each macro and micro nutrient are required for the proper functioning of all immune organs directly or indirectly. Probiotics are mixture of live microorganism swhich are beneficial to animal's health when given in proper amounts. These live organisms occupy the entire length of the gut and protects against pathogensentry and colonisation by a process called"competitive exclusion" or "bacterial antagonism" or "bacterial interference" [43] and strengthens the intestinal mechanical barrier. Prebiotics are said to be indigestible feed ingredients that selectively stimulating the growth of bacteriain colon [44]. Symbiotics, a combination of probiotics and prebiotics stimulates for phagocytosis and releases cytokines like TNF-alpha, IFN gamma and antibody production [45] also increase the antibody response to vaccines [46].

Genetic selection

Advances in environmental modifications and nutritional management in part alleviate the impact of thermal stress on animal performance during the hotter seasons. However, long-term strategies have to be evolved for adaptation to climate change. Differencesin thermal tolerance exist between live stock species provide clues or tools to select thermotolerant animals using genetic tools. The identification of heat-tolerant animals within high-producing breeds will be useful only if these animals are able to maintain high productivity and surviv ability when exposed to stressful conditions. Cattle with longer hair coats and darker colours are less adapted to hot environments than those with shorter hair, hair of greater diameter and lighter coat colour [47].

HSP and adaptation of cattle to thermal stress

Numerous studies have been done to identify geneticpolymorphism in HSP70 genes of cattle. The HSPs provide fortification against the adversative effects of heat and chemical or abnormal stresses [48]. The HSPs provide signalling to the immune system to encourage increased killing of pathogenic bacteria by neutrophils and macrophages and other innate immune cells against invading bacteria. The HSP70i, an inducible form ofHSP70, having a molecular weight of 70kDa has been proposed as a forecaster for thermotolerance at the cellular level in live stocks pecies. Investigations carried out to find out the association between the heat shock response of mononuclear cells in bloodand SNPs at the 5' UTR of HSP 70.1 yielded in understanding the importance of these mutation sites as molecular markers.

Conclusion

In the global climate change scenario, thermal stress is the predominant stress affecting live stock production, can also alter the immune functions of animals to bring down their production. The stress hormones –cytokine interactions are responsible for altered immune functions during thermal stress. Cell mediated immunity is adversely affected by thermal stress. It had also adversely affected the passive immunization and vaccination and gut function. Besides, various managemental and nutritional interferences genetic selection, biomarker like expression of HSPs were needs to be explored to prevent the harmful impacts of thermal stress on animal's health and welfare.

References

1. World Bank. (2009). Minding the stock: bringing publicpolicy to bear on livestock sector development.Report no. 44010-GLB.Washington DC.

2. FAO. (2009). State of food and agriculture—Livestockin the balance. Rome, FAO.

3. Wittmann, E. J., & Baylis, M. (2000). Climate change:effects on Culicoides transmitted viruses andimplications for the UK. The Veterinary Journal, 160(2), 107-117.

4. Sejian, V., Maurya, V. P., & Naqvi, S. M. K. (2011).Effect of thermal stress, restricted feeding andcombined stresses (thermal stress and restrictedfeeding) on growth and plasma reproductivehormone levels of Malpura ewes under semiaridtropical environment. Journal of AnimalPhysiology and Animal Nutrition, 95(2), 252–258.

5. Rivington, M., Matthews, K. B., Buchan, K., Miller,D., & Russell, G. (2009). Investigating climatechange impacts and adaptation options usingintegrated assessment methods. Aspects ofApplied Biology, 93, 85–92.

6. Shinde, A. K., &Sejian, V. (2013). Sheep husbandryunder changing climate scenario in India: Anoverview. Indian Journal of Animal Science,83(10), 998–1008.

7. Moberg, G. P., &Mench, J. A. (2000). The biology ofanimal stress: Basic principles and implicationsfor animal welfare. USA: CABI Pub.

8. Dhabhar, F. S. (2009). Enhancing versus suppressiveeffects of stress on immune function: implicationsfor immunoprotection and immunopathology.Neuroimmunomodulation,

16(5), 300–317.

9. Nardone, A., Lacetera, N., Bernabucci, U., & Ronchi,B. (1997). Composition of colostrum from dairyheifers exposed to high air temperatures duringlate pregnancy and the early postpartum period.Journal of Dairy Science, 80(5), 838–844.

10. Stott, G. H., Wiersma, F., Menefee, B. E., &Radwanski,F. R. (1976). Influence of environment onpassive immunity in calves. Journal of DairyScience, 59(7), 1306–1311.

11. Martin, S. W., Schwabe, C. W., &Franti, C. E. (1975).Dairy calf mortality rate: characteristics of calfmortality rates in Tulare County, California.American Journal of Veterinary Research, 36(8),1099–1104.

12. Hall, H. T. B. (1998). Diseases and Parasitesof Livestock in the Tropics. Harlow, Essex:Longman Scientific and Technical.

13. Basu, A. K., &Bandhyopadhyay, P. K. (2004). Theeffect of season on the incidence of ticks. Bulletinof Animal Health and Production in Africa,52(1), 39–42.

14. Singh, K. B., Nauriyal, D. C., Oberoi, M. S., &Baxi,K. K. (1996). Studies on occurrence ofclinicalmastitis in relation to climatic factors. IndianJournal of Dairy Science, 49(8), 534–536.

15. Sirohi, S., &Michaelowa, A. (2007). Sufferer andcause: Indian livestock and climatic change.Climatic Change, 85(3-4), 285-298.

16. Lambert, G. P., Gisolfi, C. V., Berg, D. J., Moseley,P. L., Oberley, L. W., &Kregel, K. C. (2002).Selected contribution: Hyperthermia-inducedintestinal permeability and the role of oxidativeand nitrosative stress. Journal of AppliedPhysiology, 92(4), 1750-1761.

17. Hall, D. M., Baumgardner, K. R., Oberley, T. D., &Gisolfi, C. V. (1999). Splanchnic tissues undergohypoxic stress during whole body hyperthermia.American Journal of Physiology, 276(5), 1195-1203.

18. Pearce, S. C., Mani, V., Boddicker, R. L., Johnson,J. S., Weber, T. E., Ross, J. W., Rhoads, R. P.,Baumgard, L. H., & Gabler, N. K. (2013). Heatstress reduces intestinal barrier integrity andfavors intestinal glucose transport in growingpigs. Plos-One, 8(8), 70215.

19. Goff, J. and K. Kimura. 2002. Factors Affecting the Health and Disease Resistance of The Transition Cow. PSU Dairy Cattle Nutrition Workshop. Grantville, PA.

20.Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, M. and Belyea, R. (2002) The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. Anim. Res., 51(6): 479-491

21. Wheelock, J.B., Rhoads, R.P., Van Baale, M.J., Sanders, S.R. and Baumgard, L.H. (2010) Effect of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci., 93(2): 644-655.

22. Kadzere, C.T., Murphy, M.R., Silanikove, N. and Maltz, E. (2002) Heat stress in lactating dairy cows: A review. Livest. Prod. Sci., 77(1): 59-91.

23. Kadokawa, H., Sakatani, M. and Hansen, P.J. (2012) Perspectives on improvement of reproduction in cattle during heat stress in a future Japan. Anim. Sci. J., 83(6): 439-445.

24. Singh, M., Chaudhari, B.K., Singh, J.K., Singh, A.K. and Maurya, P.K. (2013) Effects of thermal load on buffalo reproductive performance during summer season. J. Biol. Sci., 1(1): 1-8

25. Hein, K.G. and Allrich, R.D. (1992) Influence of exogenous adrenocorticotropic hormone on estrous behavior in cattle. J. Anim. Sci., 70(1): 243-247.

26. Roth, Z., Meidan, R., Braw-Tal, R. and Wolfenson, D.(2000) Immediate and delayed effects of heat stress on folliculardevelopment and its association with plasma FSHand inhibin concentration in cows. J. Reprod. Infertil.,120(1): 83-90.

27. Alamer, M. (2011) The role of prolactin in thermoregulationand water balance during heat stress in domestic animals.Asian J. Anim. Vet. Adv., 6(12): 1153-1169.

28. Rutledge, J.J. (2001) Use of embryo transfer and IVFto bypass effects of heat stress. Theriogenology, 55(1):105-111.

29. Bilby, T.R., Baumgard, L.H., Collier, R.J., Zimbelman, R.B.and Rhoads, M.L. (2008) Heat stress effects on fertility:Consequences and possible solutions. In: The Proceedingsof the 2008 South Western Nutritional Conference.

30. Khodaei-Motlagh, M.M., Zare, Shahneh, A., Masoumi, R.and Fabio, D. (2011) Alterations in reproductive hormonesduring heat stress in dairy cattle. Afr. J. Biotechnol., 10(29):5552-5558.

31. Edwards, J.L. and Hansen, P.J. (1996) Elevated temperatureincreases heat shock protein 70 synthesis in bovine two-cellembryos and compromises function of maturing oocytes.Biol. Reprod., 55(2): 340-346.

32. Wolfenson, D., Roth, Z. and Meidan, R. (2000) Impaired reproduction in heat stressed cattle: Basic and applied aspects. Anim. Rep. Sci., 60-61: 535-547.reproduction in heat stressed cattle: Basic and appliedaspects. Anim. Rep. Sci., 60-61: 535-547.

33. Fear, J.M. and Hansen, P.J. (2011) Developmental changesin expression of genes involved in regulation of apoptosisin the bovine preimplantation embryo. Biol. Reprod., 84(1):43-51

34. Hu, Y., Jin, H., Du, X., Xiao, C., Luo, D., Wang, B.,& She, R. (2007). Effects of chronic heat stresson immune responses of the foot-and-mouthdisease DNA vaccination. DNA and Cell Biology,26(8), 619-626.

35. Jain, N. C. (1993). Essentials of veterinary hematology.USA: Lea and Febiger.Janeway, C. A., &Medzhitov, R. (2002). Innateimmune recognition. Annual Review ofImmunology, 20(1), 197–216.

36. Atrian, P. and Shahryar, H.A. (2012) Heat stress in dairy cows. Res. Zool., 2(4): 31-37

37. Sordillo, L. M., Shafer-Weaver, K., & DeRosa, D.(1997). Immunobiology of the mammary gland.Journal of Dairy Science, 80(8), 1851–1865.

38. Smith, K. L., Hogan, J. S., & Weiss, W. P. (1997).Dietary Vitamin E and selenium affect mastitisand milk quality. Journal of Animal Science,75(6), 1659-1665.

39. Aziz, C. S., &Klesius, P. H. (1986). Depressedneutrophil chemotactic stimuli in supernatants ofionophore-treated polymorphonuclear leukocytesfrom selenium deficient goats. American Journalof Veterinary Research, 47(1), 148-151.

40. Grasso, P. J., Scholz, R. W., Erskine, R. J., &Eberhart,R. J. (1990). Phagocytosis, bactericidal activityand oxidative metabolism of milk neutrophilsfrom dairy cows fed selenium-supplementedand selenium-deficient diets. American Journalof Veterinary Research, 51(2), 269-274.

41. Lukasewycz, O. A., &Prohaska, J. R. (1990). Theimmune response in copper deficiency. Annalsof the New York Academy of Sciences, 587(1),147–159.

42. Paulrud, C. O. (2005). Basic concepts of the bovineteat canal. Veterinary Research Communications,29(3), 215–245.

43. Popovic, Z. (2004). Performance and Udder HealthStatus of Dairy Cows influenced by organicallybound Zinc and Chromium. Proceedings ofthe 20th Annual Symposium on NutritionalBiotechnology in the Feed and Food Industries,Lexington, KY, USA.

44. Gibson, G. R., &Roberfroid, M. B. (1995). Dietarymodulation of the human colonic microflora:introducing the concept of prebiotics. Journalof Nutrition, 125(6), 1401–1412.

45. Kabir, S. M. L., Rahman, M. M., Rahman, M. B., &Ahmed, S. U. (2004). The dynamics of probioticson growth performance and immune responsein broilers. International Journal of PoultryScience, 3(5), 361–364.

46. Hassanpour, H., ZamaniMoghaddamb, A. K.,Khosravi, M., &Mayahi, M. (2013). Effectsof synbiotic on the intestinal morphology and humoral immune response in broiler chickens.Livestock Science, 153(1), 116–122.

47. Bernabucci, U., Lacetera, N., Baumgard, L.H., Rhoads, R.P.,Ronchi, B. and Nardone, A. (2010) Metabolic and hormonalacclimation to heat stress in domesticated ruminants. J.Anim. Sci., 4(7): 1167-1183.

48. De Maio A (1999) Heat shock proteins: facts, thoughts, and dreams.Shock 11(1): 1-12.

49. Lloyd, A. B., Cumming, R. B., & Kent, R. D.(1977). Prevention of Salmonella typhimuriuminfection in poultry by pre-treatment of chickensand poults with intestinal extracts. AustralianVeterinary Journal, 53(2), 82-87.