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Quantitative expression of hepatic toll-like receptors 1–10 mRNA in Osmanabadi goats during different climatic stresses

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ABSTRACT

A study was conducted to establish the impact of heat stress, nutritional stress and the combined effect of both stresses (heat and nutrition) on the expression of toll-like receptors (TLRs) genes in liver samples of Osmanabadi goats. Twenty-four adult male Osmanabadi goats (average body weight 16.0 kg) were divided into four equal groups of six each: control (C), heat stress (HS), nutritional stress (NS) and combined stress (CS). The study was conducted over a 45 day period. The C and HS goats had *ad libitum* access to their feed while NS and CS goats were restricted feed (30% intake of C) to induce nutritional stress. The HS and CS goats were exposed to solar radiation for six hours a day between 10:00 h–16:00 h to induce heat stress. The animals were slaughtered at the end of the study and their livers were sampled for different TLRs gene expression assay. Among the different TLRs studied, TLR1, TLR3, TLR6, TLR7, TLR8 and TLR10 mRNA expressions were significantly ($P < 0.05$) higher in HS group as compared to other groups (C, NS and CS). The significantly higher levels of TLR1, TLR3, TLR6, TLR7, TLR8 and TLR10 mRNA expression in HS groups indicated that, when nutrition is not compromised, heat stressed animals were able to maintain their immune functions against heat shock proteins. This suggests that improving nutrition during heat stress condition may be highly beneficial to maintaining the immune status against heat shock proteins of the goats. The higher expression of TLR8 and TLR10 in the HS group indicates that these two genes may act as the immunological markers of heat stress in goats.

1. Introduction

The Livestock sector is the source of livelihood for about 1.3 billion world population (FAO, 2009) contributing about 53% of world agricultural GDP (World Bank, 2009). Among livestock, small ruminants are mainly reared by small, marginal and landless farmers of developing countries (Mengesha and Tsega, 2012). The native goat species are hardy animals with disease resistance mechanisms that allow them to survive in the harsh climatic conditions in tropical and sub-tropical regions.

Small ruminants especially sheep and goats have an important role in the socio-economic well-being of people in developing countries in the tropics in terms of providing nutrition, income and savings, insurance against emergencies, cultural and ceremonial purposes (Kosgey et al., 2008). Goats play a vital role in securing the livelihood of poor and marginal farmers (Escareno et al., 2013). They play a crucial role in the economy and provide a valuable contribution for stable households in developing nations.

Animals reared in the tropical environments are frequently subjected to multiple stressors due to high temperature combined with high relative humidity (Abi-Saab and Saleiman, 1995), inadequate feed and fodder with low-quality nutrients and unavailability of drinking water (Sejian, 2013). These stressors impair production, reproduction (Martin et al., 2004; Sejian et al., 2011) and also compromise the immune system, thus increasing the animal's susceptibility to diseases (Deng et al., 2012; Meng et al., 2013). The bottom line is that these stresses don't occur individually rather cumulatively in the changing climate scenario. Simulation of such stressors cumulatively under controlled conditions is quite difficult (Blanc et al., 2001) but such studies are required as the livestock sector is currently facing the threat of climatic change and global warming.

Innate immunity is one of the preliminary, evolutionary conserved mechanisms that enable the differentiation between self and non-self components through Pattern Recognition Receptors (PRRs). Toll-like Receptors are one among them and widely been studied which recognize specific signature molecules in microbes.

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The presence of these receptors in important organs and its expression in response to particular stimuli is one of the factors determining the disease resistance capability of an animal (Tirumurugaan et al., 2010). The liver is the primary metabolic organ where the protein digestion takes place. The liver also prevents various pathogens and antigens from entering the gastrointestinal tract and systemic circulation. Therefore, there is a balancing mechanism which harmonizes the immune functions in liver and helps to avoid immune-mediated damage to the organ (Knolle and Gerken, 2000).

Research efforts are needed to quantify immune responses to different environmental stresses, particularly in light of the changing climate where there are sudden outbreaks of different diseases in livestock. Therefore, an effort has been made in this study to determine the impact of environmental stresses on immune response in goats. The study was conducted with the primary objective of establishing the expression pattern of TLR genes in liver samples collected from Osmanabadi goats exposed to heat stress, nutritional stress and combined stresses (heat and nutritional).

2. Materials and methods

2.1. Location

The experiment was carried out at the National Institute of Animal Nutrition and Physiology experimental livestock farm, Bengaluru, India which is located in southern Deccan plateau of the country at longitude 77° 38′E and the latitude of 12° 58′N and at an altitude of 920 m above mean sea level. The average annual maximum and minimum ambient temperature ranges between 15–36 °C. The mean annual relative humidity (RH) ranges between 20 and 85%. The annual rainfall in this area ranges from 200 to 970 mm with an erratic distribution throughout the year. The average annual minimum and maximum temperature ranges between 15 and 22 and 27–34 °C respectively. The average annual RH ranges between 40 and 85%. The experiment was carried out during April–May. The temperature and RH variations during the study period (April–May) ranged between 24 and 38 and 30–38% respectively under hot semi-arid environment. The temperature-humidity Index (THI) values were calculated as per method described by McDowell (1972). Accordingly the formula used was $THI = 0.72\ (T_{db} + T_{wb}) + 40.6$ where, T_{db} = Dry bulb temperature in °C; T_{wb} = Wet bulb temperature in °C. The THI values 72 and less is considered comfortable; THI values between 75 and 78 is considered stressful and THI above 78 considered extreme distress. Fig. 1 describes the THI both during morning and afternoon in inside and outside the animal shed.

2.2. Animals

Osmanabadi goats are a hardy dual purpose (meat and milk) breed, originating from the semi-arid areas of central tropical India. The average body weights of adult male and female animals are 34 kg and 30 kg respectively. The breed is considered useful both for meat and milk. Average daily milk yield varies between 0.5 and 1.5 kg over a lactation length of about 4 months. In favourable conditions, the does will breed regularly twice per year and twinning is common in this breed.

The study was conducted with 24 (one-year old) Osmanabadi bucks weighing between 15–20 kg. The animals were housed in well-ventilated sheds made up of galvalume sheet roofing at the height 2.4 m and open from the side and maintained under proper hygienic conditions. Prophylactic measures against goat diseases like goat pox, peste des petits ruminants, enterotoxaemia, endo and ectoparasitic infestations were carried out as prescribed by the health calendar of the institute to ensure that the animals were in a healthy condition throughout the study.

Table 1
The ingredients and chemical composition of concentrate mixture and hybrid napier hay fed to Osmanabadi kids.

Attribute	Concentrate mixture (kg/100 kg)	Napier hay (*Pennisetum purpureum*)
Ingredients		
Maize	36	–
Wheat bran	37	–
Soybean meal	25	–
Mineral mixture	1.5	–
Salt	0.5	–
Chemical composition (%)		
Dry matter	92.9 ± 0.079	94.0 ± 0.289
Organic matter	95.9 ± 0.190	95.4 ± 0.298
Crude protein	19.6 ± 0.176	6.21 ± 0.098
Ether extract	1.82 ± 0.183	1.49 ± 0.026
Total ash	4.10 ± 0.190	4.64 ± 0.298
Fibre fractions (%)		
Neutral detergent fibre	40.4 ± 1.400	82. 9 ± 0.881
Acid detergent fibre	11.1 ± 0.239	64.6 ± 1.950
Acid detergent lignin	2.14 ± 0.029	12.3 ± 0.651
Nutritive value		
Total digestible nutrients%[a]	72.2	55.0
Digestible energy (kJ/kg)[a]	13.3	10.1
Metabolizable energy (kJ/kg)[a]	10.9	8.28

[a] Calculated values.

2.3. Technical program

The study was conducted over a period of 45 days. The bucks were randomly allocated into four equal groups of six each: control (C), heat stress (HS), nutritional stress (NS) and combined stress (CS). The animals were stall-fed with a diet consisting of 60% roughage (hybrid napier) and 40% concentrate (maize 36 kg, wheat bran 37 kg, soya bean meal 25 kg, mineral mixture 1.5 kg and common salt 0.5 kg/100 kg of feed). The chemical composition of the diet was described in Table 1. The C and NS bucks were maintained in the shed in thermo-neutral condition while HS and CS bucks were exposed outside to summer heat stress between 10:00 h–16:00 h to expose them to heat stress. C and HS bucks were provided with *ad libitum* feeding while NS and CS bucks were provided with restricted feed (30% of the intake of C) to induce nutritional stress. All four groups of animals were fed and watered individually throughout the study period. All cardinal weather parameters were recorded both inside and outside the shed. At the end of the study period, the animals were slaughtered and their liver samples were collected under aseptic condition. The study was conducted with the approval (F. No. 25/51/2015-CPCSEA) from the committee for the purpose of control and supervision of experiments on animals (CPCSEA) for subjecting the animal to both heat and nutritional stresses and to slaughter the animals for organ collection for gene expression.

2.4. Slaughter and tissue collection

The animals were slaughtered on day 46 of the experiment in a humane manner and their liver samples were collected in sterile containers containing RNA shield (Zymo Research, California, USA) and stored at −80° C until analysed.