NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 52, 2019 (3): 119–126

ISSN 1337-9984
E-ISSN 1338-0095

EFFECTS OF HEAT STRESS ON PHYSIOLOGICAL PARAMETERS AND SERUM CONCENTRATION OF HSP70 IN INDIGENOUS BREEDS OF SHEEP IN NIGERIA

Mabel Omolara AKINYEMI*, Osamede Henry OSAIYUWU, Andrew Enahoro EBOREIME

Department of Animal Science, University of Ibadan, Ibadan, Nigeria

ABSTRACT

Heat stress is one of the most challenging environmental conditions affecting livestock production especially in the tropical regions of the world. The present study was conducted to examine the physiological response and HSP70 secretion in four extensively managed indigenous sheep breeds with little access to shade. Rectal temperature (RT), Skin temperature (ST), Respiration rate (RR) and Heart rate (HR) were taken from 565 adult rams comprising 139 Uda, 88 Yankassa, 221 Balami and 117 West African Dwarf sheep in early morning and midafternoon at the peak of the dry season in Ibadan, South West Nigeria. Extracellular heat shock protein 70 (HSP70) concentration was determined by ELISA. At Temperature Humidity Index (THI) > 82 significant differences were observed between the early morning and midafternoon readings in ST in all the breeds, in RT for WAD and Yankassa and RR in Uda and WAD. In the pooled readings there was a significant difference ($p < 0.05$) between Yankassa and other breeds studied in RR. The concentration of HSP70 ranged from 69.17 to 210.71 $ng.mL^{-1}$ with the highest value recorded for Uda. The investigated breeds differ in their response to heat stress.

Key words: heat stress; sheep; heat shock protein; skin temperature; rectal temperature

INTRODUCTION

Tropical regions are characterized by high levels of temperature and relative humidity which adversely affect animal production (McManus *et al.*, 2009; Naqvi and Sejian, 2010). Thermal stress is a major constraint on animal productivity in the region as it impairs general well-being, growth, protein metabolism, energy and mineral balances, reproduction and productivity. The effect of heat stress is often aggravated when it is accompanied by high ambient humidity (Abdel-Hafez, 2002; Marai, 2007), resulting in increased tissue catabolism in the fat depots and lean body mass and a decrease in anabolic activity occasioned by a decrease in voluntary intake of essential nutrients (Marai and Habeeb, 1998; Marai *et al.*, 2007)

The magnitude of heat stress, defined as the sum of external forces that disperse body temperature from set point, is caused by combined effects of ambient temperature and relative humidity. Several indices have been used to determine the degree of heat stress affecting farm animals, the most common of which is Temperature Humidity Index (THI) (Dikmen and Hansen, 2009). THI value ≤ 74 is considered normal, 75 to 78 is alered status, 79 to 83 is danger status while values above 84 are an emergency (LCI, 1970).

In response to stress, mammals set physical, biochemical and physiological processes into play to counteract the negative effects of heat stress and maintain thermal equilibrium (Silanikove, 1992). This thermoregulatory mechanism ensures the survival and relative ability of the animals to adapt

***Correspondence:** E-mail: larakinyemi@gmail.com
Mabel Omolara Akinyemi, Department of Animal Science, University of Ibadan, Ibadan, 200284, Nigeria
Tel.: +1 2152526984, +2348055125806

Received: January 11, 2019
Accepted: March 13, 2019

to the environment. These thermoregulatory characteristics include respiration rate, rectal temperature, skin temperature, pulse rate and heart rate.

Heat shock proteins (HSPs), a large protein family highly conserved across evolutionary lines, allow cells to adapt to environmental changes and play an important roles in environmental stress tolerance and thermal adaptation (Parsell and Lindquist, 1993; Hoffman *et al.*, 2003). These molecular chaperones, which encompass several families, are classified according to molecular weight and play important physiological roles to help cope with heat stress. Among the members of this family, HSP70 is the most temperature sensitive and is induced by various physiological, pathological and environmental stressors (Beckham *et al.*, 2004). In response to heat challenge, blood flow is redirected to the periphery for enhanced heat dissipation and blood flow to the intestines is reduced, resulting in cell damage occasioned by the reduction in the supply of O_2 and nutrients leading to loss of intestinal barrier integrity (Doklandy *et al.*, 2006, Cronje, 2007) and increased intestinal permeability. This often facilitates the penetration of endotoxins, thereby causing inflammatory response (Shapiro *et al.*, 1986; Lambert, 2009). Extracellular HSP70 has been reported to promote inflammatory immune response (Doklandy *et al.*, 2006), thus changes in HSP70 may be an indication of response to thermal challenge.

Guerriero and Raynes (1990) reported that HSP70 was the main heat shock protein synthesized when lymphocytes of different livestock species were exposed to heat stress *in vitro*.

Sheep (*Ovis aries*) is one of the oldest domesticated species (Pedrosa *et al.*, 2005) and is widely distributed throughout the world due to its high plasticity and adaptability to withstand poor nutrient diets and tolerance to extreme climatic conditions (Kijas *et al.*, 2009). These animals have developed adaptive mechanism that allows their survival at high ambient temperature and humidity. However, despite their tolerance, the productivity is often reduced due to heat stress (Marai *et al.*, 2007).

In Nigeria the four predominant breeds are Balami, Uda, Yankassa and West African Dwarf (WAD). These animals are raised under the traditional system where they are grazed from one location to the other in search of fodder and tethered under the hot sun in open markets with little or no shade to attract buyers.

With the current trend in climate change and its impact on global warming, the performances of these animals are adversely affected due to heat stress in the tropical environment such as Nigeria. The need to urgently address this global trend has become imperative; one vital tool to address this challenge is through careful identification and selection of well-adapted breeds with appreciable tolerance to heat stress. The aim of this study was to compare the thermotolerant ability of the four sheep breeds as indicated by plasma concentration of HSP70 and to also evaluate the physiological response of indigenous sheep breed to elevated climatic stress.

MATERIALS AND METHODS

This study was undertaken at the Akinyele Local Government Area of Ibadan, Oyo State, South West Nigeria (7°22'39" N, 3°54'21" E, and 181 m above mean sea level) at the peak of the dry season (January to April 2015).

Five hundred and sixty-five (565) adult rams of Uda (139), Yankasa (88), Balami (221) and West African Dwarf (117) were used for this study. The animals were sampled from an open market where they are sold. The animals were 1.5 to 3 years old and were judged to be clinically healthy and free from physical abnormalities.

Data on the meteorological variables (Ambient Temperature and Relative Humidity) were monitored and recorded. The Temperature Humidity Index (THI) was calculated from the ambient temperature (AT) and the relative humidity (RH) by the following formula according to Amundson *et al.*, 2006:

$$THI = 0.8 \times AT\ °C + (RH, \%) \times (AT\ °C\text{-}14.4)/\ 100) + 46.4$$

Rectal temperature, Skin temperature, Respiratory rate, Heart rate and Pulse rate were measured in each of the animals studied. Heart rate was measured by palpitation of the jugular artery using a stethoscope. Respiratory rates were recorded by counting the number of flank movements and reported as breaths per minute. Rectal temperatures were recorded in degrees

Celsius using a standard digital thermometer. Pulse rate was determined for each animal by placing fingertips on the femoral artery of the hind limb and counting the number of beats per minute. Skin temperature was measured using non-contact infrared thermometer (VMR Scientific Horiba) on the shaved portion on the back of the animal. Readings were taken in the early morning hours (8:00) and in the afternoon (14:00).

Handling of the animals, which can be classified as a stressor, was minimized when recordings were taken.

The climatic data – ambient temperature (AT, °C) and relative humidity (RH %) – were recorded using a digital hygrometer throughout the study.

Heat Shock Protein 70 (HSP70) Analysis

Blood samples for HSP70 determination was collected from 50 animals per breed from the jugular vein into 5 mL tubes without anticoagulant and was allowed to clot. The samples were then centrifuged at 1400 x g for 10 minutes. Serum separator tube was used to separate the serum. The serum samples were then frozen and stored for laboratory analysis. Serum Hsp70 concentration was measured according to the manufacturers protocol of a commercially available HSP70 Enzyme-linked immunosorbent assay (ELISA) kit (USCN Life Science Inc, Wuhan, China).

Statistical Analysis: Descriptive statistics for the physiological variables was generated for each breed. The data generated were subjected to analysis of variance (ANOVA) using the SAS package (2008). Means generated were separated using DMRT of the same software. Values obtained for the serum concentration of HSP70 for each breed were subjected to analysis and the differences were considered as significant at $P < 0.05$.

RESULTS

The prevalent physiological parameters recorded in the early morning and midafternoon hours in the peak of the dry season in Ibadan, Oyo State Nigeria at THI > 82 are documented in this study (Figure 1).

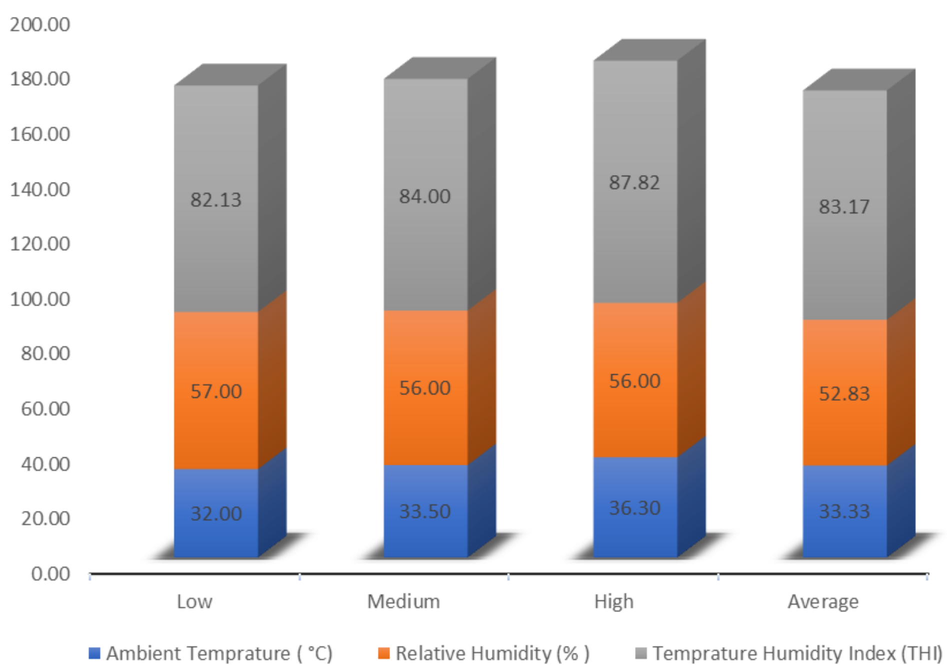

Figure 1. Meteorological Data during the experimental period

The temperature humidity index THI suggests that the animals were exposed to considerable heat load during the period of the study. The observed physiological parameters of extensively reared animals as affected by heat stress in this study are presented in Table 1 and 2. Significant differences were observed in the ST between early morning and midafternoon readings in all the breeds sampled. Differences in RT and RR were also significant in WAD and Yankassa and Uda and WAD respectively. The average daily value of rectal temperature (RT) was highest in WAD and the lowest value was recorded for Yankassa. However, there was no significant difference in the values obtained for Balami and WAD. Pulse rate was significantly higher in WAD (95.41), the lowest value (87.40) recorded for Yankassa was not significantly different from 90.08 observed in Uda. There was no significant difference among the values recorded for heart rate as the values ranged from 98.61 in Uda to 102.24 in WAD.

Table 1. Effect of Heat Stress on Physiological Parameters of Sheep Breeds at THI ≤ 82

Parameters	Balami	UDA	WAD	Yankasa
RT (°C)	38.34 ± 0.52^{a}	38.16 ± 0.43^{b}	38.43 ± 0.51^{a}	38.14 ± 0.48^{b}
PR (beat.min^{-1})	91.24 ± 12.72^{b}	90.08 ± 11.11^{bc}	95.41 ± 18.78^{a}	87.40 ± 13.12^{c}
HR (beat.min^{-1})	100.82 ± 16.11	98.61 ± 14.75	102.24 ± 18.48	98.72 ± 14.09
RR (breath.min^{-1})	92.38 ± 22.28^{b}	88.84 ± 17.19^{b}	90.65 ± 15.40^{b}	107.39 ± 20.93^{a}
ST (°C)	37.28 ± 0.65^{ab}	37.34 ± 0.50^{a}	37.18 ± 0.32^{b}	37.23 ± 0.76^{ab}

Rectal temperature (RT), Pulse rate, Heart rate (HR), Respiration rate (RR), Skin temperature (ST). Mean ± SD.
Mean with different superscript along the same row are significantly different (P < 0.05).

Table 2. Physiological parameters in the studied population as influenced by time of the day

	RT		PR		HR		RR		ST	
Breed	Early	MidDay	Early	MidDay	Early	MidDay	Early	MidDay	Early	MidDay
Balami	38.21	38.31	87.69^{b}	91.06a	95.76^{b}	100.34^{a}	93.26	91.06	36.60^{b}	37.30^{a}
Uda	38.10	38.15	87.97	90.23	95.78	98.76	78.08^{b}	88.86^{a}	37.15^{b}	37.34^{a}
WAD	38.17^{b}	38.42^{a}	91.72^{b}	95.61^{a}	98.07	102.40	80.12^{b}	90.37^{a}	37.02^{b}	37.17^{a}
Yankassa	37.76^{b}	38.13^{a}	86.50	87.39	97.27	98.71	103.23	107.38	36.72^{b}	37.22^{a}

Rectal temperature (RT), Pulse Rate, Heart rate (HR), Respiration rate (RR), Skin temperature (ST).
Mean with different superscript along the same row are significantly different (P < 0.05).

The result showed no significant difference (P > 0.05) in RR between Balami, Uda and WAD but Yankasa was significantly different (P < 0.05). The highest value was recorded for Yankasa and the lowest for Uda.

Serum HSP70 differ significantly in the breeds studied (p < 0.05). The concentration ranged from 69.17 to 210.71 ng.mL^{-1}, the highest concentration value recorded for Uda while the lowest was for the WAD (Table 3).

Table 3. Effect of Heat Stress on Heat Shock Protein 70 (HSP70) Serum Concentration in Nigerian Indigenous Sheep Breeds

Breed	HSP70 mean (ng.mL^{-1}) ± SE
Uda	210.71 ± 38.75^{a}
Yankasa	173.28 ± 36.24^{a}
Balami	82.13 ± 19.01^{b}
WAD	69.17 ± 19.50^{b}

Mean with different superscript along the same column are significantly different (P < 0.05).

DISCUSSION

In this study, physiological parameters at THI 82 indicate that the animals were heat stressed. Readings taken in the early morning hours (8:00) were compared with readings taken in the afternoon (14:00) to evaluate the effect of the change in temperature on physiological parameters. The increase of the body core temperature and rectal temperature (RT) have been considered good indicators of heat stress in animals (Alamer and Al-Hozab, 2004). RT observed in this study ranged from (38.07 to 38.63 °C at 14:00) and is similar to the values reported by Fadare *et al.* (2012) in heat-stressed WAD sheep. However, Buswat *et al.* (2000) reported slightly higher RT values of 39.6, 39.7 and 39.7 °C in Yankassa, Uda and Balami breeds at the peak of the hot season in Bauchi, Northern Nigeria. The disparity in the values may be due to the differences in the climatic region of the study locations. RT values in the early morning (8:00) and midafternoon (14:00) were significantly different in WAD and Yankassa breeds. Rectal temperature is often used as a representative measurement of the core body temperature and has been reported to be perhaps the most reliable indicator of heat stress as it drives other heat stress alleviating mechanisms (Gerbremedhin *et al.*, 2008). This important physiological mechanism offers a valuable window into the stress faced by vulnerable internal organs during periods of extreme hyperthermia and increases when the body is unable to counteract the effect of excessive heat load (Gagnon *et al.*, 2010). Alhidary *et al.* (2012) and Lallo *et al.* (2011) also reported high RT values in goats following exposure to high ambient temperature.

Pulse rate increases on exposure to high environmental temperature (Aboul-Naga, 1987) and appears to signal the immediate response of sheep to the environmental stress (Butswat *et al.*, 2000). This increase leads to a rise in blood flow from the core to the surface to allow for more heat to be lost by sensible and insensible means thus as ambient temperature increases pulse rate and blood circulation increases to transfer heat from the core to the surface (Marai *et al.*, 2007). This trait has been reported to be significantly higher in the summer months. In this study pulse rate ranged from 87.40 ± 13.12 and 95.41 ± 18.78 and higher than the values reported for heat stressed Balami, Uda and Yankassa by Butswat *et al.* (2000. However, the least PR value observed in Yankassa in this study was also reported by Butswat *et al.* (2000) The observed accelerated pulse rate (PR) could be due to the redistribution of blood to the peripheral tissues during heat exposure in sheep and goat as reported by Silanikove (2000b). These findings support the previous reports on other sheep breeds (Marai *et al.*, 2009, McManus *et al.*, 2009b).

Heart rate in the studied population ranged from 98.61 ± 14.75 to 102.24 ± 18.48 however no significant difference was observed among the breeds. Significant difference was only observed in the Balami breed between the early morning and midafternoon readings. This trait has been reported to accelerate during the peak hour of the heat load in animals with unrestricted access to water due to cutaneous blood flow (Alexiev *et al.*, 2004.).

Silanikove (2000a) reported that the respiration rate was a practical and reliable measure of heat load and indicated that respiration rate above 80 breaths per minute is an indication of severe heat stress. RR for all the breeds was above the basal respiration rate in sheep (25-30 breaths per min; Hales and Brown, 1974). Significant differences were observed in the early morning and midafternoon readings in Uda and WAD breeds. The observed respiratory rates of Balami, Yankasa, Uda and West African Dwarf (WAD) sheep in this study ranged from (88.84 ± 17.19 to 107.39 ± 20.93 breaths per minute) indicating that the animals were exposed to severe heat stress. This finding is in agreement with Kumar (2005) who also reported that increased respiratory rate (RR) is the first response when animals are exposed to environmental temperature above the thermoneutral zone. Alamer and Al-Hozab (2004) stated that respiration rate can be used as an indicator of heat stress, and to estimate the adverse effects of environmental temperature. The values reported in this study are higher than that reported by Butswat *et al.* (2000) (62.2, 64.9 and 66.0 breaths per minute) in three of the four breeds sampled in this study. This can be attributed to the high relative humidity that characterizes the location of this study. Relative humidity determines the rate at which sweat is evaporated from the skin, increase in RH makes it difficult for the circulating air to absorb sweat from the skin of the animal.

Thus when animals are exposed to high temperature and high relative humidity their ability to lose heat by evaporation is impaired. Thus, heat stressed animals in areas of high humidity which characterizes southern Nigeria may have higher respiration rate (Hales and Brown, 1974).

The mammalian skin provides an excellent pathway for heat dissipation from the body surface to the environment. Thus, skin temperature is the result of the adjustment of skin to blood flow and heat regulation. Skin temperature of sheep differs according to season of the year and time of the day and it becomes higher with an elevation in ambient temperature (Marai *et al.*, 2007). ST readings for 8:00 and 14:00 were significantly different in all the breeds studied. It is corroborated by Marai *et al.* (2007) that ST of sheep differs according to season of the year and time of the day and increases with an elevation in temperature. In this study ST follows the same trend as RT with higher ST recorded in the Uda sheep however the value was not significantly different from that obtained for Yankassa and Balami. The observed range for ST in the studied population is 37.18 to 37.34 °C higher than the values reported by Catanherrra *et al.* (2010) in some Brazilian sheep breeds. but comparable to the range reported by Marai *et al.* (2007) in Ossimi breed. The values obtained for the indigenous sheep under hot conditions could be attributed to prevalent heat stress and more importantly to the shade deprivation experienced by the animals in this study, which has been reported to cause vasodilatation of skin capillary bed and consequently an increase in the blood flow to the skin surface to facilitate heat dissipation (McManus *et al.*, 2009b).

Hsp70 in particular has been shown to respond to both acute, short term stress (Tomanek and Sanford, 2003) and chronic, long term stress (Helmuth and Hofmann, 2001), and its level appears to be the best predictor of heat tolerance (Sorte and Hofmann, 2005). Cellular and extracellular heat shock proteins have been reported to be associated with stress and higher levels of secretion linked to increased resistance (Sorensen *et al.*,2003; Kristensen *et al.*, 2006). In the present study, there were higher levels of Hsp70 expression in the Uda (210.71 ± 38.75) and Yankasa (173.28 ± 36.24) than that of Balami (82.13 ± 19.01) and WAD (69.17 ± 19.50). Uda had the highest HSP70 level and suggests a better physiological coping mechanism in the breed to the prevalent environmental stress. These findings are in agreement with Horowitz (2001) that the changes in HSP transcription are due to intermediate messengers responding to changes in ambient heat but not body temperature, it would appear that once animals receive a significant stress challenge the HSP response is elicited; and according to Xiao *et al.* (2002) reported that HSP70 can confer transient protection against the adverse effects of subsequent heat and chemical or abnormal stresses. The observed difference in the secretion of HSP70 among the breeds in the studied population following heat stress is also similar to the reported increase in the expression of HSP70 when animals are exposed to hot conditions, (Liu *et al.*, 2010) and Cao *et al.* (2009) reported that higher heat stress level led to higher concentration of HSP70 in the testis and epididymis of mice. It has been postulated that differences in HSP concentrations between species may be due to differences in their thermo-tolerance (Agnew and Colditz, 2008). This may also apply within a species. The range in HSP70 concentration in the present study is higher than the range (0.03 to 2.85 $ng.mL^{-1}$) for Pelibeuy and Suffolk sheep breeds reported by Romero *et al.*, 2013 and for Angus steers (0.54 to 19.75 $ng.mL^{-1}$) reported by Gaughan *et al.* (2014). The differences in the HSP concentration among individual animals in the current study may reflect within-breed and among-breed variations with respect to thermo-tolerance or stress in general.

The differences in HSP concentration may reflect breed variations, however since a relatively small population was used in the study, further research work will be needed in the indigenous sheep breeds.

CONCLUSION

The physiological parameters recorded and HSP70 secretion during the dry season in this study indicate that the prevailed environmental condition during this season has adverse impact on the indigenous sheep breeds. The effect of heat stress on the extensively managed sheep should be minimized for their optimum performance through the provision of regular supply of clean water and adequate shade. Further research on the expression of heat shock proteins at the thermoregulatory organs to determine the thermotolerance ability of the breeds is recommended.

REFERENCES

Abdel-Hafez, M. A. M. (2002). *Studies on the reproductive performance in sheep*. Ph.D. thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Aboul-Naga, A. I. (1987). *The role of aldosterone in improving productivity of heat-stressed farm animals with different techniques*. Ph.D. thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Alamer, A. & Al-Hozab, A. (2004). Effect of water deprivation and season on feed intake, body weight and thermoregulation in Awassi and Najdi sheep breeds in Saudi Arabia. *Journal of Arid Environment*, 59, 71–84.

Alhidary, I. A., Shini, S., Al Jassim, R. A. M. & Gaughan, J. B. (2012). Physiological response of Australian Merino wethers exposed to high heat load. *Journal of Animal Science*, 90(1), 212–220.

Agnew, L. L. & Colditz, I. G. (2008). Development of a method of measuring cellular stress in cattle and sheep. *Veterinary Immunology and Immunopathology*, 123, 197–204.

Alexiev, J., Gudev, D., Popova-Ralcheva, S. & Moneva, P. (2004). Thermoregulation in sheep. IV. Effect of heat stress on heart rate dynamics in shorn and inshorn ewes from three breeds. *Zhivotnovodni-Nauki*, 41(1), 16–21.

Amundson, J. L., Mader, T. L., Rasby, R. J. & Hu, Q. S. (2006). Environmental effects on Pregnancy Rate in Beef Cattle. *Journal of Animal Science*, 84(12), 3415–3420.

Beckham, J. T. , Mackanos, M. A., Crooke, C., Takahashi, T., O'Connell- Rodwell, C., Contag, C. H. & Jansen, E. D. (2004). Assessment of cellular response to thermal laser injury through bioluminescence imaging of heat shock protein 70. *Photochemistry and Photobiology*, 79, 76–85.

Butswat, I. S., Mbap, S. T. & Ayibantoye, G. A. (2000). Heat tolerance of sheep in Bauchi, Nigeria. *Tropical Agriculture (Trinidad)*, 77(4), 265–268.

Castanheira, M., Parva, S. R., Louvandini, H., Landim, A., Fiorvanti, M. C. S., Dallago, B. S., Correa, P. S. & Mcmanus, C. (2010). Use of heat tolerance traits in discriminating between groups of sheep in central Brazil. *Tropical Animal Health and Production*, 42, 1821–1828.

Cao, W., Huang, P., Zhang, L., Wu, H. Z., Zhang, J. & Shi, F. X. (2009). Acute heat stress increases HSP70 expression in the testis, epididymis and vas deferens of adult male mice. *National Journal of Andrology*, 15(3), 200–206.

Cronje, P. B. (2007). Gut health, osmoregulation and resilience to heat stress in poultry. In: *Proceeding of the 19th Australian Poultry Science Symposium*, Sydney, New South Wales, Australia, 12 – 14 February 2007, p. 9–13.

Dikmen, S. & Hansen, P. J. (2009). Is Temperature-Humidity Index the best indicator of heat stress in lactating dairy cows in a subtropical environment. *Journal of Dairy Science*, 92(1), 109-116.

Doklandy, K., Moseley, P. L. & Ma, T. Y. (2006). Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. *American Journal of Physiology, Gastrointestinal and Liver Physiology*, 290, G204–212.

Fadare, A. O., Peters, S. O., Yakubu, A., Sonibare, A. O., Adeleke, M. A., Ozoje, M. O. & Imumorin, I. G. (2012). Physiological and haematological indices suggest superior heat tolerance of white-coloured West African Dwarf sheep in the hot humid tropics. *Tropical Animal Health and Production*, 45, 157–165.

Gagnon, D., Lemire, B. B., Casa, D. J. & Kenny, G. P. (2010). Cold-Water Immersion and the Treatment of Hyperthermia: Using 38.6 °C as a Safe Rectal Temperature Cooling Limit. *Journal of Athletic Training*, 45(5), 439-444.

Gebremedhin, K. G., Hillman, P. E., Lee, C. N., Collier, R. J., Willard, S. T., Arthington, J. E. & Brown Brandl, T. M. (2008). Sweating rates of dairy cows and beef heifers in hot conditions. *Transactions ASABE*, 51, 2167–2178.

Guerriero, J. R. V. & Raynes, A. D. (1990). Synthesis of heat shock proteins in lymphocytes from livestock. *Journal of Animal Science*, 68, 2779–2783.

Hales, J. R. S. & Brown, G. D. (1974). Net energetic and thermoregulatory efficiency during panting in the sheep. *Comparative Biochemistry and Physiology*, 49A, 413–422.

Helmuth, B. & Hofmann, G. E. (2001). Microhabitats, thermal heterogeneity and physiological gradients of stress in the rocky intertidal zone. *Biological Bulletin*, 201, 374–384.

Hoffmann, A. A., Sorensen, J. G. & Loeschcke, V. (2003). Adaptation of Drosophila to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology*, 28, 175–216.

Kijas, J. W., Townley, D., Dalrymple, B. P., Heaton, M. P. & Maddox, J. F. (2009). A Genome Wide Survey of SNP Variation Reveals the Genetic Structure of Sheep Breeds. *Plos One*, 4(3), e4668.

Kristensen, T. N. & Løvendahl, P. (2006). Physiological responses to heat stress and their potential use as indicators of reduced animal welfare in Jersey calves. *Acta Zoologica Sinica*, 52, 681–689.

Kumar, V. (2005). *Effect of thermal stress management on nutritional, physiological and behavioural responses of buffalo heifers*. PhD. Thesis. Deemed University, Indian Veterinary Research Institute, Izatnagar.

Lallo, C. H. O., Paul, I. & Bourne, G. (2012). Thermoregulation and performance of British Anglo-Nubian and Saanen goats reared in an intensive system in Trinidad. *Tropical Animal Health and Production*, 44(3), 491–496.

Lambert, G. P. (2009). Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. *Journal of Animal Science*, 87(E. Suppl.), E101–E108.

LCI (1970). *Patterns of transit losses*. Livestock Conservation Inc., Omaha, NE.

Liu, Y. X., Li, D. Q., Cui, Q. W., Shi, H. X. & Wang, G. L. (2010). Analysis of HSP70 mRNA level and association between linked microsatellite loci and heat tolerance traits in dairy cows. *Hereditas (Beijing)*, 32(9), 935–941.

Marai, I. F. M., Bahgat, L. B., Shalaby, T. H. & Abdel-Hafez, M. A. (2007a). Fattening performance, some behavioral traits and physiological reactions of male lambs fed concentrates mixture alone with or without natural clay, under hot summer of Egypt. *Journal of Annals of Arid Zone*, 39, 449–460.

Marai, I. F. M., El-Darawany, A. A., Fadiel, A., Abdel & Hafez, M. A. M. (2007b). Physiological traits as affected by heat stress in sheep: A review. *Small Ruminant Research*, 71, 1–12.

McManus, C., Paluda, G. R., Louvandini, H., Gugel, R., Sasaki, L. C. B. & Paiva, S. R. (2009). Heat tolerance in Brazilian sheep: Physiological and blood parameters. *Tropical Animal Health and Production*, 41, 95–101.

NAQVI, S. M. K. & SEJIAN, V. (2010). Physiological adaptation of sheep to hot environmental conditions with special references to climate change. In: *Climate change and stress management: sheep and goat production*: 1st Ed, Hakyawar D.B, Naqvi S.M.K, Tripathi BN, eds): Satish, Delhi, pp: 259–282.

Parsell, D. A. & Lindquist, S. (1993). The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annual Review of Genetics*, 27, 437–496.

Pedrosa, S., Uzun, M., Arranz, J. J., Gutierrez-Gill, B. & Primitivo, F. S. (2005). Evidence of three maternal lineages in near eastern sheep supporting multiple domestication events. *Proceedings of the Royal Society B-Biological Sciences*, 272: 2211–2217.

Romero, R. D., Pardo, A. M., Montaldo, H. H., Rodríguez, A. D. & Hernández-Cerón, J. (2013). Differences in body temperature, cell viability, and HSP-70 concentrations between Pelibuey and Suffolk sheep under heat stress. *Tropical Animal Health and Production*, 45(8), 1691–1696.

SAS (2008). *Statistical Analysis System SAS Stat Version 9.0.* SAS Institute Inc, Cary.

Shapiro, Y., Alkan, M., Epstein, Y., Newman, F. & Magazanik, A. (1986). Increase in rat intestinal permeability to endotoxin during hyperthermia. European *Journal of Applied Physiology and Occupational Physiology*, 55, 410–412.

Silanikove, N. (1992). Effects of water scarcity and hot environment on appetite and digestion in ruminants: A review. *Livestock Production Science*, 30, 175–193.

Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science*, 67, 1–18.

Sorte, C. J. B & Hoffman, G. E. (2005). Thermotolerance and heat shock protein expression in Northeastern Pacific Nucella species with different biogeographical ranges. *Marine Biology*, 149, 985–993.

Sorensen, J. G., Kristensen, T. N. & Loeschcke, V. (2003). The evolutionary and ecological role of heat shock proteins. *Ecology Letters*, 6, 1025–1037.

Tomanek, L. & Sanford, E. (2003). Heat-shock protein 70 (Hsp70) as a biochemical stress indicator: an experimental field test in two congeneric intertidal gastropods (Genus: Tegula). *Biology Bulletin*, 205, 276–284.

Xiao, C., Chen, S., Li, J., Hai, T., Lu, Q., Sun, E., Wang, R., Tanguay, R. M. & Wu, T. (2002). Association of HSP70 and genotoxic damage in lymphocytes of workers exposed to coke-oven emission. *Cell Stress Chaperones*, 7(4), 396–402.