Journal of Animal & Plant Sciences, 2011. Vol. 11, Issue 3: 1474-1480
*Publication date: 31/10/2011, http://www.biosciences.elewa.org/JAPS; **ISSN 2071 - 7024***

Comparative assessment of erythrocyte osmotic fragility of apparently healthy goat and cattle during the hot-dry and harmattan season in Makurdi, Nigeria

Adenkola, A.Y., Agbendeh, J., Okpe, J.
[1] *Department of Physiology Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria.*

Corresponding author address E-mail: aadenkola@yahoo.com Tel +2348054977696
Key words: Erythrocyte osmotic fragility, Cattle, Goat, Harmattan Season, Hot-dry Season

1 SUMMARY

The experiments were conducted with the aim of determining which of the two seasons, hot-dry or harmattan, is more stressful to goat and cattle in the Southern Guinea Savanna zone of Nigeria, using Erythrocyte Osmotic Fragility (EOF) as an index. A total of two hundred and twenty animals of both sexes were used for the study, comprising of 110110 goats and 110 cattle. One hundred and ten animals during the hot-dry season and 110 animals during harmattan. Approximately 5 mls of blood was collected from each animal during slaughtering into a sample bottle containing 2 mg/ml of sodium salt of ethylene diaminetetra acetic acid (NaEDTA) as an anticoagulant. After collection the samples were transferred in an ice pack immediately to Physiology Laboratory department of Physiology, Pharmacology and Biochemistry, University of Agriculture, Makurdi, where erythrocyte osmotic fragility test was carried out on the blood using the standard method. The meteorological data recorded during the study period were collected and collated from the Nigeria Meteorological Centre (NIMET) Makurdi. The maximum ambient temperature recorded during the hot-dry season was 38.06 ± 0.39^0 C which was significantly (P < 0.05) higher than 34.53 ± 0.35^0 C obtained during the harmattan season, while the maximum relative humidity obtained during the hot-dry season was 61.00 ± 3.05 % and was significantly higher (P < 0.05) than the value of 46.00 ± 0.32^0 C obtained during the harmattan season. The results indicated a higher value which was significantly (P < 0.05) different, was obtained in goat at percent sodium chloride concentration of 0.4 %, 0.5 %, 0.6 %, 0.7 % during harmattan season which now shifted the fragiligram towards left. In conclusion harmattan season characterized by high ambient temperature (AT) and dusty-wind affects the erythrocyte osmotic fragility of cattle and goat. However goat' erythrocytes were more susceptible to osmotic lysis than those of cattle indicating that goats were more stressed during the harmattan season than cattle. This study therefore showed that harmattan season was more stressful, hence the animal should be subjected to minimal environmental stress during this period, in order to improve their health and productivity.

2 INTRODUCTION

Livestock play an important role in Nigeria agriculture contributing about 12.7 % of the gross domestic product (GDP). Livestock subsector provides an important role in the Nigerian economy not only in terms of its contribution of GDP, but also contribute substantial supplies of animal protein (FDLPCS, 1992; Oluwafemi 2009). The productivity and welfare of these livestock, however, are dependent on the environment in which they live (Bouraoui, *et al.*, 2006) which may

have effects directly on livestock's health and productivity.

In the tropical conditions of Nigeria, the climatic season comprises of rain and dry season and the dry season occur between November and May (Igono *et al*., 1982) with a mean monthly rainfall of less than 51mm (Walter, 1969). The dry season comprises of harmattan and hot-dry season. The harmattan season in Nigeria is a cold-dry season occurring between the month of November and February (Igono *et al.,* 1982; Oladele *et al.,* 2003) and it is characterized by high ambient temperature (AT) in the afternoon hours of the day and relatively AT of about 10^0 C in the evening and early morning hours of the day. During the season, the wind is cold-dry and dust laden (Igono and Aliu, 1982). The hot-dry season is also characterized by high AT and RH and long duration of sunshine. These seasons (hot-dry and harmattan) constitutes environmental stress to livestock (Adenkola, *et al*., 2010). Stress is the responses of the body to extraneous stimuli that disturb the normal physiological equilibrium or homeostasis (Khansari *et al*., 1990; Mstl and Palme, 2002). Under this stressful environmental condition, the concentration of antioxidant vitamins decreases, lipid peroxidation as a result of free radicals formation which increases in the plasma and tissues leading to damage of cell membrane (Sahin *et al*., 2001). Free radicals are known to play a pivotal role in tissue damages as well as adverse effects on erythrocyte (Avellini et al., 1995; Adenkola and Ayo, 2009).

There is paucity of information on the relationship between meteorological and the erythrocyte osmotic fragility (EOF) of goat and cattle reared in the Southern Guinea Savannah zone of Nigeria. This parameter has been demonstrated to be an important index of quantifying oxidative stress indirectly in livestock (Adenkola, 2010). The aim of the present study was to determine which of the two seasons, hot-dry or harmattan, is more stressful to goat and cattle in the Southern Guinea Savanna zone of Nigeria, using EOF as an index.

3 MATERIALS AND METHODS

3.1 Experimental Site: This study was conducted during the harmattan season in Makurdi Benue state, Nigeria. Makurdi (07^0 N, 08^0 37/ E) is located along River Benue, which is very warm, with daily temperature ranging from 26.5 – 42^0 C. The area has an annual rainfall of 1,317 – 1,323 mm which spans 6 -7 months (Adenkola *et al*., 2010).

3.2 Experimental Design: A total of two hundred and twenty (220) animals were used for the study, comprising of110 goats and 110 cattle during the hot-dry and harmattan season respectively. Approximately 5 mls of blood was collected from each animal during slaughtering into a sample bottle containing 2 mg/ml of sodium salt of ethylene diaminetetra acetic acid (NaEDTA) as an anticoagulant. After collection the samples were transferred in an ice pack immediately to Physiology Laboratory department of Physiology, Pharmacology and Biochemistry, University of Agriculture, Makurdi, where erythrocyte osmotic fragility test was carried out on the blood using the method of Faulkner and King (1970).

3.3 Meteorological Data: The meteorological data recorded during the harmattan season (December to January) and during the hot-dry season (February to April) which include ambient temperature (AT) relative humidity (RH), rainfall, sunshine hour per day, wind speed and wind direction were collated for the period from Nigerian Meteorological Centre (NIMET) Makurdi.

3.4 Erythrocyte Osmotic Fragility Determination: Sodium chloride solution was prepared according to Faulkner and King (1970) in concentration ranging from 0.1 to 0.85 at pH 7.4. A set of 10 test tubes (containing 5 ml of sodium chloride solution) where arranged serially in a test tube rack to analyze each sample. The test tubes were labeled with corresponding sodium chloride concentrations. One ml pipette was used to transfer 0.02 ml of blood into each of the ten test tubes. The content was then mixed by gently inverting the test tubes for about 3 times. The test tubes were allowed to stand at room temperature (26 - 27^0 C) for 30 minutes. The contents of the test tubes were maintained at pH 7.5 thereafter the contents of the test tube were centrifuged at 1,500 g for 20 minutes. The supernatant of each test tube was transferred into a cuvette. The concentration of haemoglobin in the supernatant solution was measured at 540 nm using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, England) by reading the absorbance. The same procedure was repeated for every blood sample used for the study. The percent

haemolysis was then calculated using the formula (Faulkner and King, 1970)

Percent haemolysis =

$$\frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 100$$

Erythrocyte osmotic fragility curve was obtained by plotting percent haemolysis against the sodium chloride concentrations.

3.5 Statistical Analysis: All data obtained were subjected to statistical analysis using Student's *t*-test. Data were expressed as Mean ± Standard error of mean. Values of $P < 0.05$ were considered significant.

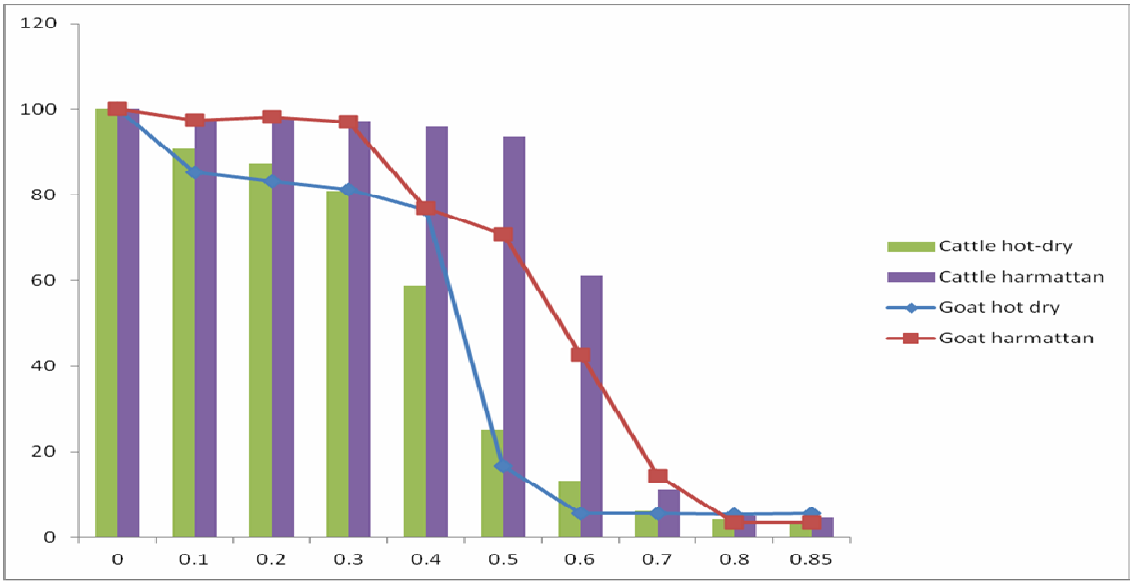
4 RESULTS

The maximum AT recorded during the hot-dry season was 38.06 ± 0.39^0 C which was significantly ($P < 0.05$) higher than 34.53 ± 0.35^0 C obtained during the harmattan season, while the maximum RH obtained during the hot-dry season was 61.00 ± 3.05 % and was significantly higher ($P < 0.05$) than the value of 46.00 ± 0.32^0 C obtained during the harmattan season. The lowest AT of 16.32 ± 0.43^0 C was recorded during the harmattan season which was significantly lower ($P < 0.05$) than the corresponding value 26.20 ± 0.15^0 C obtained during the hot-dry season. The wind speed was significantly ($P < 0.05$) higher during the harmattan season with a value of 42.00 ± 2.60 m/ sec than 2.60 ± 0.00 m/ sec recorded during the hot-dry season. The wind direction fluctuates from east to north- east during the hot-dry season to predominantly north eastern direction during the harmattan season and this was accompanied with moderate breeze and maximum dust. There was no significant ($P < 0.05$) difference in the sunshine duration during the two seasons (Table 1). The minimum EOF value of 3.47 ± 0.48 % recorded in cattle at 0.8 % NaCl concentration while the corresponding value in the goat was 4.96 ± 0.67 %. A higher value which was significantly ($P < 0.05$) different was obtained in goat at percent sodium chloride concentration of 0.4 %, 0.5 %, 0.6 %, 0.7 % (Figure 1) which now shifted the fragiligram towards left.

Table 1: Meteorological Parameters during the Study Period

Meteorological Parameters	Hot - Dry	Harmattan
Ambient Temperature Maximum	38.06 ± 0.39*	34.53 ± 0.35*
Ambient Temperature Minimum	26.20 ± 0.15*	16.32 ± 0.43*
Rainfall (mm)	12.6 ± 0.00	0.00
Relative Humidity High (%)	61.00 ± 3.05*	46.00 ± 0.32*
Relative Humidity Low (%)	35.00 ± 4.58*	22.5 ± 3.50*
Sunshine (hr/ day)	7.90 ± 0.67*	8.44 ± 0.34
Wind Speed (m/sec)	2.60 ± 0.00*	42.00 ± 2.60*
Wind Direction	North East	North East

Journal of Animal & Plant Sciences, 2011. Vol. 11, Issue 3: 1474-1480
*Publication date: 31/10/2011, http://www.biosciences.elewa.org/JAPS; **ISSN 2071 - 7024***



5 DISCUSSION

5.1 Meteorological Data during Harmattan Season: The high AT recorded during the period of study (hot-dry and harmattan) are predominantly outside the thermo-neutral zone of 25^0 C – 30^0 C established for ruminants (Tarr, 2007) and this was not conducive for their normal thermoregulation. The meteorological data also indicated that there was no rainfall what so ever especially during the harmattan period and that harmattan period was characterized by maximum dusty and windy condition (Igono *et al*., 1982; Adenkola *et al*., 2009). It has been observed that temperature and moisture of air are two major environmental factors controlling stress in livestock (Bouraoui *et al*., 2002; St-Pierre *et al*., 2003.) Meteorological results obtained during the present study agrees with the previous findings that the harmattan season is thermally stressful to livestock (Adenkola and Ayo 2006; Adenkola *et al*., 2009) of all the three seasons in (harmattan, hot-dry and rainy season) in the Southern Guinea Savannah zone of Nigeria. This is because AT could be very low in the early morning hour and late evening while it is very high in afternoon hours this poses a lot of challenges to homeostatic controlling mechanism of the system. This stressful harmattan season could lead to increasing physiological stress associated with failure of several homeostatic systems, tissue dysfunction and damage, metabolic derangements and activation of the hypothalamus pituitary axis (HPA) as well as generation of free radicals which may impair homeostatic mechanisms resulting into pathological changes (Teeter *et al*., 2005) as well as causing irritation and some degree of psychomotor disturbances (Rakesh and Amit, 2004).

5.2 Erythrocyte Osmotic Fragility of Goat and Cattle: The normal functionality of the erythrocyte is based on its ability to maintain its membrane integrity. The compromization of the erythrocyte membrane integrity resulting in increased EOF may have arisen from the increased lipoperoxidative changes which lead to the destruction of erythrocytes being more in the animals during harmattan than hot-dry season. This is evidenced by increase in haemolysis which is more during the harmattan season apparently leads to massive release of transition metals like iron (Fe^{2+}) and copper (Cu^{2+}) which act as powerful free radical generators when found as free ions in high concentration. It has been established that environmental stress factors have been shown to cause oxidative stress (Sahin *et al*., 2001), including the harmattan stress factors, and they induce generation of free radicals in large amounts (Adenkola and Ayo, 2009). When free radicals production exceed the capacity of natural tissue antioxidants enzymes (Miller and Brzezinska-Slebodzinzka, 1993; Nazifi *et al*., 2009) or as a result of depletion of endogenous antioxidant enzymes due to increased lipoperoxidative changes (Gultekin *et al*., 2001; Mansour and Mossa, 2009). The oxidative modification of the erythrocyte membrane has been shown to increase the fragility of the RBC (Langsdorf and Zydney, 1993). Increase free radical

generation in the body has been shown to cause lipid peroxidation of cytomembranes, resulting in cell injury and, consequently, death (Padayatty *et a*l., 2003; William *et al.,* 2008), including the erythrocytes (Sumikawa *et al.* 1993; Avellni *et al*., 1995; Adenkola and Ayo, 2009) thereby exposing the erythrocyte to destruction by macrophages (Lichtensteiger and Vimir, 2003). Lipid peroxidation, which is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) when it happens in biological membranes leads to impairment of membrane function and structural integrity (Gutteridge and Halliwell, 1988). The results of this study agree with the findings of Avellini *et al.* (1995) who showed that free radicals play a vital role in tissue damage and have adverse effects on erythrocytes. Although free radicals were not measured in this study it has been established that free radicals are generated in animals subjected to stress (Elsna, 1991; Altan *et al*., 2003; Tauler *et al*., 2003). The constant exposure to high oxygen tension, high level of iron and richness in PUFA (Kollanjiappan *et al*., 2002) coupled with their inability to possess nucleus and other organelles (Dorđevic *et al*., 2008) have made erythrocyte a centre of free radical attack. Process of lipid peroxidation decreases hydrophobic characteristics of bi-layer membrane of erythrocytes, altering affinity and interaction of proteins and lipids, thereby impairing the functioning and homeostasis of erythrocytes membrane (Dargel, 1991).

In conclusion harmattan season characterized by high AT and dusty-wind affects the EOF of these animals' during harmattan than during hot-dry season hence their erythrocytes were more susceptible to osmotic lysis during this season, indicating that the animals were more stressed during the harmattan season. This study therefore showed that harmattan season was more stressful, hence the animal should be subjected to minimal stress during this period, in order to improve their health and productivity.

6 REFERENCES

Adenkola AY. and Ayo JO: 2006. Effect of ascorbic acid on diurnal variations in rectal temperature of piglets during the harmattan season. *Proceedings of 11th Annual Conference of . Animal Science Association of Nigeria.* 11: 9 – 12.

Adenkola AY. and Ayo JO: 2009. Effect of eight hours road transportation stress on erythrocyte osmotic fragility of pigs administered ascorbic acid during the harmattan season. *Journal of . Cell and Animal Biology* 3(1): 004 - 008.

Adenkola AY, Ayo JO. and Sackey AKB: 2009. Ascorbic acid-induced modulation of rectal temperature in pigs during the harmattan season. *Journal of Thermal Biology* 34 (13): 152 – 154.

Adenkola AY: 2010. Physiological and behavioral responses of pigs administered with ascorbic acid and transported by road during the harmattan season Ph.D dissertation submitted to Ahmadu Bello University Zaria 200pp.

Adenkola AY, Kaankuka FG, Ikyume TT, Ichaver IF. and Yaakugh IDI: 2010. Ascorbic acid effect on erythrocyte osmotic fragility, haematological parameters and performance of weaned rabbits at the end of rainy season in Makurdi, Nigeria. *Journal of Animal and Plant Science* 9(1): 1077 - 1085.

Altan O, Pabuccuoglu A, Konyalioglu S. and Bayracktar H: 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Science* 44: 54 - 55.

Avellini L, Silverstrelli M. and Gaitti A: 1995. Training-induced modifications in some biochemical defenses against free radicals in equine erythrocytes. *Veterinary Research Communications* 19: 179 - 184.

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, *Animal Research* 51: 479-491.

Dargel R: 1991. Lipid peroxidation—a common pathogenic mechanism? *Experimental Toxicology and Pathology* 44:169 – 81.

Dorđevic NZ, Barbic GM, Markovic SD, Ognjanovic BK, Štajn AS, Žikic RV. and Saicic ZS: 2008. Oxidative stress and changes in antioxidative defense system in erythrocytes of preeclampsia in women. *Reproductive Toxicology*, 25: 213 - 18.

Elsna EF: 1991. Oxygen radical- biochemical basis for efficacy. *Klinische Klerchenscrift*, 69: 949 - 956.

Faulkner WR. and King JW: 1970. *Manual of Clinical Laboratory Procedures*. Published by the Chemical Rubber Company, Cleveland, Ohio, 354 pp.

FDLPCS, Federal Dept of Livestock and Pest Control Services: 1992. National Synthesis Resource Inventory and Management. *Nigeria Livestock Resources*. 11.

Gultekin F, Delibas N, Yasar S. and Kilinc I: 2001. *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. *Archives of Toxicology* 75: 88 - 96.

Gutteridge JM, Halliwell B (1988). Oxygen radicals and tissue injury. *FASEB Journal* 13: 9 -19.

Igono MO, Aliu YO: 1982. Environmental profile and milk production in Friesian-Zebu crosses in Nigeria Guinea Savanna. *International Journal of Biometeorology,* 26: 115 – 120.

Igono MO, Molokwu ECI. and Aliu YO: 1982. Body temperature responses of Savanna Brown goat to the harmattan and hot-dry season. *International Journal of Biometeorology,* 26: 225 – 230.

Kerr LD, Inoue J, Verm IM. (1992). Signal transduction: the nuclear target. *Current Opinion in Cell Biology*, 4: 496 - 01.

Khansari DN, Murgo A. and Faith RE: 1990. Effects of stress on the immune system. *Immunology Today, 11: 170.*

Kolanjiappan K, Manoharan S, Kayalvizhi M: 2002. Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. *Clinica Chimica Acta* 326: 143 – 49.

Langsdorf LJ. and Zydney AL: 1993. Effect of uremia on the membrane transport characteristics of red blood cells. *Blood* 81: 820 - 827.

Lichtenstiger CA, Vimr ER: 2003. Purification and renaturation of membrane neuraminidase from Haemophilus parasuis. *Veterinary Microbiology*

Mansour SA. Mossa AH: 2009. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. *Pesticide Biochemistry and Physiology* 93: 34 - 39.

Miller JK, Brzezinka-Slebodzinska E: 1993. Oxidative stress, antioxidants, and animal function. *Journal of Dairy Science* 76: 2812 – 2823.

Mstl E. and Palme R: 2002. Hormones as indicator of stress. *Domestic Animal Endocrinology* 23: 67 - 74.

Nazifi S, Saeb M, Baghshani H. and Saeb S: 2009. Influence of road transportation during hot-summer conditions on oxidative status biomakers in Iranian dromedary Camels (*Camelus dromedarius*). *African Journal of Biochemical Research* 3: 282 – 287.

Oladele SB, Ayo JO, Ogundipe SO. and Esievo KAN: 2003. Seasonal and species variations in erythrocytes osmotic fragility of indigenous poultry species in Zaria, Northern Guinea Savannah zone of Nigeria. *Bulletin of Animal Health Production Africa* 51: 204 - 214.

Oluwafemi RA: 2009. Cattle production and marketing in Nigeria, the impact of diseases. A case study of Maiakiya, Assakio and Shinge cattle markets in Lafia Local Government Area of Nasarawa State. Nigeria. *International Journal of Veterinary Medicine* 6(1).

Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee J, Chen S, Dutta A, Dutta SK. and Levine M (2003). Vitamin C as antioxidant: Evaluation of its role an disease prevention. A review. *Journal of American College of Nutrition* 22: 18 – 35.

Rakesh KS, Amit KR: 2004. An assessment of changes in open field and elevated plus-maze behavior following heat stress in rats. *Iranian Biomedical Journal* 8(3): 127 – 133.

Sahin, K, Sahin N, Onderci M, Yaraliogu S. and Kucuk O: 2001. Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress. *Veterinary Medicine (Czechoslosvakia)* 46: 140 - 144.

Sidhu P, Garg ML, Dhawan DK: 2004. Protective effects of zinc on oxidative stress enzymes in liver of protein deficient rats. *Nutrición Hospitalaria*, XIX **(6)**: 341 - 47.

Sumikawa K, Mu Z, Inoue T, Okochi T, Yoshida T. and Adachi K: 1993. Changes in erythrocyte membrane phospholipids composition induced by physical training and physical exercise. *European Journal of Applied Physiology* 67: 132 - 137.

St-Pierre NR, Cobanov B. and Schnitkey G: 2003. Economic losses from heat stress by US livestock industries. *Journal of Dairy Science* 86: 52-77.

Tarr B: 2007. Cold stress in cows. Fact sheet of ministry of agriculture food and rural affairs Ontario. Agdex No420/5.1.

Tauler P, Aquilo A, Gimelo I, Fuentaspina E, Tur JA. and Pons A: 2003. Influence of vitamin C diet supplementation on endogenous antioxidant defences during exhaustive exercise. *European Journal of Physiology* 446: 658 - 664.

Teeter RG, Belay T, Deyhim F. and Cason JJ: 2005. Heat stress steals nutrition and genetic potential. Department of Animal Science, Stillwater, Oklahoma.

Walter MW (1969). Length of the rainy season in Nigeria. *Nigeria Geographical Journal* 10: 123 – 128.

Williams CA, Gordon ME, Betros CL, McKeever KH: 2008. Apoptosis and antioxidant status are influenced by age and exercise training in horses. *Journal of Animal Science* 86: 576 - 583.

Yoshioka G, Imaeda N, Torimoto Y, Ohtani T. and Hayashi K: 2004. Influence of transport stress on serum cortisol and thyroid hormones in pigs with halothane gene. *Animal Science Journal* 75: 451 – 456.