RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences
(P-ISSN 1595-093X/ E-ISSN 2315-6201)
Biobaku *et al*/*Sokoto Journal of Veterinary Sciences* (2016) 14(2): 43-52.
http://dx.doi.org/10.4314/sokjvs.v14i2.6

Effects of xylazine on physiological and biochemical parameters of Sahel bucks exposed to twenty-eight hours road transportation

KT Biobaku[1], BM Agaie[2], KI Onifade[2], IA Odetokun[3]*, BS Okediran[4], SA Ameen[5], MS Ismaila[2], M Adam[6] & LO Raji[7]

1. *Department of Veterinary Pharmacology & Toxicology, University of Ilorin, Ilorin.*
2. *Department of Veterinary Pharmacology & Toxicology, Usmanu Danfodiyo University, Sokoto.*
3. *Department of Veterinary Public Health & Preventive Medicine, University of Ilorin, Ilorin .*
4. *Department of Veterinary Physiology & Biochemistry, University of Ilorin, Ilorin.*
5. *Department of Veterinary Medicine, University of Ilorin, Ilorin.*
6. *Department of Veterinary Pathology, University of Ilorin, Ilorin.*
7. *Department of Theriogenology and Production, University of Ilorin, Ilorin*

*Correspondence: Tel.: +2348030886018, E-mail: odetokun.ia@unilorin.edu.ng

Abstract

Thirty two apparently healthy animals were used in the study with four bucks per group. There were eight groups in all and two stocking densities. The experimental treatment groups were xylazine at 0.01 mg/kg intramuscularly administered (IM), xylazine at 0.015 mg/kg (IM), xylazine at 0.020 mg/kg (IM) and a control none treated group. Each of the treatments had low and high stocking rates respectively. Thus, 16 animals each were experimented upon for the high and low stocking rates. Xylazine was administered prior and midway into the experimental journey. Physiological parameters taken were: respiratory and heart rates, rectal temperature and excitability score. Biochemical parameters analyzed were: alanine aminotransferase (ALT) aspartate amino transferase (AST), glucose, cholesterol, and protein. The electrolytes analyzed were Ca+, Mg++, Na+ K+ and Cl- . Antioxidative stress markers assayed were glutathione transferase, superoxide dismutase, malonyldialdehyde. Full blood count and thyroid hormones [triiodothyronine (T3) and tetraidothyronine (T4)] were also determined using ELISA. The results show there was no significant ($P>0.05$) changes at all doses except for cholesterol where the dose of (0.015mg/kg) of xylazine produced a significantly ($P<0.05$) higher value when compared to the control, and the other treated groups. The serum Na+ and Cl- were significantly higher in the group treated with 0.01 mg/kg of xylazine (155.51±15.11 and 121.32±36.90 mg/dl) compared to the control. Xylazine at 0.015 mg/kg and 0.02 mg/kg dose caused a reduction in the Cl- levels. Xylazine treatment might have improved adaptability in long term transportation.

Keywords: Antistress markers, Biochemistry, Bucks, Physiology, Transportation
Received: 02-03- 2016 Accepted: 14-07-2016

Introduction

Stress is a physiological disturbance that is imposed by a stressor, such as threatening of animal by harmful situation (Gregory, 2004). It is associated with suffering when there is mental distress (Gregory, 2004), following physical trauma, during disease, and psychological or emotional conflicts (Kannan *et al.,* 2000). Transportation stress in animals results in psychological and physiological changes, which could be induced by restraint of loading, social disorder; starvation and thirst (Kannan *et al.,* 2002; Ayo *et al.,* 2006; Minka & Ayo, 2010). These stress predisposing factors aforementioned might have a detrimental influence on welfare and quality of meat product. Previous studies by Blokhuis *et al.* (2000) suggested that it is important to promote the welfare of animals by attenuation of stress due to transportation. Codes and legislations concerning animal welfare, which protect animals from potentially stressful stimuli that may compromise their welfare are either non-existent

in many countries of the world or not enforced appropriately (Ali *et al.,* 2006).

Fear responses in a particular situation are difficult to predict because they depend on how the animals perceived their handling or transport experience (Zavy *et al.,* 1992). The amygdala in the brain is probably the central fear system that is directly involved with fear behaviour and the acquisition of conditioned fear, while the subcortical pathway is associated with fear and extinguishing fear condition (Davis, 1992). There is no universal agreement on what constitute stressful stimuli in animals, method of quantifying the animal's response to stressful stimuli and the ways by which stress can be ameliorated (Ali *et al.,* 2006).

The classes of psycho-active drugs used in alleviating stress in domestic animals include: α-2-adrenoceptor agonists, benzodiazepines, opiates and some supplements. The α-2-adrenoceptor agonist used include: xylazine, medetomidine and detomidine while benzodiazepines such as diazepam and midazolam, opioids such as morphine and butorphanol; and nutritional supplements such as vitamin C, tryptophan, and magnesium are used (Minka & Ayo, 2008; Adenkola *et al.,* 2009). Other drugs that could be used are the phenothiazines: acepromazine, chlorpromazine (Wanamarker & Massey, 2009).

In a study by Sanhouri *et al.* (1991), xylazine (0.01mg/kg) was able to suppress plasma cortisol (one of the markers of stress) concentration. Brearley *et al.,* (1990) showed that xylazine increased blood glucose level and suppressed respiratory and heart rates. Xylazine pretreatment however did not alter the cortisol response to corticotrophin releasing factor (CRF) suggesting that xylazine must have acted above the pituitary level when blocking the cortisol response to transport stress (Sanhouri *et al.,* 1991). It was also postulated that under resting condition, the hypothalamus is suppressed under the influence of xylazine, thus stimulation of cortisol secretion in response to stress could be inhibited by α -2-adrenoceptor agonist (Sanhouri *et al.,* 1992).

This study is aimed at evaluating the stress ameliorative effect of xylazine in Sahel bucks subjected to 28 hours stress of road transportation when adhering to standard regulations (low stocking as stipulated in the Animal Disease Control Act of 1988, Federal Republic of Nigeria) for caprine transportation alongside non-standard (high stocking) on physiological and biochemical parameters.

Materials and Methods

Experimental animals

Thirty-two apparently healthy Sahel bucks were used for the experiment. Their ages ranged between one and a half and two years, weighed between 10 and 14 kg and had overall universal body condition score of 3. The animals were purchased from livestock markets in Tangaza, Gada, Kware and Achida of Sokoto State. The animals were clinically examined and acclimatized for two weeks before the commencement of the study. They were prophytically treated against helminthes using albendazole (Albenzole, Agbara Industries, Lagos, Nigeria) and antibacterial cover with a combination of penicillin and streptomycin (penstreptomycin®, Kepro, Holland) at manufacturer's recommended doses. The bucks were not restrained and the stocking rate in the pen was $2m^2$/goat so that the animals could be free and would not be predisposed to stress. The bucks were fed groundnut hay, cowpea husk and corn residue and were provided with clean water *ad libitum.* The animals' basal vital parameters and blood samples for basal haematologic parameters determinations, were obtained while weights were taken once weekly during the two weeks of acclimatization.

Study location and bio-meteorological monitoring from study area

The study began at the livestock farm unit of Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria located along 13.1^{o}N and longitude $5^{o}13^{'}$E 350m above sea level in the semi-arid zone of North-western Nigeria. The goats were transported during the harmattan season in the month of January. The experimental journey ended in Federal University of Agriculture, Abeokuta, Ogun State; which is located along latitude 7.1° N, longitude $3.4^{o'}$E at an altitude of 76m above sea level. Abeokuta is located in the rain forest zone of South-western Nigeria. The distance travelled during the experimental journey was 969km at an average speed of 40km/hr.

The ambient temperature and wind speed were measured using wet and dry bulb thermometers and digital anemometer (Model N492203, China) respectively. The average values of wet to dry bulb temperatures obtained were 30.0^{o}C and 36.0^{o}C while the wind speed was 3.71m/s in Sokoto at the commencement of the journey. Mid-way into the journey, the temperatures obtained were 27^{o}C and 28^{o} C while the wind speed was 1.3m/s at Jebba. The temperatures were 23^{o} C and 26^{o}C and a wind speed of 0.8m/s in Abeokuta at termination of the journey.

Experimental design

Thirty two animals were used for the study and four bucks per group using two stocking densities. The animals were stocked using a standard (low stocking rate) which is in conformity with the Animal Disease Control Act of 1988 of Nigeria and standard international regulations for transportation of goats, and high stocking rate which was not in conformity with standard international regulation but being the routine way that goats were transported by most marketers in Nigeria. The treatment groups were xylazine at 0.01mg/kg, 0.015mg/kg, and 0.020mg/kg intramuscularly administered and an untreated control group. Each treatment applied to low and high stocking rates groups with 4 animals each.

Loading, stocking, experimental journey, and ethics

Prior to transportation, a health certificate was obtained from the Veterinary unit, Ministry of Forestry and Animal Health, Sokoto State. This is done to ensure strict adherence to guidelines governing animal transportation welfare by road as previously adopted by Minka & Ayo, (2010).

The Sahel bucks were handled with care and were loaded by four people between 09:00 to 11:00h. The animals at low stocking density (rate) were stocked at 0.30m^2per animal; while the animal grouped high stocking density was stocked at 0.15m^2 per animal. The floor of the truck was cushioned using sorghum leaves and saw dust to avoid the animals coming in contact with their urine and faeces. This was done to reduce the risk of animals sliding, physical injuries and also minimize the risk of transmission of diseases.

During the journey, at Jebba town located along latitude 9.1°N and longitudes 4.8° E, the low stocking density animals were rested for 3hours after being subjected to 12 hours journey and fed with groundnut hay and wheat bran and given water *ad libitum*. This is in conformity with the Animal Diseases Control Act of 1988. Animals that were transported at high stocking density were rested but not fed; in order to create an experimental condition similar to the way the livestock marketers treat their animals during transportation in this part of the country.

Administration of xylazine

Xylazine (XYL-M2® Berendonk Drug Company, Belgium) an injectable solution was used in this study and was administered intramuscularly (IM). The dose of 0.01mg/kg which was the lowest dose of xylazine was based on a previous study of Sanhouri *et al.* (1992). In addition, other graded doses were also used. The xylazine was administered at the beginning of the experimental journey at Sokoto and mid-way into the experimental journey at Jebba.

Measurement of vital physiologic parameters and weight

All the vital parameters were taken pre-loading at Sokoto; at Jebba and after off-loading of the animals at Abeokuta. Rectal temperature was measured using a thermometer, while a stethoscope and time set was used to assess heart and respiratory rates using standard procedures respectively. Excitability score was also assessed as previously reported by Ayo *et al*. (2006) but slightly modified using the score of 1- 4. The lower score means calmness and the higher score indicates anxiety. The score was also assessed pre-slaughter at Abeokuta after the animals had been rested.

Blood collection, haematological and biochemical analysis

Blood samples were collected from each animal preloading at Sokoto, midway into the journey, when resting the animals at the veterinary quarantine post and after offloading the animals from the truck at Abeokuta. Twenty one gauge hypodermic needles and syringes were used for blood collection from the jugular vein after disinfecting the skin using 10% methylated spirit. Five milliliters of blood were collected in separate bottles with lithium heparin or ethylene diamine tetraacetic acid (EDTA).

Full blood count was carried out using the method previously described by Schalm & Jain (1986). The parameters determined were red blood cell count (RBC), erythrocytes sedimentation rate (ESR), haemoglobin concentration (HbC), packed cell volume (PCV) and erythrocytes indices such mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) using the method of Schalm & Jain (1986). Total white blood cell, differential count and the neutrophils – lymphocytes ratio (N:L) were also determined using the methods previously adopted by Ayo *et al.* (2006), Minka & Ayo, (2010) and Chauhan & Agarwal, (2006). Randox® commercial test kits (Randox®, England) were used to determine the biochemical parameters using standard spectrophotometeric procedures. The parameters determined were alanine aminotransferase (ALT) and aspartate amino transferase (AST). Glucose was determined using glucometer (Roche Diagnostics, Germany), cholesterol and total protein using standard procedures. The electrolytes (Na^+, Ca^{2+}, Mg^{2+}, K^+ and Cl^-) tests were carried using commercial test kit (Randox®, England). Antioxidative stress markers assayed were glutathione-s-transferase (GST) using the method of Habig *et al*. (1974). Superoxide dismutase (SOD) was assessed as previously

described by Zhou *et al.* (1986) and Malondialdehyde enzyme assessment was done using the thiobarbituric acid method (Buege & Aust, 1978; Mohammed *et al*., 2007). Thyroid hormones, namely triiodothyronine T3 and tetraidothyronine T4, were assayed with Biorex test kit (Biorex Diagnostics Ltd, UK) using ELISA as previously described by Muzzaffar & Gharib (1998).

Statistical analysis

The values of physiologic parameters were analyzed using Analysis of variance (ANOVA) at the three phases of the journey, while the statistical sum of means of biochemical parameters at the three phases (prior, midway and after journey) were compared using (ANOVA) on SPSS, version 16. All P values < 0.05 were considered significant.

Results

The mean values of physiological parameters in the bucks prior, midway and post transportation are presented in Table 1. Parameters presented include: heart rate, respiratory rate, temperature, excitatory score and the differences in values of respective physiologic parameters prior, midway into the journey and at the end of journey respectively. Also presented in Table 1 is the statistical assessment of the rate of interaction of various physiologic parameters in relation to stocking rates and various pharmacologic influences of treatment and dose. No statistical significance ($p > 0.05$) were observed among these interactions.

The results in Table 2 indicate that none of the parameters studied manifested any significant ($P > 0.05$) change at all the doses except for cholesterol, where the dose (0.015 mg/kg) of xylazine produced a significantly ($P < 0.05$) higher value when compared to the control, and the other treated groups. The serum Na^+ and Cl^- were significantly higher in the group treated with 0.01mg/kg of xylazine (155.51±15.11 and 121.32±36.90 mg/dl respectively) compared to the control. Xylazine at 0.015mg/kg and 0.02mg/kg doses caused a reduction in the chloride levels when compared to the control (Table 3).

Table 1: Effects of xylazine dose and stocking rate on physiologic parameters of Sahel bucks exposed to long term stress of transportation stress

	Dose				Stocking rate			
Parameters	0.01mg/Kg	0.015mg/kg	0.02mg/kg	SEM	High	Low	SEM	Interaction
HR (prior) (beats/mins)	88.71	85.00	83.00	2.54	83.00	88.71	2.09	NS
HR (midway)[beats/mins]	85.00	78.25	85.75	5.55	84.16	81.83	4.52	NS
HR(end)[beats/min]	98.63	92.88	93.88	2.814	97.92	92.33	2.30	NS
RR (cycles/min) (prior)	39.17	39.17	38.17	1.09	33.38	36.83	1.22	NS
RR(cycles/min)[midway]	17.50	20.13	18.13	0.99	19.41	17.50	0.80	NS
RR(cycles/min)[E of jo]	35.13	38.88	40.50	2.27	35.75	40.58	1.85	NS
Temp.c^0 (prior)	39.18	39.18	39.66	0.19	38.19	38.13	0.12	NS
Temp.c^o(midway)	39.98	39.10	39.93	0.205	39.175	40.158	0.168	NS
Temp. c^o (end)	39.18	38.70	39.37	0.189	39.43	38.75	0.154	NS
Excitatory score (prior)	3.92	4.04	4.04	3.88	4.00	3.88	0.16	NS
Excitatory score (midway)	1.38	1.500	2.00	0.18	1.92	1.33	0.15	NS
Excitatory score(E of jo)	3.88	3.88	4.00	0.08	4.00	3.83	0.083	NS

E of jo – end of Journey

NS - not significant

*Significant ($p<0.05$), HR: Heart rate, RR: Respiratory rate, Temp: Temperature

Table 2: Effects (mean±SD) of varying doses of xylazine on some biochemical parameters of Sahel bucks under long duration transportation stress

		Xylazine doses		
Parameters	Control	0.0/0mg/kg	0.015mg/kg	0.02mg/kg
ALT(IU/l)	112.53±25.04	122.64±49.71	91.07 ±17.19	96.54 ±12.26
AST (IU/l)	224.07±45.30	206.80±61.31	223.31 ±49.46	190.70±50.00
Glucose (mg/dl)	82.22 ±15.48	73.22 ±20.28	78.61 ±19.39	82.77±21.15
Cholesterol (mg/dl)	90.52±49.57 [b]	81.72±25.41 [c]	115.56 ±66.4 [a]	84.96±24.94 [c]
Total Protein (g/l)	73.68±12.04	69.88±14.69	71.71±16.15	67.08±8.95

Means bearing different superscripts abc along the same row differ significantly ($p<0.05$)

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

All the treated groups had significantly higher MDA values than the control. The values of the MDA were not dose dependent since 0.01mg/kg of xylazine gave a concentration of 0.14±0.08M^{-1} CM^{-1} while the 0.015mg/kg and 0.02mg/kg respectively of xylazine produced MDA values of 0.09± 0.08 and 0.11±0.01 M^{-1} CM^{1} as presented in Table 4.

The effect of different doses of xylazine on haematological parameters of Sahel bucks exposed to long term transportation stress is presented in Tables 5 and 6. There were no significant differences in haematological parameters observed in this study. The MCHC however, was the only parameter (of Sahel bucks exposed to long term transportation stress following pretreatment with various doses of xylazine) which showed significant ($P < 0.05$) higher difference between the control and the treated groups.

The differential counts showed a significant increase in the values of neutrophils (percentage) Table 6. With the administration of Xylazine at the dose of 0.015 mg/kg, significant increase in neutrophil to 39.27±2.37% was observed, followed by a neutrophil increase to 38.78±7.52% at the dose of 0.01mg/kg. At the highest dose, a percentage neutrophil value of 34.00±9.09% was observed compared to control values (30.36±4.94%). The same trend was observed for the increase in the values of neutrophils-lymphocytes ratio (N:L) and lymphocytes in the treated groups. All the values were higher in

Table 3: Effects (mean±SD) of various doses of xylazine on some serum electrolytes of Sahel bucks exposed to long duration transportation stress

		Xylazine dose		
Parameters	Control	0.01mg/kg	0.015mg/kg	0.02mg/kg
Calcium (mg/dl)	9.63 ±0.79	9.44 ±0.59	9.32 ±0.64	9.60 ±0.71
Magnesium(mg/dl)	2.73±0.47	2.65 ±0.57	2.90 ±0.58	2.7 ±0.64
Sodium (mg/dl)	147.83±11.39^{b}	155.5±15.11^{a}	145.59±10.91^{b}	146.05±12.1^{b}
Potassium (mg/dl)	5.30 ±1.14	6.44 ±0.58	4.97 ±1.69	4.82 ±1.97
Chloride (Mmol/l)	102.69±25.84^{b}	121.32±36.90^{a}	91.05 ±9.58^{c}	97.12±25.15^{c}

Means bearing different superscripts abc along the same row differ significantly ($p<0.05$)

Table 4: Effects (mean±SD) of various xylazine doses on antioxidative stress markers and thyroid hormones of Sahel bucks exposed to long duration transportation stress

		Xylazine doses		
Parameters	Control	0.01mg/kg	0.015mg/kg	0.02mg/kg
GST (µl /ml)	1.42 ±0.54	1.34 ±0.32	1.27 ±0.35	1.39 ±0.46
MDA ($M^{-1}CM^{-1}$)	0.05±0.005^{d}	0.14 ±0.08^{a}	0.09 ±0.08^{c}	0.11 ±0.01^{b}
SOD (Iµ)	33.21±11.24	42.18 ±16.41	39.47 ±13.82	40.13 ±16.42
T_4 (µg/dl)	9.31±4.16	9.74 ±5.69	8.89 ±4.78	8.50 ±6.55
T_3 (ngl/ml)	2.81 ±1.25	1.76±1.14	5.79±94.28	2.27 ±2.10

Means bearing different superscripts abc along the same row differ significantly ($p<0.05$)

GST: Glutathione-S-Transferase

MDA: Malondialdehyde, SOD: Superoxide dismutase, T_3: Triiodotyronine, T_4: Tetraiodotyronine

Table 5: Effects (mean±SD) of various xylazine doses on erythrocytes and erythrocytic indices of Sahel bucks exposed to long duration transportation stress

Parameters		Doses of xylazine		
	Control	0.01mg/kg	0.015mg/kg	0.02mg/kg
RBCX (10^{6}/µl)	9.31 ±2.32.	11.05 ±0.32	11.22 ±0.35	11.22 ±0.35
PCV (%)	26.72±4.97	27.05 ±5.82	26.83±3.84	26.94±5.36
ESR (MM/hr)	6.78±11.24	7.11 ±16.41	6.24 ±13.82	6.88±16.42
Hb (g/dl)	9.18 ±1.69	9.53 ±1.82	9.72±1.41	9.61 ±1.36
MCH(pg)	8.27 ±3.16	9.00 ±2.13	8.782 ±2.61	8.82 ± 2.30
MCHC (g/dl)	32.90±6.71^{c}	35.98±6.80^{a}	34.09±4.26^{b}	34.65 ±4.94^{b}
MCV(fl)	26.49±9.38	25.15 ±5.21	26.09 ±7.30	24.7 ±6.09

Means bearing different superscripts abc along the same row differ significantly ($p<0.05$)

RBC: Red Blood Cell Count

PCV: Packed Cell Volume

ESR: Erythrocytes Sedimentation Rate

Hb: Haemoglobin Concentration

MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration

Table 6: Effects (Mean±SD) of varying doses of xylazine on leucocytes and differential leucocytes count of Sahel bucks exposed to long duration transportation stress

		Doses of administered xylazine		
Parameters	Control	0.01mg/kg	0.015mg/kg	0.02mg/kg
WBC(10^3/µl)	1.13 ±3.35	1.23 ±4.12	1.43±3.31	1.56±3.33
Neutrophils (%)	30.36±4.97[c]	38.78±5.82[a]	39.27±3.84[a]	34.00 ±5.36[b]
Lymphocytes (%)	58.22±6.79[d]	75.53±6.28[a]	60.11±2.2 [c]	63.22±63.22[b]
N:L	0.60 ±0.10[a]	0.68±0.21[a]	0.63 ±0.08[a]	0.49±0.22[b]
Eosinophils (%)	1.09 ±1.37	1.44 ±1.58	0.83 ±1.15	1.00 ± 1.37
Basophils (%)	0.74 ±0.71	1.27 ±0.80	1.10±0.50	0.83 ±4.94
Monocytes (%)	0.61 ±0.84	0.67 ±0.84	0.67 ±1.36	0.72 ± 1.36

Means bearing different superscripts abc along the same row differ significantly ($p<0.05$)
WBC: White Blood Cell Count, N: L: Neutrophils – Lymphocytes Ratio

the treated groups when compared to the control except for the values of the neutrophils-lymphocytes ratio at 0.02 mg/kg xylazine that were lower than that of the other treated groups and the control.

Discussion

Xylazine is an alpha-2 receptor agonist that acts on both the peripheral and central nervous systems, producing sedation, analgesia and muscle relaxation (Ali & Al-Qarawi, 2002). The stressful stimuli might involve the adrenergic system and therefore alpha-2 adrenergic agonist could be used to alleviate stress as previously reported by Brearley *et al.* (1990) and Sanhouri *et al.* (1992). Xylazine has been a popular sedative for use in food animal practice (Lin & Riddel, 2003). The antistress activity is associated with its depressant effect of the central nervous system, thus, affecting the neuroendocrine functions mainly related to inhibition of sympathetic outflow and decreasing plasma level of catecholamines (Hokfelt *et al.,* 1975). Xylazine antistress activity is based on inhibition of adrenocorticotropic hormone and cortisol by acting centrally above the pituitary level, alleviating stress of transportation in food animal (Sanhouri *et al.,* 1992).

Sedation which was brought about by the central nervous system is depressive effect especially in goats may be utilized to counteract the sympathoadrenal discharge of anxiety during transportation, since goats are sensitive to low doses of xylazine (Dehghani *et al.,* 1991). Similarly, the analgesic effect of xylazine is good (Aithal *et al.,* 1996). Its analgesic activity is due to its action on the autonomic and central nervous system and since pain of the extremities occurs during loading and transportation, the therapeutic value of this drug could be exploited (Mogoa, 1990). It was suggested that xylazine in animals subjected to stress may manifest prolonged analgesic effect than those that are not (Fayed *et al.,* 2003), thus the value of the drug. Xylazine was therapeutically utilized. Previous work in animals transported also showed they suffered associated muscular soreness (Minka & Ayo, 2010), the muscle relaxant effect of xylazine may help in alleviating such adverse effect hence its usage in this study. The result on heart rate gave an insight into xylazine modulating effect on the parameter, thus the drug may improve survivability of animals under transportation, due to its modulating effect as suggested by Khan *et al.* (1999). Similarly, there was a non-significant decrease in heart rate of goats in low density stocking. This may suggest that the heart rate modulation could be due to the peripheral, effect of the drug on the sinoatrial node since peripherally the drug would have acted on the contractile tissue of the heart and alpha-1 adrenoceptors of the vascular smooth muscle Mogoa *et al.* (2001). The effect of xylazine on the cardiovascular system could have decreased the sympathoadrenal stimulation causing a decrease in catecholamines surge thereby reducing the rhythmicity observed in the heart (Ali & Al-Qarawi, 2002). It could also be that xylazine may have influenced a decrease in the sympathetic outflow from the central nervous system. This decrease in the neurotransmitter could be at the pre and post junctional neurons directly causing a decrease in heart rate, by inhibition of the norepinepherine from the sympathetic nervous system and the decrease in the acetylcholine from the parasympathetic nerves of the heart. The assumption is that the long journey undergone by the animals in this study resulted in change in their behavior, possibly hyperexcitability which was counteracted by xylazine. The various doses of xylazine influenced the decrease in heart rate due to the sensitivity of caprine specie to this drug as a result of the alpha-2D receptors. The sensitivity of the caprine alpha-2D receptor to xylazine might further explain the sedative effect which would probably lead to the decrease in heart rate. The effect of xylazine in this study on the heart rate was not dose-dependent. This finding is not in agreement with the study of Sanhouri *et al.*

(1991). The difference could be the variation of the route of administration which was intravenous in the previous study while intramuscular route was used in this study. The duration of transportation was shorter in the previous study and duration could have been a factor in the differences observed in this study.

Xylazine at sedative doses did not influence the respiratory rate in animals subjected to long term transportation stress. The trend of changes in the respiratory rates in this study was in line with the findings of Saleh (1993), but contrary to the findings of O' Hair *et al.* (1986) who reported a mean increase in respiratory rate in sheep. This could be due to species difference and routes of administration and variation in the distribution of the adrenoceptor effect in animals. Ali *et al.* (2006) had earlier reported the effect of sympathetic nervous system on the adrenoceptors which might have influenced the varying trends of respiratory activity thus alleviating the detrimental respiratory, psychological and central nervous system depressive effect. In the same vein, the findings of Fereidoon *et al.* (2005) showed non-significant changes in the respiratory rate when the combination of ketamine – xylazine was used in goats. The breed of goats used in this study might also have influenced the findings, as Sahel goats were used unlike in the other studies where Nubian breed was used. Xylazine treatment however had a significant influence on the temperature of transported goats. It has been documented that the influence of xylazine is dependent on environmental temperature (Mogoa *et al*., 2001). The findings in this work were also similar to that of Fayed *et al*. (2003) who reported a non-significant temperature changes in stressed and unstressed cattle. In contrast, Mohammed & Yelwa (1993) reported a dose dependent effect on temperature. The difference in the effect of xylazine on temperature may be because the animals were transported for a long duration, therefore the effect of the sympathoadrenal system coupled with various biochemical and endocrine nervous changes might have counteracted the direct effect of the drug, thus maintaining the temperature within a physiologic range. The condition of the animals, the doses and routes of administration used in this study could also account for the observed differences. The sedative doses employed (0.01mg/kg and 0.015mg/kg) and the higher dose used might have influenced the thermoregulatory centre of the brain in the hypothalamus due to the central nervous system effect of xylazine. The dose used and the environmental condition might account for the effect of xylazine on body temperature as documented by Prajapathi *et al.* (1994). The disparity in our findings with results by Mohammed and Yelwa (1993) may be due to changes in ambient temperature along the Savannah zone in the Northern to rain forest in the South western part of Nigeria. There was a significant decrease in the excitatory score due to xylazine treatment. This decrease was due to the inhibition of catecholamines discharge from the autonomic and central nervous systems. This is coupled with the peripheral depressive effect of alpha-2 adrenoceptors agonists on the adrenal medulla which may have resulted in a decrease in excitatory score. This can also be due to the sympatho-adrenal system being overwhelmed by the effect of xylazine and decrease in the production of catecholamines from the neurons thus decreasing the hyperactivity during transportation. This effect of xylazine is in agreement with the works of Ali *et al.* (2006) and Sanhouri *et al.* (1992). The cholesterol level increased significantly in the group that was administered with medium dose of xylazine. This might be due to reduced metabolic rate due to the sedative effect of the drug, which can interfere with the cascade of events owing to the sympatho-adrenal conversion of cholesterol and fatty tissue to other energy sources during the fright-flight response. The lower dose (0.010mg/kg) of xylazine also increased significantly ($P < 0.05$) Na^+ and Cl^- in the Sahel bucks. It was suggested that this is due to the effect of the drug on the receptors of the nephron. This is in agreement with observations of Sanhouri *et al.* (1991), who reported that sedative dose of 0.01mg/kg of xylazine could offers stress alleviating effect, arising from nervous and non-nervous dependent factors. However the changes in the ion concentrations were not dose dependent. The malondialdehyde was significantly higher at the lower dose of 0.01mg/kg indicative of an indirect effect of the prooxidant-antioxidant activity in stress induction during long term transportation. We imply that xylazine has no antioxidative effect.

We found that in spite of the graded doses of administered xylazine, there was a persistent increase in the neutrophils. At a higher dose of 0.02mg/kg it alleviated stress, and improved the neutrophils-lymphocytes ratio. The various experimental doses in this study did not significantly affect the erythrocytes. However the MCHC was influenced by the dose of 0.01mg/kg by increasing the value when compared to other xylazine doses. Adenkola *et al.* (2011) suggested that improvement observed in the erythrocytes index might be due to the antistress effect of xylazine. However, Dehghani *et al.* (1991) observed significant decrease in PCV, RBC and haemoglobin concentration due to sedative doses

of xylazine administered intravenously. This shows that the route of administration is a factor to be considered in xylazine administration in order to alleviate transportation stress. Also, the induced stress in the goats might trigger some associated neuroendocrine mechanisms that could be amongst the reasons for the observed differences.

The leucocytic parameters (white blood cell count and various differential white blood cell counts) were influenced by xylazine. The medium dose of xylazine (0.015mg/kg) increased the neutrophils more than the two other doses (0.01mg/kg and the 0.02mg/kg). Xylazine may have decreased the cortisol surge in the central nervous system, which possibly induced the cell-mediated response of the cellular component of the immunity. This could improve the immunity of the animal in transit and prevent further stress due to diseases. Neutrophils-lymphocytes ratio was also improved by xylazine especially at 0.02mg/kg dose. In this study, an alpha-2 adrenoceptor agonist was used while in the previous study β-2 agonists were used. The species, duration of administration, dose, and the stress level pre-slaughter might also be amongst the reasons for the differences in the findings.

Xylazine treatment in animals subjected to stress significantly increased serum Na^+ K^+ and Cl^- at a low dose of 0.01mg/kg when compared to other treatment groups. Minka *et al.* (2010) documented a decrease in the levels of electrolytes at transportation. In this study, xylazine might have ameliorated this electrolyte deficit sequel to stress and this could be advantageous to transported goats as it will aid the physiologic compensatory mechanisms to stress and decrease deterioration of meat quality. The calming effect of xylazine in the bucks helped in decreasing the effect of the excitatory neurotransmitters in the central nervous system and this minimized the detrimental effect of stress, allowing for physiologic adaptation. This probably is the reason for the limitation of the adverse effect of the high stocking rate on the animals. Other works showed that high stocking rate had detrimental effects on animals and their products (Kannan *et al.,* 2002), though there is dearth of information on the effect of CNS acting drugs on stocking density. However, it was shown in our study that a sedative drug such as xylazine could produce therapeutic effect that can alleviate stress of transportation and those related to stocking density.

Transportation of livestock involves physical demand for energy that is required to carry out enormous contraction and relaxation of muscles which can produce a negative impact on muscles (Minka & Ayo, 2010). This could be in the form of production of free radicals, reactive oxygen species (ROS), nitrogen oxygen species (NOS) and E-type prostaglandin and their release into circulation especially during stress. The anti-oxidative biomarkers assessed in this study (superoxide dismutase, glutathione S-transferase and malondialdehyde) showed that xylazine has no significant influence on antioxidative enzymes and malondialdehyde. There was significant decrease in SOD, GST and catalase levels and an increase in the levels of MDA, AST and ALT in experimental rats exposed to stress of immobilization (Zaidi *et al.,* 2005). Bhogade *et al.* (2008) showed a similar trend of antioxidative stress biomarkers variation in patients subjected to stress. When compared to baseline values, there was a similar trend of non-significant decrease in SOD enzymes in the transported animals in our study. Also, GST was not significantly affected in long term induced transport stress when compared to the non-treated group subjected to transportation stress. This implies that the low dose of xylazine only improved the anti-oxidative status of the animals non-significantly when compared to other treated groups.

Xylazine could have calmed the animals to reduce the level of production of free radicals stimulated by psychological release of cortisol. Sanhouri *et al.* (1992) suggested that xylazine decreased cortisol level above the production level of the pituitary gland. The decreased cortisol level further reduced the production of reactive oxygen species that could be detrimental to the body. Xylazine significantly influenced the antistress endogenous MDA, and did not lower the level of MDA. The effect of xylazine on the antioxidative stress makers shows that xylazine may not have radicals mopping effect. This conclusion agrees with the works of Ali *et al.* (1987) and Ali & Qarawi (2005) in which they suggested that the antistress effect of xylazine was dose-dependent. The low dose response may suggest species sensitivity to the xylazine. Dehghani *et al.* (1991) reported the dose-dependent sensitivity – in terms of biochemical parameters – to sedative effect of xylazine in caprine and feline species.

In this study xylazine did not significantly affect the triiodothyronine (T_3) levels when values of animals treated and subjected to long-term transportation stress were compared with the control. It was reported that triiodothyronine (T_3) and tetraiodothyronine (T_4) concentration may be altered by various stress induced factors (Gomez *et al.,* 1999). However, the trend of alteration of thyroid hormones was not consistent and is dependent on species, sex, nutrition, environmental factors, health status and the conditions of the animal (Chao *et al.,* 1999). In addition, there was no specific trend on the effect

of xylazine on the surge of thyroid hormones in various species (Chao *et al.,* 1999). In a study in white tailed deer, xylazine had no significant effect on the activity of thyroid hormones T_3 and T_4. The stress induced by long term transportation is possibly the cause of disparity observed in our results when compared to previous reports. It could be deduced that the breed of goat used in this study are hardier when compared to other Nigerian goat breeds. They are well adapted to stress due to varied loading and stocking conditions. Conclusively, xylazine treatment might have improved adaptability in long term transportation, hence the non-influence of stocking on the varied treatment regimes.

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