

Antistress evaluation of xylazine and ascorbic acid doses using biomarkers and chevon characteristics sequel to stocking and 28-hours road transportation

¹*Biobaku, K.T., ³Ameen, S. A. and ²Jibir, M.

¹Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin

²Department of Animal Science, Usmanu Danfodiyo University, Sokoto.

³Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ilorin, Nigeria.

*Corresponding author's e mail: biobaku.kt@unilorin.edu.ng

Target audience: Small ruminant farmers, livestock marketers, meat scientists and researchers

Abstract

Transportation of animals cannot be avoided in animal husbandry and when they are subjected to psychological and physiological stress could compromise product. Thirty-two apparently healthy Sahel bucks were subjected to an experimental journey. Four groups were randomly assigned into control none treated, xylazine (0.02 mg/kg) and ascorbic acid (200 mg/kg), xylazine (0.01 mg/kg) and ascorbic acid (400 mg/kg), xylazine (0.015 mg/kg) and ascorbic acid (300 mg/kg) each group had four animals each in both high and low stocking density sub-groups. The xylazine was administered intravenous while ascorbic acid was administered orally. Group (0.02mg/kg) xylazine and (200mg/kg) ascorbic acid at high stocking rate significantly ($p < 0.05$) increased the dressing percentage, while low stocking rate decreased using the same combination significantly ($p < 0.05$) decreased dressing percentage. Group of (0.015mg/kg) xylazine and (300mg/kg) ascorbic acid at high stocking rate significantly ($p < 0.05$) increased the excitatory score at slaughter. There were also interactions of treatment on excitatory score, dressing percentages and triiodothyronine. This means that the treatment using the two drugs influenced these parameters. It infers the antistress treatment influenced some meat characteristics. Conclusively, groups (0.02mg/kg) xylazine and (200mg/kg) ascorbic acid, and (0.015mg/kg) xylazine and (300mg/kg) ascorbic acid improved some meat characteristics in bucks, withdrawal period of xylazine must be considered.

Key words: Doses; Xylazine-ascorbic acid; Improved; Chevon; Stress

Description of Problem

Transportation is one of the husbandry practices that is common though often neglected, since animals are moved for sale, production or slaughter (1). Long term road transportation of animals is a practice that is consistently taking place in Sub-Saharan region of Africa (2). There is little or no

consideration given to likely effect of transportation stress on animals meat characteristics in some countries in Tropics (3). A number of methods have been adopted by the government in Nigeria to reduce transport stress in animals. They include legislation on stocking density, creation of livestock control post and trade animal

certification (4). Unfortunately, the legislation is weak and often not enforced and animals are left at the mercy of transporters and merchants (3,5). With regards to the control post, the infrastructure appears to have collapsed with adequate and qualified manpower to man such posts where available. In other parts of the world, drugs are used to alleviate transportation stress in animals with benefits as long as withdrawal period is observed (3,6). This process exerts psychological and physical stress on the animals and this has been reported to be detrimental to their welfare and may affect the meat quality. (4,7).

The detrimental effects of long term road transportation and extreme environmental temperatures were previously studied (8,9). The findings from these investigations would be of value to farmers, livestock marketers and other stakeholders in the livestock industry (including the consumers) since stress has been reported to affect health and meat characteristics in food animals. The use of drugs that could help in alleviating stress of transportation therefore would be valuable if used judiciously.

The present study will investigate the benefit of combining a supplement which is ascorbic acid with a centrally acting drug and an α -2 adrenoceptor agonist xylazine on stress of transportation in goats. Goats are a source of cash for many farmers in Nigeria (10,11). This work could improve animal health, quality of goat meat and profitability of livestock enterprise.

The objective of the study is to evaluate three doses of co-administered xylazine and ascorbic acid combinations were co-administered on stress biomarkers and some chevon characteristics at two stocking rates.

The aim of the study may further provide an insight on use of therapeutics in rendering welfare animal transportation to improve chevon.

Materials and Methods

Experimental animal and Design

Thirty-two apparently healthy Sahel bucks were used in this research. Four groups were randomly assigned into control none treated, xylazine (0.02 mg/kg) and ascorbic acid (200 mg/kg), xylazine (0.01 mg/kg) and ascorbic acid (400 mg/kg), xylazine (0.015 mg/kg) and ascorbic acid (300 mg/kg) each group had four animals each in both high and low stocking density sub-groups. The age range of the animals were between 18- 24 months all with the same body condition score of 3 over the scale of 5 and were within the weight range of 9 to 15kg. The animals were purchased from livestock markets in Sokoto state, Nigeria. The animals were acclimatized for two weeks in the Animal House of Department of Agriculture, Usmanu Danfodiyo University, Sokoto. Animals were dewormed prophylactically with albendazole (Bamizole[®], Nigeria) and were administered with Penstreptomycin (Kepro[®], Holland) against secondary bacterial infections. They were fed and maintained on cowpea husk, wheat bran (Crown[®], Nigeria) and water was provided *ad libitum*. Animal welfare procedure was in accordance with Usmanu Danfodiyo University, Sokoto, research ethical approval.

Usmanu Danfodiyo University, Sokoto, Nigeria located along 13.1°N and latitude 5°13'E 350m above sea level in the Semi-arid zone of North-west Nigeria. The goats were transported during the Harmattan season in the month of January. The induction of

transportation stress began at Sokoto and ended at the Federal University of Agriculture, Abeokuta, and Ogun State; which was located along latitude 71° 0'N, longitude 32°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 is 996km at a speed of 40km/hr as previously adopted (2,5). This distance gives insight of the journey covered by goat marketers when transporting food animals across the country.

Table 1: Effects of Co-administered Xylazine and Ascorbic acid Doses on Stress Biomarkers of Sahel bucks Exposed to Stocking and 28 –hours Road Transportation

Biochemical Parameters	Control	Dose						SEM	Interactions
		X YL 0.01mg/kg and ASC 400mg/kg		X YL 0.02mg/kg and ASC 200mg/kg		X YL 0.01mg/kg and ASC 400mg/kg			
		High	Low	High	Low	High	Low		
SOD (Iµl) p	40.47	42.07	36.90	42.100	42.68	40.46	38.60	5.47	NS
SOD (Iµl) m	199.52	21.40	20.57	17.20	22.17	19.93	15.83	3.196	NS
SOD (Iµl) e	41.73	44.70	42.60	47.63	37.97	39.40	38.10	3.07	NS
GSTµl/ml p	1.39	0.96	1.39	1.81	1.27	1.55	1.36	0.16	NS
GSTµl/ml m	1.05	1.02	1.37	0.85	1.16	1.06	0.85	0.15	NS
GSTµl/ml e	0.88	0.73	0.73	1.59	1.57	1.43	1.40	0.60	NS
MDA(M ⁻¹ Cm ⁻¹) p	0.09	0.08	0.07	0.08	0.07	0.08	0.07	0.07	NS
MDA(M ⁻¹ Cm ⁻¹)m	0.09	0.05	0.08	0.05	0.04	0.08	0.08	0.06	NS
MDA(M ⁻¹ Cm ⁻¹) e	0.09	0.06	0.07	0.04	0.05	0.06	0.08	0.06	NS
T ₃ (ngl/ml) p	2.60	3.20	2.23	2.10	4.40	0.80	2.87	1.14	NS
T ₃ (ngl/ml) m	3.22	1.90	2.27	4.30	2.73	3.33	4.77	1.03	NS
T ₃ (ngl/ml) e	4.41	10.63	2.13	0.60	7.00	4.60	1.47	1.57	*
T ₄ (µg/dl) p	5.21	4.57	13.90	2.40	2.83	3.97	3.60	2.89	NS
T ₄ (µg/dl) m	5.03	5.67	2.83	0.70	8.50	3.13	9.37	2.51	NS
T ₄ (µg/dl) e	10.43	8.80	4.37	17.00	12.57	7.4	12.37	3.70	NS

GST: Glutathione-S-Transferase ; MDA: Malondialdehyde; SOD: Superoxide Dismutase T3: Triiodotyronine; T4: Tetraiodotyronine; XYL: Xylazine ASC: Ascorbic acid. Phases of the experimental journey P (prior),m(midway) and e (after) , * : P<0.05 (interaction)

Administration of Xylazine and Ascorbic acid

The choice of this drug and supplement with dosages were as previously suggested (2, 12). Xylazine (XYL-M2[®], Belgium) was administered intramuscularly while the ascorbic acid (Ascormed[®], Nigeria) tablets were administered orally. Both were co-administered at pre-loading point and midway in to the experimental journey 12 hours into the journey as previously adopted by (12,13)

Ethics, Loading of Animals and Experimental Journey

A health certificate to reaffirm the health condition of the was collected from the Ministry of Forestry and Animal Health, Sokoto State, Nigeria. This is in adherence to the Animal disease Act (14). The experimental Sahel bucks used were acclimatized and not predisposed to psychological and physiological stress before to the experimental journey as previously adopted (3). The Sahel bucks were

handled carefully and loaded onto the transporting truck in the early hours of the morning at about 9.00 am.

The stocking ratio density of floor area was 2:1. This is in concordance with the standard as previously stipulated (14). This is a comparison of the stocking rate used routinely by marketers. The floor area of the loading vehicle used was 10.70 m² and 5.30 m² floor areas for low and high density stocking respectively giving a floor area of 4.02 m × 2.01 m (low density stocking) and 2.84m x 1.40 m (high density) as previously adopted (2,13). The loading truck used floor was cushioned using sawdust, sand and sorghum leaves to prevent the goats coming in contact with urine and faeces and injury at transportation as previously done (3,13).

Blood Collection and Stress Biomarkers Analysis

After ensuring proper restraint, a 21 gauge needle and syringe was used and after swabbing with methylated spirit and cotton wool to ensure asepsis. Blood samples were collected from the juglar vein in sample bottles containing lithium heparin from each animal at prior to loading at Sokoto, midway exactly 12 hours into the journey when resting the animals midway at the veterinary quarantine post at Jebba. Blood Sample was also collected after offloading at Abeokuta as previously adopted (2). The blood samples were analysed for enzymatic antioxidative stress markers such as glutathione -S transferase, superoxide dismutase using Randox[®] commercial test kit using as previously adopted (3).The malondialdehyde was also assessed using thiobarbituric acid reacting substances to determine the extent of

lipid peroxidation as previously adopted (15).Thyroid hormones namely triiodothyronine (T3) and tetraiodothyronine (T4) were also assayed using Biorex[®] test as previously adopted (3).

Carcass preparation and chevon quality assessment

The animals were rested and a slaughter schedule was prepared in which animals on the regimen of xylazine were rested for 4 days. This was carried out to ensure adherence to international rules for withdrawal period of the α -2 adrenoceptor agonist the xylazine to allow drug residues attain the daily acceptable concentration (12,13). The halal method of slaughter was adopted (7).The experimental bucks were prepared by shaving as being the method used in the Southwestern Nigeria as previously described (7). The cranial aspect of the slaughtered animal was separated at the atlanto-occipital joint and the fore and hind limb dissected at the carpal and tarsal joints as procedures for evacuating goat carcass as previously conducted (16, 17).The animals were subsequently evisceration and internal offals the carcass removed. But it is important to note that carcass still beard the weight of the skin in this study is considered the dressed carcass as in previous studies (5,13). The carcass weight without the internal organs was considered was the empty body weight (5). The dressing percentage was deduced mathematically by dividing the carcass weight by the live weight multiplied by one hundred (18).Cooking loss test was also conducted using a chop from the *semi membranous* muscle as conducted (13, 17). The percentage loss in weight deduced by mathematical extrapolation after observing the

weight difference as previously adopted (18). The ultimate pH of the meat was determined as previously adopted (19,20). Water holding capacity was determined using the filter paper method (21) and as adopted in previous recent studies (5,7). Meat colour evaluation was carried out using the *rectus abdominis* muscle as previously conducted (22) and as previously modified (17).

Result and Discussion

Table 1 below shows no influence (P>0.05) of the co administered of combinations of xylazine and ascorbic acid on all stress biomarkers. There was however, an interaction of the treatment on T₃ which significantly decreased its level (P <0.05) at high stocking rate. The effect of stress on the thyroid hormones in this study had shown that transportation stress causes an influence on the level of thyroid hormone the T₃. This could be due its principal role of the thyroid

gland in response to stress and metabolic changes. This might also be due to the influence of the thyroid stimulating hormone in the anterior pituitary gland whose surge was influenced by the central depressive effect of xylazine on the hypothalamo-hypophyseal axis. Breed disposition and other environmental influences could also be a contributory factor to the decrease in T₃. In a previous study of the effect of xylazine on the thyroid gland there were no significant changes on the level of the T₃ and T₄ in the animals. Some other researches in goats showed similar results (7,23). The variations in the findings could be due to the long road transportation stress encountered by the animals and specie variation. The administration of ascorbic acid in the combination with the xylazine could also further influence the physiologic changes towards adaptation and envisaged influence on the T₃ in long road stress.

Table 2: Effects of Combinations of Co-administered Xylazine and Ascorbic acid on Chevon Characteristics of Sahel bucks Exposed to Stocking and 28 –hours Road Transportation Stress

Meat Character istics	Control Distilled water	Combinations			SEM	Stocking rate			Interaction
		Xylazine (0.02mg/kg) and ASC (200mg/kg)	Xylazine (0.01mg/kg) and ASC (400mg/kg)	Xylazine 0.015mg/kg and ASC (300mg/kg)		High	Low	SEM	
S (%)	19.52	21.43	14.28	15.29	4.30	18.60	15.41	3.50	NS
D (%)	73.69 ^b	80.12 ^a	74.77 ^b	72.00 ^c	2.38	78.79 ^a	73.12 ^b	1.94	*
E S	3.67	4.00 ^a	4.00 ^a	3.50 ^a	0.00	4.00 ^a	3.67 ^b	0.00	*
EBW (Kg)	7.95 ^b	9.53	10.39	8.23	0.58	9.44	9.33	0.48	NS
WHC (%)	69.00 ^a	45.62	42.13	46.98	2.91	44.72	45.10	2.38	NS
pH	5.87	5.87	5.80	5.82	0.21	5.63	6.02	0.47	NS
Col (l)	106.67 ^a	80.00	86.66	80.00	3.85	80.00	84.44	3.14	NS
CL (%)	41.07	43.88	44.98	42.73	0.73	42.19	45.48	0.59	NS

S %: Shrinkage Percentage; D % : Dressing Percentage; E S: Excitatory score at slaughter; EBW: Empty body weight; WHC: Water holding capacity; Col: Colour ; CL : Cooking loss. NS: not significant; * (P<0.0 5)

The mean values of various combinations of xylazine and ascorbic acid are presented in the table 2 below. There were interactions between treatment and stocking rate on dressing percentage and excitatory score of slaughter parameters. In Figure 1 and 2 below showed the interactions of the treatment of co administered of xylazine and ascorbic acid on dressing percentage and excitatory score at slaughter. This is due to the effect of ascorbic acid on the metabolism and rejuvenating effect on the animals transported and the muscle relaxing effect of the anxiolytic agent, xylazine on the animals. The various co-administered combination at different doses elicited their effect to bring about the improvement of animal welfare and improving the dressing weigh percentage. This is associated with pain relieving and sedating effect of xylazine. It could also be due to the fact that xylazine tends to decrease the demand for food by decreasing the gastrointestinal motility. This is in line with the previous study (24) found decrease in motility sequel of administration of medetomidine (a drug of the same class as xylazine) in ruminants. This would decrease the appetite at transportation and brings calmness. This pharmacological properties elicited could be associated with xylazine's agonistic effect on the α -2 adrenocetors. Previous authors (23), explained that the goat possess receptors which are very sensitive to the alpha agonists this attributed to the α -2D subclass which causes a lot of physiological manifestations such as marked respiratory depression, influence on the electrolytes via the presence of the receptors in the uriniferous tubules of the kidney all aimed in counteracting and restoring homeostasis. This is in line with the findings as

suggested (7), this couple with the supplementation of ascorbic acid most likely be the reason of the interactions of various treatment at stocking rates on the dressing percentage and excitatory score at slaughter.

0.02mg/kg of xylazine and 200mg/kg of ascorbic acid at high stocking increased the dressing percentage, while low stocking decreased using the same drug combination decreased dressing percentage. In the other combination doses: (0.01mg/kg of xylazine and 400mg/kg of ascorbic acid) and (0.015mg/kg of xylazine and 300mg/kg of ascorbic acid). Combination of 0.015mg/kg of xylazine and 300mg/kg of ascorbic acid at high stocking rate increased the excitatory score at slaughter while at low stocking rate the excitatory score at slaughter was decreased. This show that at standard stocking the later doses was more effective in improving the dressing percentage. The combination of (0.02mg/kg) of xylazine and (200mg/kg) of ascorbic acid did not improve the dressing percentage at low stocking, but the dressing percentage increased at high stocking density. There is no clear explanation for this but it could be explained that at high stocking rate, the rate of absorption of ascorbic acid was high because of the envisaged decrease in the level of ascorbate in the plasma. But despite the high demand the vitaminergic receptors might be influenced by the physiologic mechanisms that were upset by stress. In the same vein it was previously speculated too that high doses of ascorbic acid simulate or procreation reactive oxygen species if stress overwhelms the cellular antioxidative mechanism. This is due to the effects of oxidative stress and production of reactive oxygen species. Previous work correlated the impact of electrolytes namely

calcium, sodium, potassium and chloride ions are influenced by ascorbic acid (7). The effect of the triiodothyronine and tetraiodothyronine which are forms of thyroid hormones might also be influenced by the supplement aiding adjustment to physiologic adaptation and metabolism. This might be the most likely reason for the improvement of the dressing percentage. Other reasons are not far away from improvement of the intermediary

metabolic pathways which would aid physiologic adaptation in counteracting the adverse effect of muscle glycogen depletion, deterioration of myocytes (muscle cell) integrity. This with the muscle relaxing effect of xylazine would elicit its effect peripherally and centrally in the brain and spinal cord which could counteract the detrimental effect of cortisol and catecholamine and its congeners.

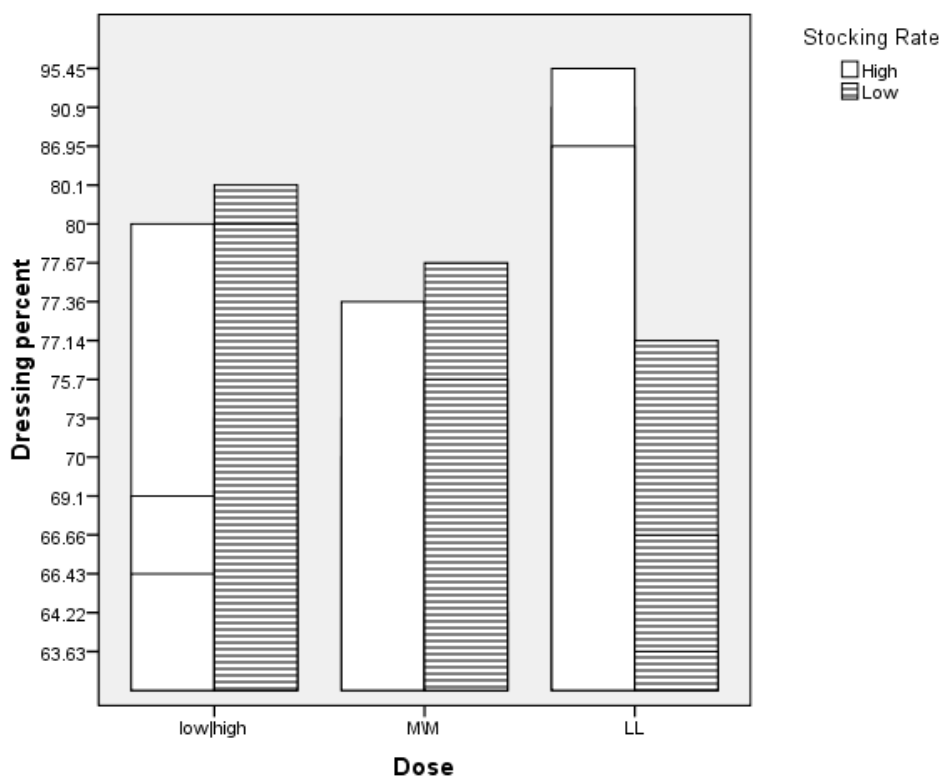


Figure 1: Interaction of various combinations of xylazine and ascorbic acid, stocking rate on dressing percent

Low/high: 0.01mg/kg of xylazine and 400mg/kg of ascorbic acid

MM: 0.015mg/kg of xylazine and 300mg/kg of ascorbic acid

LL: 0.02mg/kg of xylazine and 200mg/kg of ascorbic acid

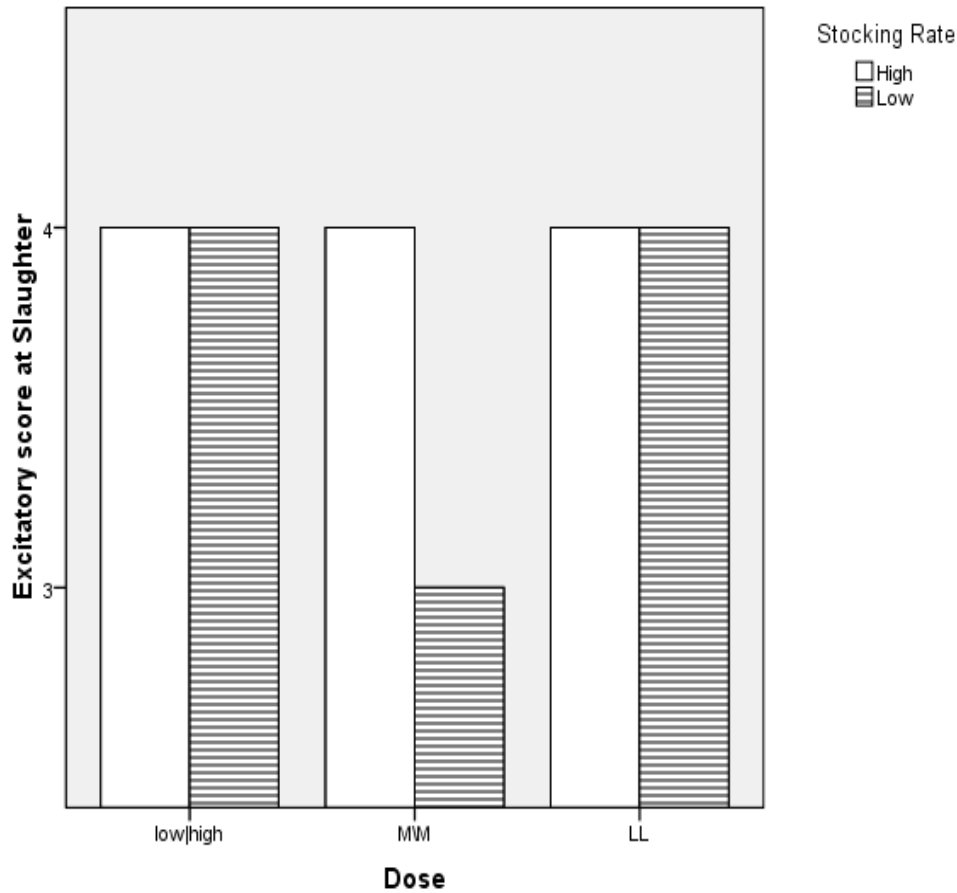


Figure 2: Interaction of various combinations of xylazine and ascorbic acid , stocking rate on excitatory score at slaughter

Low/high: 0.01mg/kg of xylazine and 400mg/kg of ascorbic acid

MM: 0.015mg/kg of xylazine and 300mg/kg of ascorbic acid

LL: 0.02mg/kg of xylazine and 200mg/kg of ascorbic acid

The combination dose of 0.015mg/kg of xylazine and 300mg/kg of ascorbic acid at high stocking rate increased the excitatory score at slaughter .This suggests that this dose is efficacious even at a high or non standard stocking density. This could be due to the

stress condition of the animal involved and with the relatively high dose of ascorbic acid it must improved the response mechanism which is linked with the synthesis of catecholamine as previously suggested (4). In the previous study the stress induced was for two hours and

the doses used are relatively lower but correlation in respect to improvement of animals excitatory score. In the previous study xylazine was not co administered and since xylazine possess sedative and hypnotic effects this could be the likely reason for the variation in the findings and the observations. The breed of goats, sex and environmental influences might also have impact on the findings when compared with the present study. At low stocking rate, the excitatory score at slaughter was decreased, this might be due to physiologic influence in animals to achieve general adaptation syndrome and the temperament of the animals since at low or standard stocking they were calmer. The combination 0.01mg/kg of xylazine and 400mg/kg of ascorbic acid and 0.02mg/kg of xylazine and 200mg/kg of ascorbic acid improved excitatory score at slaughter at low density stocking (standard stocking rate). This could be associated with doses of the combined drugs. This combinations with the low stocking density might have influence the sympathetic and parasympathetic systems to alleviate stress.

Conclusions and Applications

1. The combination 0.02mg/kg xylazine and ascorbic acid at 200mg/kg improved dressing percentage of bucks exposed to high stocking and 28-hours stress. This would eventually be profitable to the farmer or marketer in terms of money value.
2. The combinations (0.01mg/kg xylazine and 400mg/kg of ascorbic acid) and (0.015mg/kg of xylazine and ascorbic acid at 300mg/kg) could be used at non standard or high and low stocking density

(standard stocking) could be used to ameliorate stress of animals to be slaughtered since it improved their excitability score this means that the meat from the treated animal would have less concentration of stress hormone (cortisol and its congeners) this would be healthier to consumers and could aid shelf life.

3. The meat characteristics that were influenced and improved upon at low stocking are the colour which had higher value to indicate higher luminosity and tended pink in colour. This would improve wholesomeness, acceptability and patronage.
4. This gives scientific evidence that strict adherence to the standard stocking or (low density stocking) would improve animal welfare at transported and meat quality.
5. The only drawback to the implementation of this study is the farmer and marketers adhering to withdrawal period of 2-4 days for the xylazine to reach it tolerable level. This drawback could be avoided by use of professionals in the livestock industry to proffer solutions, enlightenment and advice the farmers.

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