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Full Length Research Paper

Ameliorative effects of ascorbic acid on rectal temperature, excitability score and liveweight of rabbits transported by road

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This experiment was performed with the aim of investigating the effects of ascorbic acid (AA) on stress due to road transportation of rabbits. Nine rabbits administered AA served as the treated animals, while seven others given sterile water were used as the controls. All the rabbits were transported by road for 2 h under standard conditions, and their rectal temperature (RT), excitability score and liveweight values were recorded before, during and after the transportation. The results showed that road transportation was stressful to all the rabbits, as evidenced by an increase in RT values of both the treated and control animals after the transportation. Post-transportation RT value in the control rabbits (39.67 ± 0.41°C) was significantly ($P < 0.05$) higher than that of the treated rabbits (39.0 ± 0.16°C). The excitability scores of the rabbits decreased considerably following road transportation, especially in the control rabbits that were not administered AA. The liveweights of both the treated and control rabbits decreased on arrival. On day 1 post-transportation, the control rabbits lost 2.70% of the pre-transportation liveweight, while the treated rabbits gained 2.37% of the pre-transportation liveweight. In conclusion, the administration of AA to rabbits prior to the commencement of the journey ameliorated the adverse effects of stress due to road transportation.

Key words: Road transportation, rectal temperature, liveweight, excitability score, ascorbic acid, rabbits.

INTRODUCTION

It has been established that road transportation is a major stress factor in farm animals, exerting deleterious effects on health, performance and, ultimately, product quality (von Borell, 2001; Minka and Ayo, 2007a; Averos et al., 2009). Behavioural, physiological and biochemical changes have been demonstrated to occur during handling, loading and transport procedures of animals (Fazio et al., 2005; Minka and Ayo, 2007a, 2008, 2009, 2010; Minka et al., 2009). Vitamin C, also known as ascorbic acid (AA), protects the cells from the damage caused by reactive oxygen species and mutagens (Kelly, 1998; Yarube et al., 2009). One of the most widely accepted functions of AA is that it acts as an antioxidant by interrupting free-radical chain reactions in the body (Powers and Jackson, 2008) and, thus, significantly decreases the levels of reactive oxygen species in the body (Dröge, 2002; Whitehead and Keller, 2003). As an antioxidant, AA can rejuvenate the antioxidant, vitamin E and indirectly prevents free-radical damage in lipids. These two antioxidant vitamins reduce the destructive process of lipid peroxidation of cytomembranes (Kanter et al., 1993; Huang et al., 2002). AA occurs at high levels in many vital organs with an active metabolism, and its concentration in various tissues is related to the dietary intake of this vitamin (Gabaudan and Verlhac, 2001). AA has been shown to reduce the risk of stress due to transportation (or journey) by road in goats (Ayo et al., 2006b; Minka and Ayo, 2007b; Minka et al., 2009), pigs (Adenkola and Ayo, 2009; Adenkola et al., 2009) and pullets (Ayo et al., 2005; Minka and Ayo, 2008, 2010). It decreases lipid peroxidation intensity *in vitro* in leucocytes obtained from calves transported by ship (Urban-Chmiel et al., 2009). Ali et al. (2006) showed that pretreatment with xylazine ameliorated stress due to 2-h

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road transportation in desert sheep and goats. The residual effects of this drug may be harmful to man; but AA is relatively non-toxic, cheap and readily available. Important indices of stress due to road transportation in livestock include rectal temperature (RT) (Ayo et al. 2005; Minka and Ayo, 2007b), excitability scores (Ayo et al., 2006b; Minka et al., 2009) and live-weight (Minka and Ayo, 2007b, 2010). Although substantial studies on the effect of transportation stress have been carried out in other livestock including poultry, there is paucity of information on the effect of transportation on the welfare of rabbits (Fuente et al., 2004; Maria et al., 2006). Besides, management strate-gies aimed at alleviating stress due to road transportation in rabbits under hot-humid conditions are currently lacking.

The aim of this study was to evaluate the role of AA on rectal temperature (RT), behavioural and liveweight changes in rabbits transported by road.

MATERIALS AND METHODS

Animals and management

Sixteen (16), apparently, healthy rabbits aged between 8 and 12 months, belonging to both sexes and weighing 800 to1200 g were used for this experiment. The rabbits were of different crossbreeds; characteristic of rabbits reared by farmers in the zone. They were purchased from a private farm in Kaduna (10° 31′ N, 7° 26′ E), Nigeria and kept for three days in the Livestock Farm, College of Agriculture and Animal Science, Kaduna. During this period, the rabbits were pre-conditioned to the environment and experimental procedure. They were reared in standard individual metallic cages of 60 × 25 cm. The rabbits were given access to pelleted feed (proximate analysis: 14.5% crude protein, 7.2% crude fibre, 7% fat, 0.8% calcium, 0.4% phosphorus) and water *ad libitum*.

Experimental sites

The study was carried out from 30th August to 9th September, 2008 during the peak of the rainy season in Kaduna and Zaria (11° 10′ N, 07° 38′ E), located in the Northern Guinea Savannah zone of Nigeria. The first (pre-transportation) phase of the experiment was performed in Kaduna, while the second (post-transportation) phase was conducted in Zaria.

Experimental procedures

After the 3-day pre-conditioning period, the rabbits were randomly divided into treated and control groups, comprising nine (6 males and 3 females) and seven rabbits (five males and two females), respectively. RT values were recorded daily from all the rabbits for three consecutive days at 07:00, 13:00 and 18:00 h (GMT +1). The RT was measured by inserting a digital clinical thermometer (Electron thermometer, COCET, China) for about 1 to 2 min into the rectum of each rabbit until alarm sound was produced, indicating the end of the reading. Dry and wet-bulb temperatures were also recorded during this period using a dry and wet-bulb thermometer (COCET, Shenzhen, Guangdong, China). The relative humidity (RH) was calculated from the values obtained using the instruction attached by the manufacturer. On day 4, at 30 min before the transportation, the treated rabbits were administered AA orally and using a gavage at the dose of 100 mg/kg (Zapadnyuk et al., 1983) dissolved in 5 ml of sterile water; while the control rabbits were administered similarly, but with only 5 ml of sterile water. Thereafter, the values of RT, liveweight and excitability score were recorded in both treated and control rabbits.

On day 4, just before loading the animals into the vehicle, the excitability scores were assessed in the rabbits by a blind observer during weighing, as an indicator of the reaction of the nervous system, according to the modified method of Ayo et al. (2006b) and Minka et al. (2009). Briefly, the reaction of each animal to handling was determined during weighing using an ordinal scale as follows: rabbits that did not struggle at all were given an excitability score of 0; those that struggled little by occasional shaking (once or twice) were given an excitability score of 1; those that struggled much (3 to 5 times of body shaking) were reported to have excitability score of 2; and those that struggled excessively by violent resistance during weighing had excitability score of 3. The rabbits were transported on asphalt road in the cabin of a Honda car (made in Japan), 1986 model, in rabbit cages from Kaduna to Samaru-Zaria, covering a distance of 80 km at a speed of about 45 km/h. The journey lasted for 2 h. During the journey, RT, dry- and wet-bulb temperature values were recorded at 30 and 90 min into the journey, and immediately on arrival in Zaria (at 2 h of the transportation), when the journey was completed. The liveweight and excitability scores were recorded in all the rabbits on arrival (immediately after the 2-h transportation), and on days 1 and 3 post-transportation. The RT, dry- and wet-bulb temperatures were recorded on days 3 and 7 after transportation at 7:00, 13:00 and 18:00 h each day (Ayo et al., 2006b). The liveweight value of each rabbit was obtained using an electronic weighing balance (Citizen Digital Balance, Switzerland) and according to a standard procedure (Minka and Ayo, 2007b).

Statistical analysis

The data obtained were expressed as mean ± standard error of the mean (mean ± SEM), and were subjected to Student's *t*-test and Pearson's correlation analysis. Values of $P < 0.05$ were considered significant.

RESULTS

Dry-bulb temperature and relative humidity values

The dry-bulb temperature (DBT) values recorded from the study period fluctuated between 20.5 and 34°C, with mean and range values of 33.0 ± 0.58°C and 13.5°C, respectively. As shown in Table 1, the DBT inside the vehicle fluctuated between 30 and 34°C, with mean and range values of 31.3 ± 0.95°C and 14°C, respectively. The RH values fluctuated between 48 and 61%, with mean and range values of 59.3 ± 4.01% and 13.0%, respectively.

Rectal temperature changes

The mean RT value was lowest at 07:00 h and highest at 18:00 h in both the treated (38.42 ± 0.04 and 39.04 ± 0.05°C, respectively; $P < 0.001$) and control rabbits, with the values of 38.30 ± 0.06 and 39.05 ± 0.05°C, respectively ($P < 0.001$). The overall mean values of RT were

Table 1. Dry-bulb temperature and relative humidity before transportation, during transportation and on arrival.

Period	Dry-bulb temperature (℃)	Relative humidity (%)
Before transportation	30	67
30 min into transportation	34	48
90 min into transportation	30	61
On arrival (2-h transportation)	31	61
Mean ± SEM	31.25 ± 0.95	59.25 ± 4.01

38.77 ± 0.03 and 38.70 ± 0.06℃ in the treated and control rabbits, respectively (P > 0.05). Immediately after loading, just before (30 min) the commencement of the transportation, the RT values recorded were 38.48 ± 0.13 and 38.64 ± 0.14℃ (P < 0.05) in the treated and control rabbits, respectively (Figure 1). The RT values obtained at 30 min (39.09 ± 0.23℃ in treated rabbits vs. 40.6 ± 0.46℃ in control rabbits) and 90 min into the journey (39.21 ± 0.13 and 39.91 ± 0.15℃ in the treated and control rabbits, respectively) were significantly (P < 0.05) higher in the controls as compared to the corresponding values obtained in the treated rabbits. On arrival, the overall mean value of RT in the treated rabbits was significantly (P < 0.05) lower than that of the control rabbits (39.00 ± 0.16 and 39.67 ± 0.41℃, respectively) (Figure 1).

The extreme minimum and maximum RT values of 38.0 and 39.6℃, respectively, were recorded both in the treated and control rabbits before the transportation. The extreme minimum and maximum RT values recorded during and after the transportation were predominantly higher in the control than in the treated rabbits (Figures 2 and 3).

Excitability scores in the transported rabbits

Before the transportation, excitability score of 2 or 3 was recorded in 44% of the treated rabbits; while in the control, 57.1% of the rabbits obtained the score of 2 and 3, respectively (Figure 4). However, immediately after the journey, percent excitability scores of rabbits decreased to 0 and 1 in the control rabbits, and the scores were recorded in 71 and 29% of the animals, respectively. Immediately after the journey, 56% of the treated rabbits had excitability score of 1, while 22% obtained excitability score of 2 or 3. A day after the transportation, the highest excitability score of 3 was recorded in 42.9 and 37.5% of the control and treated rabbits, respectively. Three days after the journey, 38.2 and 57.1% of the treated and control rabbits, respectively had excitability score of 2. The excitability score of 3 was recorded in 12.5 and 14.3% of the treated and control rabbits, respectively three days post-transportation (Figure 4).

Alteration in liveweights of transported rabbits

The liveweight values of both treated and control rabbits decreased immediately after transportation, with percentage losses of 1.7 and 0.67% in the treated and control rabbits, respectively (Table 2). On day 1 after transportation, the treated rabbits gained 2.37% liveweight, while the control rabbits further lost 2.70% liveweight. On day 3 after transportation, the treated and control rabbits gained liveweight of 5.13 and 2.16%, respectively, when compared to the corresponding pre-transportation liveweights.

DISCUSSION

The relatively high DBT (20.5 to 34.0℃) and RH (48 to 91%) obtained from the study period indicated wide fluctuations and that the values were above the thermoneutral zone of DBT of 13 to 20℃ and RH of 55 to 65% for the rabbit (Marai and Rashwan, 2004). The DBT and RH values recorded from the study period were considerably higher than the optimum values for rabbit production, and the wide fluctuations in the parameters showed that the environmental conditions were thermally stressful and may have adverse effects on the rabbits. Similarly, the high DBT values (30 to 34℃) during the transportation showed that the temperature inside the vehicle was higher than the upper limit of the optimum air temperature of 20℃ (Marai and Rashwan, 2004).

The RT values recorded in both the treated and control rabbits demonstrated a distinct diurnal fluctuation, which agrees with the previous findings in rabbits (Ayo et al., 2006a), goats (Minka and Ayo, 2007a) and ostrich chicks (Minka and Ayo, 2007b). Diurnal fluctuations in livestock have been shown to be driven by a biological clock in the brain, particularly in the hypothalamus (Piccione and Caola, 2002). Although the pre-transportation RT values obtained in the treated and control rabbits were not significantly (P > 0.05) different, the values rose significantly 30 min into the transportation in both treated and control rabbits, indicating that road transportation was stressful to all the rabbits. Similar results on the negative effect due to road transportation stress on rabbits were obtained by Fuente et al. (2004) and Maria

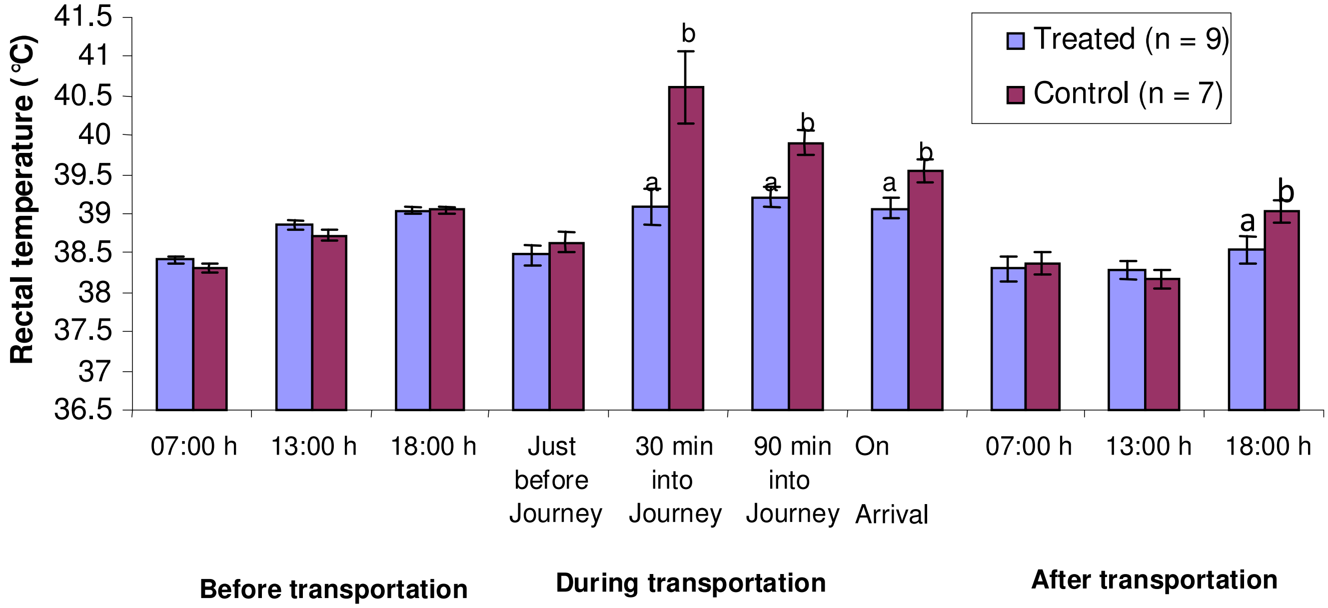

Figure 1. Fluctuations in mean rectal temperature of rabbits before, during and after transportation. Values are mean ± SEM. Values in the same time of recording with different superscript alphabets are significantly ($P < 0.05$) different.

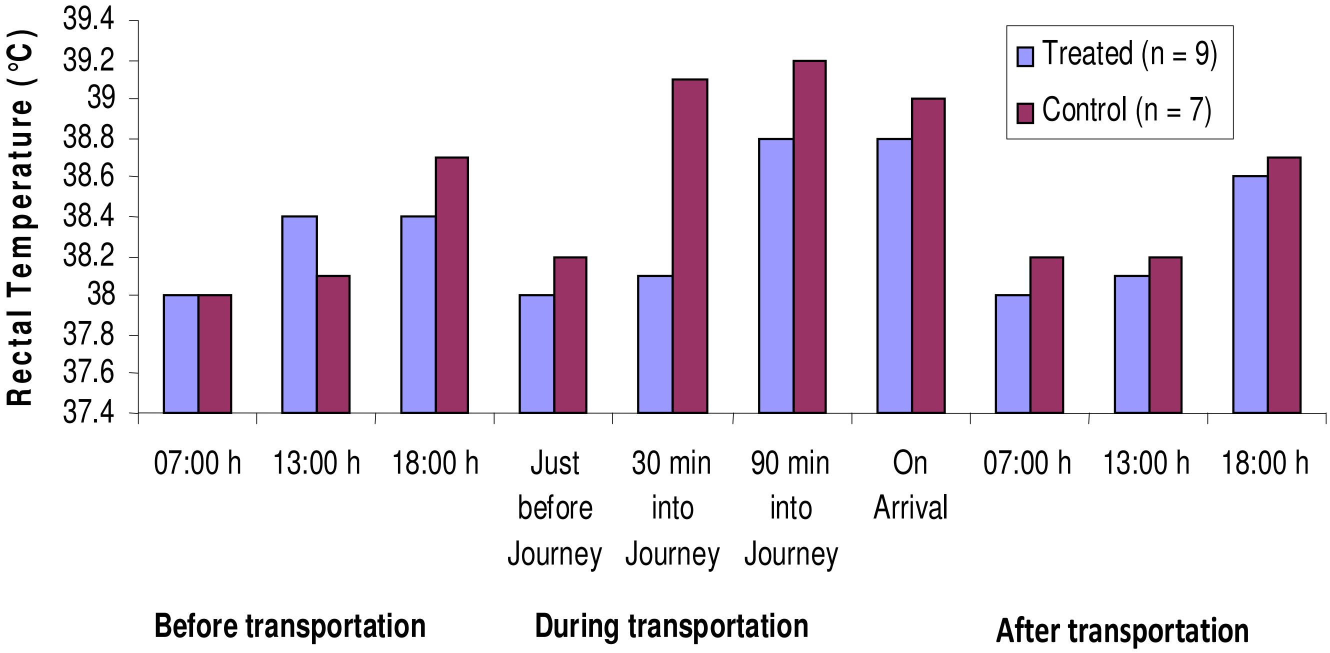

Figure 2. Fluctuations in minimum values of rectal temperature of rabbits before, during and after transportation.

et al. (2006). The results further demonstrated that AA began to manifest its action as early as 30 min of its administration, corresponding to the time when the transportation lasted 30 min. This finding agreed with the results of the pharmacokinetic study on vitamin C that AA exerts its effects following 30 min of its oral administration in humans (Hickey et al., 2008). The results of this study further showed that AA exerted a hypothermic effect in treated rabbits, which agree with the previous findings of Ayo et al. (2005) in pullets and Minka and Ayo (2007b) in goats during the hot-dry season. The findings of this study, for the first time, demonstrated that AA reduced

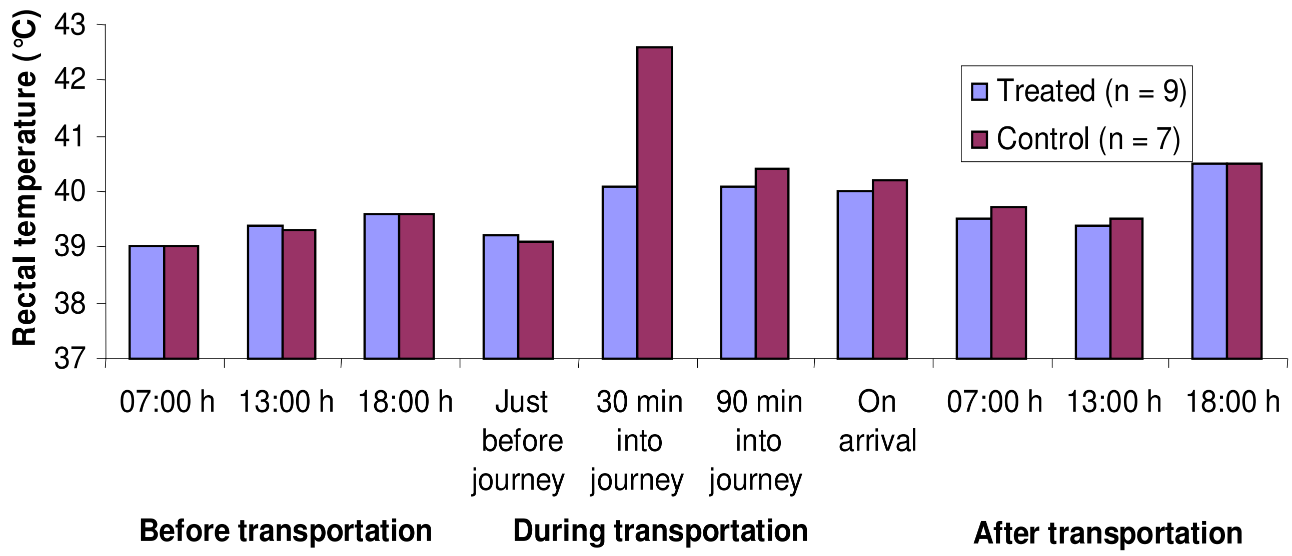

Figure 3. Fluctuations in maximum values of rectal temperature of rabbits before, during and after transportation.

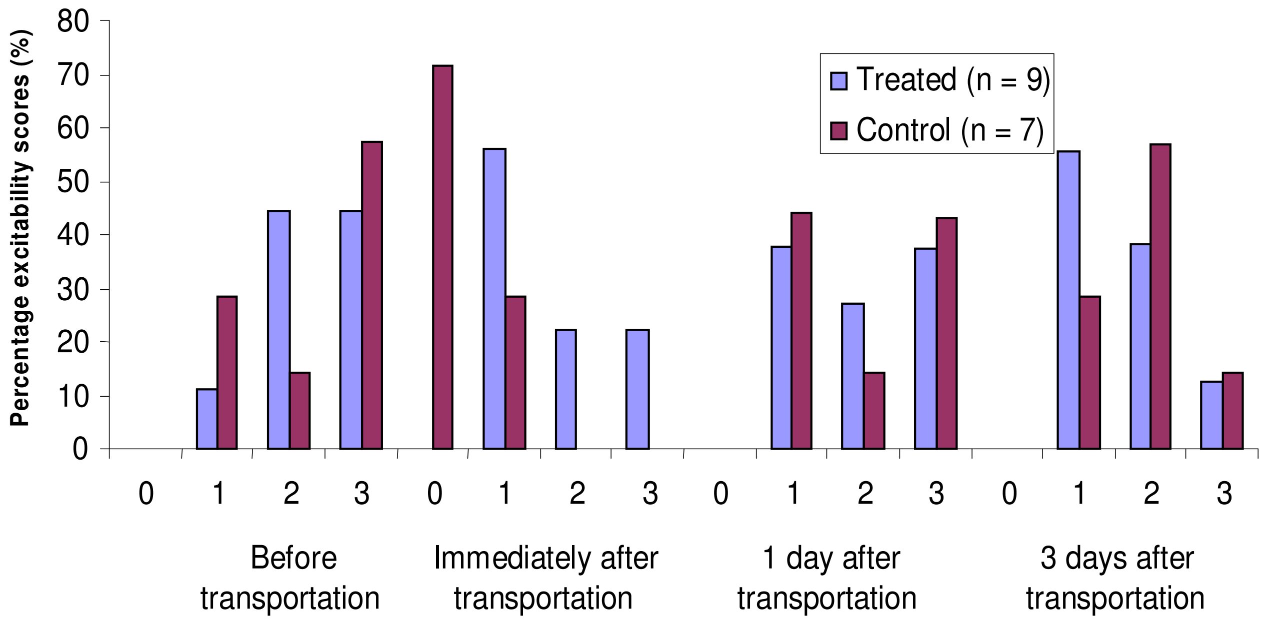

Figure 4. Percentage excitability scores of rabbits before transportation (15 min), immediately after transportation and on days 1 and 3 after transportation.

the adverse effects of road transportation stress in rabbits, and that the vitamin is beneficial when administered to the animals before the commencement of the journey.

The fact that the overall mean value of RT obtained in the treated rabbits was signifi-cantly lower than the corresponding value recorded on day 3 in the control rabbits indicated that the antioxidant, AA had a prolonged effect on relative maintenance of the RT values, even three days after the journey. This finding disagreed with those obtained in goats (Minka and Ayo, 2007b) and pullets (Minka and Ayo, 2008) that AA action diminished three days post-transportation and, thus, exerted a transient effect on RT values in the transported animals. The differences in the results may be due to seasons and species. Minka and Ayo (2007a, 2007b, 2008) conducted their studies on goats during the hot-dry season.

The results of this study showed that the excitability

Table 2. Effects of ascorbic acid on percentage changes in liveweight of rabbits transported by road.

Period	Treated rabbit (n = 9)		Control rabbit (n = 7)	
	Liveweight mean ± SEM (g)	% Change as compared to pre-transportation liveweight	Liveweight mean ± SEM (g)	% Change as compared to pre-transportation liveweight
Before transportation	892.78 ± 50.25	0.00	798.71 ± 39.33	0.00
Immediately after transportation	877.56 ± 49.29	- 1.7	793.33 ± 42.28	-0.67
Day 1 after transportation	914.00 ± 56.95	+2.37	777.14 ± 46.59	-2.70
Day 3 after transportation	938.63 ± 48.29	+5.13	815.29 ± 40.29	+2.16

scores of the rabbits decreased considerably after road transportation, especially in the control rabbits that were not administered AA. This finding is in agreement with that of Ayo et al. (2006b), who demonstrated that AA increased excitability scores in goats transported by road. The fact that AA increased the excitability scores of rabbits post-transportation further confirmed that AA protects the cholinergic receptors from free-radical-induced damage (Venkatesham et al., 2005). This finding is in agreement with the results obtained by Chakraborti et al. (2008), who showed that pre-treatment with anti-oxidants consistently reversed stress-induced neuro-behavioural changes in rats. The results of this study demonstrated that road transportation in rabbits induced neuro-behavioural stress that was transient, and AA administration ameliorated the fatigue observed in the transported rabbits.

Although the mechanism of action of AA in ameliorating road transportation stress in rabbits was not elucidated in this study, it has been established that AA is a potent antioxidant vitamin (Tauler et al., 2003; Whitehead and Keller, 2003; Powers and Jackson, 2008) that scavenges reactive oxygen species in the body. Furthermore, Karanth et al. (2000, 2004) showed that AA is a vitaminergic neurotransmitter released from the hypo-thalamus and involved in thermoregulation, and that the release was mediated by nitric oxide. The findings suggested that AA exerts both peripheral and central effects on the amelioration of adverse effects of free radical-induced changes in animals subjected to stress situations. This requires further investigation.

The decrease in liveweight of all the rabbits recorded in this study after the journey further showed that road transportation was stressful to the animals, even with AA pre-treatment. This finding is in agreement with the reports of Ayo and Oladele (1996), Fuente et al. (2004) and Minka and Ayo (2008, 2009) that stress factors, including road transportation, have adverse effects on the well-being of animals. The fact that on day 1 after transportation, the treated rabbits gained liveweight, but a further loss in liveweight was obtained in the control rabbits, demonstrated the ameliorative effects of AA against the adverse effects of road transportation stress. This finding is in agreement with the established findings of Ayo et al. (2005), Minka and Ayo (2007b), Adenkola and Ayo (2009) and Adenkola et al. (2009) that AA ameliorates the adverse effects of road transportation stress in livestock. It is recommended that AA be administered to rabbits before commencement of transportation in order to alleviate the adverse effects of road transportation stress. AA is cheap, readily available, safe and easy to administer by farmers.

Conclusions

AA reduces RT values, liveweight loss and increases excitability scores in rabbits transported by road. Its administration prior to the commencement of the journey ameliorated the adverse effects of road transportation stress in rabbits. The results of this study demonstrated that measures aimed at ameliorating the adverse effects of the thermal environmental conditions may also be beneficial to rabbits transported during the late rainy season.

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