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Haematological and serum biochemical changes in pigs administered with ascorbic acid and transported by road for four hours during the harmattan season

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This study was conducted with the aim of investigating the effect of ascorbic acid (AA) on haematology and serum biochemical responses of pigs transported for short journey (4 h) during the harmattan season. Sixteen pigs administered with AA at a dose of 250 mg/kg *per os* and individually served as experimental animals, and 13 others each administered orally with sterile water served as control animals. The animals were then transported for 4-h at a speed of 40- 50 km/h covering a distance of 140 km. Blood samples were taken early in the morning a day before transportation, immediately after and a week after transportation. The leucocyte count ($15830.25 \pm 1063.08 \times 10^3/\mu\text{l}$) dropped ($P < 0.05$) in the experimental pigs after transportation and the value was significantly ($P < 0.05$) lower than the value of $22010.69 \pm 1722.00 \times 10^3/\mu\text{l}$ obtained in the control pigs after the journey. The neutrophils: lymphocyte ratio obtained in the control animals increased from 0.61 ± 0.04 before transportation to 0.79 ± 0.17 immediately after transportation. There was a significant ($P < 0.05$) increase in total protein post-transportation in all experimental and control groups, but the increase was higher in the control than the experimental group. Alkaline phosphatase and aspartate amino transferase values in the control pigs increased immediately after transportation. This study indicates for the first time the beneficial effect of AA administration on haematology of pigs transported by road during the harmattan season for short journey of 4 h. It is, therefore, recommended that pigs be administered with AA before transportation by road during the harmattan season in order to reduce the risk of adverse effects of transportation stress on health.

Key words: Ascorbic acid, harmattan season, haematological and biochemical parameters, pigs, road transportation.

INTRODUCTION

Road transportation is a critical phase in animal production and utilization (Odore et al., 2004; Buckham et al., 2008a). The intensity and specialization of livestock production and the demand for livestock to be marketed and slaughtered outside places where they are being produced have necessitated animal transport all over the world (Ayo and Oladele, 1996; Kannan et al., 2000;

Perez et al., 2001) and it is often considered as one of the main causes of stress (Giovagnoli et al., 2002; Pineiro et al., 2007; Adenkola et al., 2008; Buckham et al., 2008b), adversely affecting production both in economic and animal welfare terms (Mormede et al., 1982). Physical and psychic exertions occurring during transport of food animals disrupt their homeostasis and metabolism, and as a result of the exertion, road transport stress increases activity of enzymes and hormones (Ayo and Oladele, 1996; Mstl and Palme, 2002; Buckham et al., 2008a). It has been shown that all stages of transporta-

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tion to which pigs are subjected before slaughter or marketing constitute stress factors. They include loading, transport, unloading, regrouping of pigs and lairage.

While pigs are undergoing these stages, they are simultaneously subjected to changes in their internal environmental conditions (Ayo et al., 1996; Santoro and Faucitano, 1996). Many pigs are transported in Nigeria using vehicle not designed for swine transportation and often in the harmattan and hot-dry season. These conditions adversely affect the health or welfare of the animals and impair their homeostatic mechanisms (Mstl and Palme, 2002; Buckham et al., 2008a) resulting in body dysfunctions which may be fatal.

Environmental stress causes oxidative stress and impairs antioxidants status *in vivo* (Sahin et al., 2001). Antioxidant supplementation, therefore, has been shown to be beneficial in attenuating the adverse effect of environmental stress (Kafri and Cherry, 1984) and stress-induced tissue damage (Sen, 2001; Minka et al., 2007a). Adenkola and Anugwa (2007) showed that ascorbic acid (AA) or vitamin C supplementation improved weight gain and better feed utilization in piglets and it is a naturally occurring antioxidant (Sahin et al., 2001) and currently is the most widely used vitamin supplement throughout the world (Naidu, 2003).

Haematological parameters are good indicators of the physiological status of animals (Hawkey and Dennett, 1989; Adenkola and Durotoye, 2004). It is also an excellent medium for the measurement of potential biomarkers, because its collection is relatively noninvasive and it encompasses an enormous range of physiological process in the body at any given time (Anderson and Anderson, 2002; Ginsburg and Haga, 2006). Currently in Nigeria, there is paucity of information on the haematology and serum chemistry of pigs transported by road, especially during the harmattan season. The harmattan season occurs in the zone between late November and early March, and it is characterized by high ambient temperature (AT) in the afternoon hours of the day and relatively AT of about 10°C in the evening and early morning hours of the day and the season is characterized by cold-dry and dust laden wind (Igono and Aliu, 1982). This zone is characterized by intensive livestock marketing and consequently, transportation.

The aim of the present paper was to investigate the impact of road transportation during the harmattan season on haematology and serum chemistry of pigs administered ascorbic acid.

MATERIALS AND METHODS

Experimental site

The experiment was performed at the Livestock Pen, Faculty of Veterinary Medicine, Ahmadu Bello University, Samaru-Zaria (11° 10' N, 07° 38' E), located in the Northern Guinea Savannah zone of Nigeria during the harmattan season.

Experimental animals and management

Twenty nine local pigs, including males and non-pregnant, non-nursing females, weighing 20-48 kg, and ages ranging from 9- 12 months were bought from different localities in Zaria environs at least two weeks before the experimental day. They were kept at a stocking density of 0.8 m² per animals in a communal pen, made of concrete floor and iron walls with asbestos roofing. The pen measured 7.50 x 2.55 m with half the length of the wall to the roof without block work, which provided adequate ventilation. The pigs were not restrained inside the pen. They were kept under an intensive system of management and fed with maize offal, brewer's waste and yam peel. They were given access to water *ad libitum*.

The pigs were pre-conditioned for two weeks before the commencement of the experiment. During the period, they were screened for haemoparasites and endoparasites by taking their blood and faecal samples for laboratory analyses. They were treated accordingly using oxytetracycline (KEPRO B. V[®], Holland) at the dose of 20 mg/kg by deep intramuscular route and thiabendazole (AGVET[®], U.S.A.) at the dose of 25 mg/kg body weight *per os*.

Experimental design

On the day of transportation, the experimental pigs (n = 16) were orally and individually administered with AA at 250 mg/kg (Chervyakov et al., 1977) dissolved in 20 ml of water, while 13 pigs which served as control were given 20 ml of sterile water. The administrations were made immediately (15 min.) before loading the pigs into the vehicle.

Food and water were withdrawn 12 h before the journey and throughout the journey period, which lasted 4 h. The vehicle traveled along Zaria-Jos road at a speed of 40-50 km/h from Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria (11° 10' N, 07° 38' E), on tarred smooth and rough road covering a total distance of 140 and back to the starting point. After completing the journey, the pigs were unloaded at the spot where they were original loaded. The animals were fed and watered as they had been prior to the journey.

Vehicle design, loading, and journey time

A standard Ford six wheeler bus, popularly used in Northern Nigeria in transportation of livestock was used to transport the pigs. The vehicle engine was in good condition, serviced and made ready for the journey. The floor of the vehicle was non-slippery and was covered with dry beddings before loading the animals. The inner compartment of the vehicle measured 3.22 x 1.67 x 1.2 m high. The sidewalls of the vehicle from the floor to the roof were made smooth with no protrusion of sharp edge and with a window, which provided adequate ventilation. Each window measured 0.8 x 0.44 m on both sides of the vehicle, was at the height of about 1.0 m from the floor. A door which measured 1.4 x 1.2 m was provided at the rear end of the vehicle. Other transportation procedures were carried out in accordance with the standard guidelines governing the welfare of pigs during road transportation (Warris, 1998; Lambooij, 2000). The pigs were stocked at the density of 0.8 m² per animal. They were made to stand inside the vehicle in rows facing direction against the direction of the vehicle movement. The journey commenced at 8:00 am.

Blood sample collections

Blood samples were taken early in the morning a day before transportation, immediately after and a week post-transportation. Ten millimeters of blood was taken aseptically from the anterior vena

cava using a 10 ml syringe and 18 gauge x 1¹/₂ inch sterile needles from each animal. Each blood sample for determination (4 ml) of haematological parameters was immediately poured inside a sample bottle, containing an anticoagulant, disodium salt of ethylene diaminetetra-acetic acid (EDTA) at the rate of 2 mg/ml of blood (Oyewale, 1992). After collection, the samples were transferred to Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, where they were analysed for packed cell volume (PCV) using microhaematocrit method, total leucocyte count using haemocytometer method as described by Schalm et al. (1975). Haemoglobin concentration and differential leucocyte count were also determined as described by Schalm et al. (1975). The remaining (6 ml) meant for serum chemistry and serum electrolyte determination was centrifuged and the serum harvested. Alkaline phosphatase was determined as described by Cheesbrough (1991), aspartate aminotransferase (AST) enzyme activity level was determined using the Reitman-Frankel AST method described by Cheesbrough (1991), Alanine amino transferase, total bilirubin, urea and total cholesterol was done as described by Cheesbrough (1991), total protein by biuret method, serum albumin by Bromocresol green method as described by Cheesbrough (1991), serum globulin was determined by subtracting serum albumin from total protein value. Serum sodium and potassium ions was determined by flame emission spectrometry as described by Cheesbrough (1991), while serum bicarbonate was done using titrimetric method as described by Cheesbrough (1991) and chloride level was analysed by the method of Schales and Schales (1941).

Statistical analysis

The data obtained were analysed using Graph Pad Prism package version 4.00 of 2003. Data were subjected to Student's *t*-test and values of *P* < 0.05 were considered significant.

RESULTS

Haematological parameters before, immediately after and 7 days post- transportation

The values of haematological parameters obtained before the journey in experimental and control pigs were not significantly different (*P* > 0.05). PCV values recorded on day 7 after the journey rose (*P* < 0.05) to 37.38 ± 1.39% in the experimental pigs, and the value was significantly (*P* < 0.05) higher than 33.54 ± 1.01% recorded in the control pigs post-transportation. The total leucocyte count dropped (*P* < 0.05) from 18920.00 ± 1200.00 × 10³/μl in the experimental pigs before the journey to 15830.00 ± 1063.00 × 10³/μl after the journey, and the value was significantly (*P* < 0.05) lower than 22010.00 ± 1722.00 × 10³/μl obtained in the control pigs after the journey. The values recorded on day 7 post-transportation in the experimental and control pigs were not significantly different. The absolute neutrophil count of 5246.93 ± 429.21 × 10³/μl obtained in the experimental pigs was significantly (*P* < 0.05) lower than the value of 8328.15 × 10³/μl recorded in control pigs immediately after the journey. Although in the control pigs the absolute neutrophil dropped to 6667.07 ± 577.30 × 10³/μl on day 7 after the journey, the value was significantly (*P* < 0.05) higher than the corresponding value of 5058.87 ± 314.44 × 10³/μl recorded in the experimental pigs (Table 1). The absolute lym-

phocyte count of 10450.00 ± 757.05 × 10³/μl obtained immediately after the journey in experimental pigs was significantly lower (*P* < 0.05) than 13510.85 ± 1032.04 × 10³/μl recorded in control pigs. Immediately after the journey the absolute monocyte count rose when compared with pre-transportation values, but the increase in the value was not significantly different in experimental and control pigs (*P* > 0.05). The value increased to 134.76 ± 43.09 × 10³/μl in the control pigs on day 7 after the journey, and was significantly (*P* < 0.05) higher than the corresponding value of 35.62 ± 16.48 × 10³/μl obtained in the experimental pigs (Table 1). The neutrophils: lymphocyte ratio obtained in the control animals increased (*P* < 0.05) from pre-transportation value of 0.61 ± 0.04 to 0.79 ± 0.17 immediately after transportation.

Serum biochemical parameters before, immediately after and 7-days after four hour road transportation

Table 2 shows the response to serum biochemical changes in experimental and control pigs. There was no significant difference, in all the parameters between experimental and control pigs, before the journey (*P* > 0.05). Immediately after the journey, the total protein in the experimental pigs did not increase significantly, from 67.94 ± 1.50 gm/dl to 69.69 ± 1.67 gm/dl, while in the control it rose (*P* < 0.05) to 71.31 ± 1.93 gm/dl from 67.62 ± 1.29 gm/dl (Table 2). Albumin: globulin ratio of 1.06 ± 0.12 obtained immediately after transportation dropped to 0.87 ± 0.06 (*P* < 0.05), 7 days post-transportation from 1.11 ± 0.09 obtained before transportation in experimental pigs. The value thus increased from 1.30 ± 0.22 immediately after transportation to 1.34 ± 0.12 in the control pigs on day 7 post-transportation. However there was a significant (*P* < 0.05) difference in the recorded value of experimental and control pigs. The recorded HCO₃⁻ value of 22.15 ± 0.75 mmol/L obtained immediately after transportation in the control pigs rose to a value of 24.15 ± 0.80 mmol/L on day 7 post-transportation (*P* < 0.05) while the corresponding value in the experimental pigs was 21.69 ± 0.84 mmol/L. The Cl⁻ value of 102.70 ± 1.17 mmol/L recorded in experimental pigs immediately after transportation decreased (*P* < 0.05) to 91.00 ± 1.79 mmol/L on day 7 post-transportation, and this value was lower (*P* < 0.05) than the corresponding value of 96.62 ± 1.22 mmol/L obtained in the control pigs.

Alkaline phosphatase value recorded in the experimental pigs dropped slightly from the pre-transportation value of 40.56 ± 2.85 I.U./L to 39.94 ± 2.85 I.U./L, while the value increased in the control pigs from the corresponding value of 40.92 ± 2.92 I.U./L before transportation to 48.23 ± 2.36 I.U./L immediately after transportation (*P* < 0.05) (Table 2). However, the value dropped close to pre-transportation value on day 7 post-transportation in the control pigs. Aspartate amino transferase increased significantly in the control pigs from 15.69 ± 1.08 I.U./L

Table 1. Haematological parameters of experimental (supplemented with ascorbic acid) and control (non-supplemented with ascorbic acid) pigs before short-term road transportation (Mean ± SEM).

Parameters	Before Short Journey		Immediately After Short Journey		Day 7 After Short Journey	
	Experimental (n = 16)	Control (n = 13)	Experimental (n = 16)	Control (n = 13)	Experimental (n = 16)	Control (n = 13)
Packed Cell Volume (%)	33.31 ± 0.95 ^{NS}	32.00 ± 0.86 ^{NS}	33.24 ± 1.29 ^{NS}	32.18 ± 1.90 ^{NS}	37.38 ± 1.39 ^{**}	33.54 ± 1.01 ^{**}
Haemoglobin concentration (gm/%)	11.07 ± 0.32 ^{NS}	10.64 ± 0.28 ^{NS}	11.05 ± 0.43 ^{NS}	11.18 ± 0.33 ^{NS}	12.43 ± 0.46 ^{NS}	12.56 ± 1.23 ^{NS}
Mean corpuscular haemoglobin concentration	33.24 ± 0.02 ^{NS}	33.39 ± 0.15 ^{NS}	33.22 ± 0.02 ^{NS}	33.23 ± 0.02 ^{NS}	12.56 ± 1.23 ^{NS}	33.34 ± 0.09 ^{NS}
Total leucocyte count (× 10 ³ /μl)	18920.00 ± 1199.93 ^{**}	18836.54 ± 1727.14 ^{**}	15830.25 ± 1063.08 ^{**}	22010.69 ± 1722.00 ^{**}	16043.75 ± 829.95 ^{**}	19090.00 ± 1263.00 ^{**}
Neutrophils (× 10 ³ /μl)	6183.25 ± 893.10 ^{NS}	6364 ± 1132.73 ^{NS}	5246.93 ± 429.21 ^{**}	8328.15 ± 788.54 ^{**}	5058.87 ± 314.44 ^{**}	6667.07 ± 577.30 ^{**}
Eosinophils (× 10 ³ /μl)	74.06 ± 30.40 ^{NS}	112.23 ± 34.26 ^{NS}	83.44 ± 22.52 ^{NS}	120.53 ± 51.56 ^{NS}	73.38 ± 27.75 ^{NS}	95.25 ± 45.25 ^{NS}
Monocyte (× 10 ³ /μl)	41.00 ± 18.45 ^{NS}	30.53 ± 16.60 ^{NS}	66.68 ± 27.95 ^{NS}	48.15 ± 25.77 ^{**}	35.63 ± 16.49 ^{**}	134.76 ± 43.08 ^{**}
Lymphocyte (× 10 ³ /μl)	12460 ± 611.09 ^{**}	11362.46 ± 479.35 ^{**}	10450.00 ± 757.05 ^{**}	13510.85 ± 1032.04 ^{**}	10950.88 ± 666.35 ^{NS}	12130.31 ± 787.60 ^{NS}
Neutrophils: Lymphocyte	0.49 ± 0.06 ^{NS}	0.61 ± 0.04 ^{**}	0.52 ± 0.04 ^{NS}	0.79 ± 0.17 ^{**}	0.47 ± 0.03 ^{NS}	0.55 ± 0.03 ^{NS}

**Values with asterisks are significantly different (P < 0.05).

pre-transportation to 21.08 ± 1.09 I.U./L immediately after the journey (P < 0.05). Similar increase was observed in the values obtained in experimental pigs, which is not significantly different (P > 0.05). The values obtained 7 days post-transportation were not significantly (P > 0.05) different in the experimental and control pigs (Table 3). The urea value recorded immediately after transportation rose (P < 0.05) in the control pigs from 4.67 ± 0.25 mg/100 ml pre-transportation to 6.79 ± 0.39 mg/100 ml. However, the value dropped to 4.14 ± 0.23 mg/100 ml in the control pigs 7 days after transportation (Table 2), which was not significantly different from the corresponding value recorded in experimental pigs (P > 0.05).

DISCUSSION

The fact that there was no change in the PCV value immediately after transportation in the present study makes it agree with the result obtained by Scope et al. (2002) who did not obtain significant change in the value of PCV of racing pigeons after 4-h road transportation. This is also similar to the

observation of Knowles et al. (1999b) who demonstrated that PCV value in transported cattle showed no consistency. The observed increase in the value of PCV on day 7 post-transportation in all the transported pigs was within the normal range for pigs while the higher value obtained in the experimental pigs may be attributed to the ability of AA to maintain the integrity of erythrocyte membrane in AA-treated group (Candan et al., 2002). The higher value of leucocytes observed immediately post-transportation in control pigs is similar to the finding of Buckham-Sporer et al. (2008) who demonstrated leucocytosis in transported young beef bulls and that it is one of the biomarkers of transportation stress. The results of the study suggested that AA prevented the release of leucocytes from their pool in the body into peripheral circulation in experimental pigs, apparently due to its inhibitory role on circulating corticosteroids which is known to increase in animals under stress and cause leucocytosis. Also a decrease in AA in the body tissues, especially in adrenal glands is known to be associated with corticosteroid release (Whitehead and Keller, 2003). The depletion of AA in the pigs was prevented in the present study by its exogenous

supply prior to transportation of pigs.

Road transportation of pigs did not alter eosinophil count significantly in experimental and control pigs. This result disagreed with those obtained in goats by New et al. (1996) and Minka and Ayo (2007), who recorded an increase in eosinophil count following road transportation. The difference in the findings may be attributed to species difference and different conditions of transportation in the animals. Monocytosis was observed following road transportation in control pigs, but not in experimental pigs. Again this finding disagreed with the results of Scope et al. (2002), who did not observe any significant changes in monocyte count in transported racing pigeons for 4 h by road. The neutrophilia observed in the present study in the control pig was apparently due to the decrease effect of AA, which has been shown to increase the release of corticosterone into the peripheral circulation from the body pools. Although the mechanism involved was not elucidated in this study, Whitehead and Keller (2003) has reported the release of corticosteroids in stressed layer chickens, which in turn is involved in the mobilization of neutrophils to peripherals circulation from the body pools. Lymphopenia obser-

Table 2. Serum biochemical parameters of experimental (supplemented with ascorbic acid) and control (non-supplemented with ascorbic acid) pigs, before and immediately (30 min) after short-term road transportation (Mean \pm SEM).

Parameters	Before Transportation		After Transportation	
	Experimental (n= 17)	Control (n =12)	Experimental (n = 17)	Control (n =12)
Total Protein (gm/dl)	67.94 \pm 1.50 ^{NS}	67.62 \pm 1.29 ^{NS}	69.69 \pm 1.67 ^{**}	71.31 \pm 1.93 ^{**}
Globulin (gm/dl)	33.81 \pm 1.83 ^{NS}	30.85 \pm 1.77 ^{NS}	35.56 \pm 2.24 ^{NS}	33.77 \pm 2.66 ^{NS}
Albumin (gm/dl)	34.75 \pm 0.95 ^{NS}	36.77 \pm 1.23 ^{NS}	34.13 \pm 1.04 ^{NS}	37.54 \pm 1.73 ^{NS}
Albumin:Globulin	1.11 \pm 0.09 ^{NS}	1.26 \pm 0.11 ^{NS}	1.06 \pm 0.12 ^{NS}	1.30 \pm 0.22 ^{NS}
Na ⁺ (mmol/L)	140.70 \pm 1.58 ^{NS}	141.90 \pm 1.71 ^{NS}	144.80 \pm 1.09 ^{NS}	144.50 \pm 1.92 ^{NS}
K ⁺ (mmol/L)	6.25 \pm 0.19 ^{NS}	6.09 \pm 0.21 ^{NS}	6.06 \pm 0.19 ^{NS}	6.14 \pm 0.16 ^{NS}
HCO ₃ ⁻ (mmol/L)	24.83 \pm 0.68 ^{NS}	24.62 \pm 0.89 ^{NS}	21.25 \pm 0.85 ^{NS}	22.15 \pm 0.75 ^{NS}
Cl ⁻ (mmol/L)	104.40 \pm 1.46 ^{NS}	104.50 \pm 1.15 ^{NS}	102.70 \pm 1.17 ^{NS}	102.70 \pm 1.72 ^{NS}
Alkaline phosphatase (I.U./L)	40.56 \pm 2.85 ^{NS}	40.92 \pm 2.92 ^{**}	39.94 \pm 2.85 ^{**}	48.23 \pm 2.36 ^{**}
Aspartate amino transferase (I.U./L)	16.75 \pm 1.04 ^{NS}	15.69 \pm 1.08 ^{NS}	17.75 \pm 1.04 ^{**}	21.08 \pm 1.09 ^{**}
Alanine amino transferase (I.U./L)	32.13 \pm 1.42 ^{NS}	32.69 \pm 1.25 ^{**}	26.06 \pm 1.85 ^{**}	26.54 \pm 2.30 ^{**}
Cholesterol (mg/100ml)	4.37 \pm 0.26 ^{NS}	4.14 \pm 0.24 ^{NS}	10.74 \pm 3.31 ^{**}	4.73 \pm 0.22 ^{NS}
Bilirubin (mg/100ml)	9.06 \pm 0.59 ^{NS}	9.23 \pm 0.74 ^{NS}	8.81 \pm 0.59 ^{NS}	8.92 \pm 0.66 ^{NS}
Urea (mg/100ml)	5.29 \pm 0.19 ^{NS}	4.67 \pm 0.25 ^{**}	5.29 \pm 0.18 ^{**}	6.79 \pm 0.39 ^{**}

**Values with asterisks are significantly different (P < 0.05).

Table 3. Serum biochemical parameters of experimental (supplemented with ascorbic acid) and control (non-supplemented with ascorbic acid) pigs on day 7 after short-term road transportation (Mean \pm SEM).

Parameters	Experimental (n = 16)	Control (n = 13)
Total Protein (gm/dl)	73.50 \pm 1.97 ^{NS}	67.77 \pm 2.00 ^{NS}
Globulin (gm/dl)	40.19 \pm 2.01 ^{**}	30.00 \pm 1.93 ^{**}
Albumin (gm/dl)	33.31 \pm 0.97 ^{**}	37.77 \pm 1.68 ^{**}
Albumin:Globulin	0.87 \pm 0.06 ^{**}	1.34 \pm 0.12 ^{**}
Na ⁺ (mmol/L)	139.40 \pm 1.40 ^{NS}	142.80 \pm 2.20 ^{NS}
K ⁺ (mmol/L)	5.46 \pm 0.13 ^{NS}	5.38 \pm 0.20 ^{NS}
HCO ₃ ⁻ (mmol/L)	21.69 \pm 0.84 ^{**}	24.15 \pm 0.80 ^{**}
Cl ⁻ (mmol/L)	91.00 \pm 1.79 ^{**}	96.62 \pm 1.22 ^{**}
Alkaline phosphatase (I.U./L)	43.75 \pm 2.63 ^{NS}	39.85 \pm 1.42 ^{NS}
Aspartate amino transferase (I.U./L)	18.75 \pm 1.24 ^{**}	19.54 \pm 1.44 ^{**}
Alanine amino transferase(I.U./L)	32.88 \pm 1.59 ^{**}	26.77 \pm 2.07 ^{**}
Cholesterol (mg/100ml)	4.79 \pm 0.25 ^{NS}	4.66 \pm 0.20 ^{NS}
Bilirubin (mg/100ml)	9.38 \pm 0.68 ^{NS}	9.46 \pm 0.79 ^{NS}
Urea (mg/100ml)	4.50 \pm 0.27 ^{NS}	4.14 \pm 0.23 ^{NS}

**Values with asterisks are significantly different (P < 0.05).

observed in the experimental pigs could be attributed to the depressive effects of road transportation on lymphoid tissues, which according to Spain (1975) results in antibody depression, and impaired migration of phagocytic cells (Spain, 1975). This is in agreement with the findings of Sudakov (1992), who showed that adrenocorticotropic hormone (ACTH) and glucocorticoids cause regression of lymphoid tissue due to stress.

The increase in neutrophil:lymphocyte ratio after transportation agrees with the findings of Rajion et al. (2001), who observed increase in the ratio following road trans-

portation in goats. The result also is in agreement with the established fact that the parameter is a good indicator of stress in goats (Rajion et al., 2001; Minka and Ayo, 2007), calves (Fraser and Brown, 1990) and broiler chicken (Zulkifli et al., 2001). Thus, an increase in neutrophil:lymphocyte ratio and a decrease in lymphocyte count obtained in the present study are consistent with the finding that neutrophilia, which occurs during stress, state stimulates the anterior pituitary gland to secrete ACTH. The circulating ACTH in turn induces the adrenal cortex to produce glucocorticoids, involved in the mobilisation of

neutrophils from body pool into the peripheral circulation. The increase in the neutrophil:lymphocyte ratio was more pronounced in control than experimental pigs because the control pigs that were not treated with AA appeared more stressed than the experimental pigs. Neutrophilia has been shown to be necessary for increase in body resistance to stress situations (Dohms and Metz, 1991). The neutrophil: lymphocyte ratio is an indication of the activity of the hypothalamus-adrenohypophyseal adreno-cortical-axis, and it increases with the degree of stress acting upon the body. The ratio has been shown to be one of the most sensitive and lasting indicators of physiological stress in poultry (Whitehead and Keller, 2003), goats and calves (Fraser and Broom, 1990).

There was a significant ($P < 0.05$) increase in total protein (TP) post-transportation in all the experimental and control groups, but the value was higher in the control than the experimental group. This finding is in agreement with the observation of Broom et al. (1999a) that pigs subjected to 24-h road transportation suffered severe dehydration as the concentration of TP increased significantly. Similar results were obtained in sheep by Knowles et al. (1996) and in calves by (Knowles et al., 1999a) and Rajesh et al. (2003). The significant increase in the value of globulin and albumin recorded on day 7 post-transportation is in agreement with the findings of Knowles et al. (1994), who reported a definite increase in total plasma protein, plasma albumin and plasma globulin due to transportation and that the concentrations of the proteins were restored relatively to normal values during the resting period in lairage.

The high AT and RH recorded during the transportation have been shown to cause heat stress, resulting in severe dehydration and, consequently, clinical haemo-concentration that may increase total protein; plasma albumin and globulin. Excessive cortisol level released during stressful conditions may also cause diuresis to complicate the already aggravated situations. Sodium, potassium and chloride are involved in various fundamental physiological processes, including the maintenance of normal osmotic equilibrium, maintenance of normal water balance and distribution, acid-base equilibrium and neuromuscular function. Non-significance in the values of these parameters post-transportation in experimental and control pigs indicated that AA administration as well as 4-h road transportation did not affect these important physiological parameters in pigs. The increase in chloride value in control pigs over experimental group on day 7 post-transportation may be attributed to an increase in capillary permeability with loss of colloidal protein into the tissues.

The increase in alkaline phosphatase and aspartate aminotransferase activities observed in control pigs immediately after transportation may be due to increased hepatocellular destruction and muscular degeneration as a result of the road transportation stress. This finding is in agreement with the results obtained by Hong et al. (2007)

who observed changes in serum enzyme activities after transporting pigs due to tissue damage in transport-stressed pigs. Sahin et al. (2002) reported that the concentration of antioxidant AA in the serum and liver decreases with stress.

The increased level of urea observed following transportation was, apparently, due to feed deprivation and elevated cortisol concentration as established by several authors (Dalín et al., 1993; Stull and Rodiek, 2000; Kannan et al., 2000; Odore et al., 2004), and also as a result of increased catabolism of protein caused by hypoglycaemia. It has been shown that stress induces AA depletion in the adrenal glands, and this is associated with corticosterone release (Sahota et al., 1995). Maintenance of high adrenal gland concentrations of AA by dietary supplementation has been found to limit the rise in circulating corticosterone concentration under stress (Pardue et al., 1985). AA is known to increase blood glucose level, which decreases in animals subjected to stressful conditions, especially during road transportation (Sauberhich, 1994; Hassanzadeh, 1997).

Conclusion

The results of the present study demonstrated for the first time the beneficial effect of AA administration on haematology and serum chemistry of pigs transported by road during the harmattan season for short journey of 4 h. It is, therefore, recommended that pigs be administered with AA before transportation by road during the harmattan season in order to reduce the risk of adverse effects of transportation stress on health.

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