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# Effect of Short-Term Road Transportation on Vital Parameters, Haematology, Enzymatic Antioxidants and Cortisol Level of Healthy Dogs (*Canis Familiaris*)

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## ABSTRACT

The study aims at determining the effect of short-term road transportation on vital parameters, haematology, enzymatic antioxidants and cortisol level of healthy dogs. Ten apparently healthy dogs weighing 8-12 kg, comprising of both sexes (3 females and 2 males), aged between 6 to 8 months were used as experimental animals. Measurements of the vital parameters and blood sample collection for hemato-biochemical parameters were taken before and immediately after short-term transportation. Rectal temperature (RT) value of 37.8ºC ± 0.3 recorded before transport was significantly ($P < 0.05$) lower than the value of 39.6ºC ± 0.1 recorded after transport, similarly, the heart rate (HR) values of 80 ± 2.8 beats per minute before transport was significantly ($P < 0.05$) lower than the value of 121 ± 8.9 beats per minute recorded after the transport. There were no significant ($P > 0.05$) changes in the haematological indices before and after transportation. The activity of SOD reduced significantly ($P < 0.05$) from 140 ± 10 U/mg to 129.8 ± 12.6 U/mg. Cortisol level increased significantly ($P < 0.05$) from 18.7 ± 3.7 pg/ml before transport to 23.1 ± 4.5 pg/ml. In conclusion, short-term transportation induced stress in dogs by increasing rectal temperature, heart rate and cortisol level. The dogs were also subjected to oxidative stress.

**Keywords:** Dogs; Stress; Transportation; Welfare

## INTRODUCTION

The domestic dog *(canis familiaris or canis lupis familiaris*) is a wolf descendent that has been domesticated (Thalmann, 2018). It has become especially adapted to human behavior as a result of their long-time interaction with humans (Axelsson *et al*., 2013). Over the millennia, dogs have been selected for a variety of behaviors and characteristics (Larson and Bradley, 2014). Hunting, herding, pulling cargo, protection, supporting police and the military, friendship, therapy, and assisting disabled people are just few of the roles they play for humans (Dewey and Bhagat, 2002). Nigerian native dogs, affectionately referred to as "mongrels" by locals, are typically brown in color (Igado, 2017) and due to its resistance to various haemoparasites (e.g. babesiosis and trypanosomiasis) that plague imported and exotic breeds, there has been an increase in the purchase and utilization of this breed of dogs in recent years (Olayemi, 2009).

Dogs are been transported for various reasons, and transportation has numerous effects on dogs which varies depending on the dog's resistance, tolerance, repeated exposure and adaptation. However, transportation has been established to affect the welfare of animals generally (Zappaterra *et al*., 2023). This research will provide information on whether or not transportation has a detrimental effect on Nigeria indigenous breeds of dogs. Physiological and haemato-biochemical parameter is important in reassuring the clinician of the general wellbeing of the patient, and an abnormality of these variables leads clinicians towards various diagnostic outcomes. Enzymatic antioxidants are very good biomarkers of oxidative stress. It is important to conduct researches on Nigerian indigenous breed of dogs in order to improve their welfare and wellbeing. The aim of the study was to determine the effect of short-term transportation on vital parameters, haematology, enzymatic antioxidants and cortisol level of healthy dogs.

### Materials and Methods

### Study Area

The experiment was carried out in Ilorin, Kwara State. It is located in the transitional zone within the forest and the guinea savannah regions of Nigeria (Lat 8° 08' 49.20" N, Log 4° 43' 12.00" E). The total annual rainfall ranges from 800 to 1200 mm in the NW and 1000–1500 mm in SE.

**Ethical Statement**

Ethical clearance for the use of dogs in this study was sought and approval was gotten from the University of Ilorin Ethical Review Committee, University of Ilorin, Nigeria (UREC/FVM/15/32TA035). The dogs were allowed to be calm prior to sample collection, and were handled by experienced clinicians. The transportation of the dogs was done humanely following the EU legislation governing transport of live animals, Directives 19/628 EEC amended by Directives 95/29/EEC (Earley *et al*., 2012).

**Animals and Management**

Ten apparently healthy dogs weighing 8-12 kg, comprising of both sexes, aged between six to eight months were acquired from a reputable source and housed in a standard pen. They were fed locally formulated dog food (rice with fish and chicken giblets) and were also given water ad libitum. Routine medical examinations including haemoparasite screenings was carried out on all the dogs and were vaccinated against rabies, canine distemper and leptospirosis.

**Experimental Design**

Measurements of the vital parameters and blood sample collection for hemato-biochemical parameters were taken before and immediately after transportation. The dogs were transported by car on a short distance travel that lasted for 2 hours. Each dog was physically restrained lightly and all the vital parameters and blood samples were taken and completed within the stipulated time.

**Measurement of Vital Parameters of Healthy Dogs**

Respiratory rate (RR) was observed according to the method of Lopedote *et al*.(2020). Briefly, the RR was taken by observation and counting the number of respiratory abdominal movements for one minute. Rectal temperature (RT) was measured according to the methods of Cugmas *et al*. (2020). Briefly, RT was recorded using a digital thermometer which was turned on and allowed to calibrate. The thermometer was then inserted slowly to about 2-3 inches into the rectum. It was held firmly in place for accurate readings and also to make retraction easier. The thermometer was placed around the walls of the rectum rather than through feacal matter where stool is felt in the rectum. The thermometer was held in place for one minute until a beeping sound was heard, indicating the reading was complete. The thermometer was not forced into the rectum whenever the dog clamps down its anal sphincter to avoid injury and pain. The thermometer was then removed and wiped clean. Heart rate (HR) was recorded according to the method of Katayama *et al*. (2016). Briefly, HR was recorded through auscultation of the heart by placing a stethoscope (Spraguay, Rappaport Type Stethoscope, England) between the second and fifth rib on the left side and counting the number of heart beats per minute. The unit of measurement used was beats per minute (bpm).

**Blood Collection**

Blood (5 mL) was aseptically collected from each dog by cephalic venipuncture, using a 5-mL syringe and 23-guage sterile needle. The blood sample was collected into two 5mL syringe: one was put in a sample bottle with anticoagulant using ethylenediaminetetraacetate (EDTA)-containing tubes for haematological analyses and the other was put in a sample bottle without anticoagulant to extract serum samples for cortisol and enzymatic antioxidant analyses.

**Determination of Haematological Parameters**

Red blood cell (RBC) and white blood cell (WBC) counts were determined using a haemocytometer. The packed cell volume (PCV) was estimated by the microhaematocrit method and haemoglobin (Hb) concentration by the cyanmethaemoglobin method (Baker and Silverton, 1985). Differential leucocyte counts were determined as described by Rizzi *et al*. (2010). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Esievo (2017). Platelets (PLT) were determined using a haematological autoanalyser (HEMAVET HV950FS, Drew Scientific, Inc., USA) (Kang *et al*., 2011).

**Determination of Cortisol**

Cortisol was determined by the use of a commercial radioimmunoassay kit (Coat-a-count cortisol, Siemens Medical Solution Diagnostics, Los Angeles, CA) according to manufacturer's protocol (Gold *et al*., 2016). The blood samples were allowed to clot at room temperature for 30 minutes and then centrifuged at 3,000 rpm for 15 minutes. Sera were carefully harvested into labelled vials and then analyzed immediately.

**Determination of Superoxide Dismutase Activity**

The activity of superoxide dismutase (SOD) was measured using the Northwest Life Science Specialties SOD kit (NWLSS™ NWK-SOD02) based on the method of monitoring the auto-oxidation rate of haematoxylin originally described by Martin *et al*. (1987) and modified to enhance reliability (Ememe *et al*., 2015). Briefly, 230 µL of assay buffer was added to wells of the microplate and then 10 µL of assay buffer (for blank) and 10 µL of serum sample were added. The wells were shaken, mixed, and incubated for 2 minutes. With the use of a multi-channel pipette, 10 µL of haematoxylin reagent was added to begin the reaction. The contents of each well were mixed quickly using the instrument's shaker function and the absorbance at 560 nm was recorded. The activity was calculated as: SOD U/mL = 1.25 x % inhibition.

**Determination of Glutathione Peroxidase Activity**

Glutathione peroxidase (GPx) activity was measured using the Northwest Life Science Specialist, Vancouver, Canada (NWLSS™) glutathione peroxidase assay kits protocol NWK-GPX01. All reagents were brought to room temperature (25°C) and diluted serum samples (50 µL) were added to the wells and then 50 µL of working nicotinamide adenine dinucleotide phosphate (NADPH) was added to each well. Working $H_2O_2$ (50 µL) was also added to each well. After 1 minute, microplate was placed in plate reader and at 340 nm wavelength it was read (Flohe and Gunzler, 1984).

To calculate GPx concentration using the NADPH absorption coefficient: the GPx concentration, expressed as mU/mL, was calculated using the GPx activity definition.

$$\text{Glutathione peroxidase} = \frac{2\,(\text{mRates} - \text{mRat}\quad).\text{VRxm}}{2.74\,.\text{Vs}}\,.\,\text{df}$$

Where $mRate_s$= -1,000 ΔA340/min of sample; $mRate_b$= -1,000 ΔA340/min of blank; 2.74 = NADPH 340 nm millimolar absorption coefficient at 1 cm path length; $V_{Rxm}$= volume of reaction mixture; $V_s$ = volume of sample; 2 = correction for 2 moles reduced glutathione oxidised to 1 mole glutathione disulphide (oxidised glutathione) per mole NADPH oxidised; df = sample dilution factor.

**Determination of Catalase Activity**

Catalase activity was determined using catalase kit (Abcam PLC, 330 Cambridge Science Park, UK). Twelve microliter of 1 µmol $H_2O_2$ was added into each well of serum samples and positive control solution and sample high control (HC) to begin the reaction. Following this, the samples were incubated at 25 °C for 30 minutes, and 10 µL of stop solution were added into each sample well to stop the reaction. 50 µL of developer mix, containing: 46 µL of assay buffer, 2 µL of OxiRed probe, and 2 µL of horseradish peroxidase solution were prepared for each well. Thereafter, fifty microlitre of the developer mix was added to each test sample, control, and standard. The samples were mixed and incubated at 25 °C for 30 minutes and then optical density of 570 nm in a plate reader was measured (Aebi, 1984).

Calculation: Signal change by catalase in the sample was ΔA = AHC – A sample. AHC was the reading of sample HC; sample A was the reading of sample in 30 minutes. $H_2O_2$ standard curve was plotted, and the ΔA was applied to the $H_2O_2$ standard curve to get B nmol of $H_2O_2$ decomposed by catalase in 30 minutes reaction. Catalase activity was calculated as follows:

$$\text{Catalase activity} = \frac{B}{30 \times V} \times \text{Sample dilution factor} = \text{nmol/min/mL} = \text{mu/mL}$$

Where B was the decomposed $H_2O_2$ amount from $H_2O_2$ standard curve (in nmol); V was the pretreated sample volume added into the well (in ml); 30 was the reaction time in 30 minutes; one unit of catalase was the amount of catalase that decomposes 1.0 µmol of $H_2O_2$ per minute at pH 4.5 at 25 °C.

**Statistical Analyses**

The RT, RR and HR values and cortisol level obtained were expressed as mean ± standard error of the mean (Mean ± SEM). Students' *t* test was used to evaluate the statistical difference of haemato-biochemical and physiological parameters before and after transportation. GraphPad Prism version 4.3 for Windows (GraphPad Software, San Diego, California, USA) was used for the analyses. Values of $P < 0.05$ were considered significant.

**RESULTS**

**Rectal temperature, heart rate and respiratory rate of healthy dogs subjected to short-term transportation**

Table 1 shows the physiological responses of healthy dogs to short- term transportation. The RT value of 37.8 ± 0.3˚C obtained before transportation increased significantly ($P < 0.05$) to 39.6 ± 0.1˚C after subjecting them to transportation. Similarly, the HR value of 121.6 ± 8.9 beats/minute obtained after transportation was significantly ($P < 0.05$) higher than the value of 80.0 ± 2.8 beats/minute recorded before subjecting the dogs to transportation. However, there was no significant ($p > 0.05$) difference in the RR recorded before and after transport.

**Table1:** Physiological responses of healthy dogs to short-term transportation

| Physiological parameters | Before transport | After transport |
|---|---|---|
| RT (˚C) | 37.8 ± 0.3[a] | 39.6 ± 0.1[b] |
| HR (beats/minute) | 80.0 ± 2.8[a] | 121.6 ± 8.9[b] |
| RR (Cycles/minute) | 32.4 ± 5.2 | 45.6 ± 4.5 |

[a,b]= Values with different superscript letters on the same row are significantly ($P< 0.05$) different
RT = Rectal temperature HR = Heart rate RR = Respiratory rate

**Haematological responses of healthy dogs to short-term transportation**

Table 2 shows the haematological parameters of healthy dogs before and after short-term transportation. The result showed that there was no significant ($p>0.05$) difference between the values of RBC (6.9 ± 0.5 ×$10^6$/µL), PCV (45.3 ± 3.6 %) and Hb (10.1 ± 0.6g/dL) before transport and the values (8.2 ± 1.1×$10^6$/µL, 47.8 ± 2.9 % and 10.3 ± 0.7 g/dL, respectively) obtained after transport. The values of white blood cell, neutrophils and lymphocytes count did not significantly ($P > 0.05$) increase from the values before transport. The ratio of neutrophil to lymphocyte was significantly ($P < 0.05$) higher in the dogs after transport when compared with the values before transport. There was no significant ($p>0.05$) difference between the values of mean cell volume, mean cell haemoglobin and platelets before and after transport.

**Effect of transportation on enzymatic antioxidants and cortisol level of healthy dogs**

Table 3 shows the enzymatic antioxidants and cortisol of healthy dogs subjected to short-term transportation. The activity of SOD reduced significantly ($P< 0.05$) from 140 ± 10 U/mg to 129.8 ± 12.6 U/mg. Similarly, the activity of GPx did not significantly ($P > 0.05$) reduce from the value of 153.2 ± 8.5 U/mg to 148.2 ± 13.3 U/mg. Also, the activity of catalase did not significantly ($p>0.05$) increase from 433.4 ± 24.9 to 434.7 ± 25 U/mg. However, cortisol level increased significantly ($P < 0.05$) from 18.7 ± 3.7pg/mL, before transport to 23.1 ± 4.5 pg/mL, after transport.

**Table2:** Haematology of healthy dogs before and after a short-term transportation**.**

| Haematological values | Before Transport | After Transport |
|---|---|---|
| Red blood cell ($\times10^6/\mu L$) | 6.9±0.5 | 8.2±1.1 |
| Packed cell volume (%) | 45.3±3.6 | 47.8±2.9 |
| Haemoglobin (g/dL) | 10.1±0.6 | 10.3±0.7 |
| White blood cell ($\times10^3/\mu L$) | 10.9±2.3 | 13.9±3.2 |
| Lymphocyte (%) | 84.2±2.3 | 88.8±1.9 |
| Neutrophil (%) | 45.3±3.6[a] | 60.9±2.9[b] |
| Neutrophil:lymphocyte | 0.6±.02[a] | 0.7±.05[b] |
| Mean cell volume (fL) | 65.8±0.2 | 66.1±1.0 |
| Mean cell haemoglobin (pg) | 14.7±0.6 | 14.2±0.4 |
| Mean cell haemoglobin concentration (g/dL) | 22.4±0.9 | 21.5±0.5 |
| Platelet ($\times10^3/\mu L$) | 81.5 ± 13.1 | 83.8 ± 15.6 |

[a,b]= Values with different superscript letters on the same row are significantly (P< 0.05) different

**Table3:** Biochemical parameters of healthy dogs subjected to short-term transportation

| Parameters | Before transportation | After transportation |
|---|---|---|
| SOD (U/mg) | 140 ± 10[a] | 129.8 ±12.6[b] |
| GPx (U/mg) | 153.2 ± 8.5 | 148.2 ± 13.3 |
| Catalase (U/mg) | 433.4 ± 24.9 | 434.7 ± 25 |
| Cortisol (pg/mL) | 18.7 ± 3.7[a] | 23.1 ± 4.5[b] |

[a,b]= Values with different superscript letters on the same row are significantly (P< 0.05) different

## DISCUSSION

The significant increase in RT and HR after transportation denotes that the dogs were subjected to stress. This agrees with the findings of Herbel *et al*. (2020) who reported an increase especially in beagle dogs after subjecting them to transportation for 2 hours. Vital parameters have been used as biomarkers of evaluating stress in animals generally (Basjaruddin, 2021). This increase denotes that during transportation of the dogs, they were exposed to stressful condition which may have resulted in significant increase in the values obtained. An increase in HR denotes an initiation of sympathetic activity of the autonomic nervous system (Kasahara *et al.,* 2021). HR variability, which is a short-term fluctuation in HR, is essentially based on the antagonistic oscillatory influences of the sympathetic and parasympathetic nervous system on the nodus sinuatrialis of the heart. It therefore, reflects the prevailing balance of sympathetic and parasympathetic (vagal) tone (Herbel *et al*., 2020), however as a limitation of the study, HR variability was not measured. There was no significant change in RR, denoting that transport supply of oxygen to the tissues was normal. This did not agree with the findings of Anoh (2018) in adult rabbits who reported an increase in RR. Transportation in this study did not affect RR. The slight increase in the value of RBC, PCV and Hb after transport denotes that the dogs were not anaemic but may be tending towards recovering from dehydration and this agrees with the findings of Minka and Ayo (2008) who reported an insignificant haematological changes in pullets subjected to transportation stress. The dogs were stable despite the fact that they were subjected to stressful condition of short-term transport. However, the slight increase observed in WBC, lymphocyte and neutrophil counts denotes that there was immune response which may be due to increased inflammation as also observed in the slight increase in platelet count, which is a marker of inflammation. The significant rise in neutrophil to lymphocyte ratio in the dogs after transport also denotes that the dogs were stressed. Neutrophil/lymphocyte ratio has been established to be a biomarker of stress (Becher *et al*., 2021). Furthermore, the increase in cortisol level after transport denotes that the dogs were stressed, however, they were able to adapt to the stressful condition as confirmed from the blood picture. Dogs have been reported to be able to adapt to transportation stress, especially if they have been subjected to it repeatedly (Herbel *et al*., 2020). The dogs used for this study have been subjected to transportation stress repeatedly, thus may be the reason why they adapted well to the stressful condition. The reduced activity of SOD confirms that the dogs were subjected to oxidative stress (Mohanambal *et al.,* 2023). The study shows that transportation induced stress in the dogs by increasing RT, HR, cortisol, immune response and inflammation, leading to dehydration and increase in oxidative stress. It is recommended that more research should be done by subjecting dogs to long-term transportation, using antioxidant to ameliorate transportation stress especially in Nigerian indigenous dogs.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

**Authors' Contributions**

OFH and OTO contributed to the study concept and design. material preparation, data collection and analyses were performed by OFH and IPM. The draft manuscript was prepared by OFH. OFH, OTO and OBS read, corrected and approved the manuscript for publication. All authors have read and approved the final manuscript.

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