



Pycnogenol improves kinematic parameters of donkeys (*Equus asinus*) subjected to packing during the dry season

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Abstract

The administration of pycnogenol (PYC) prior to physical exertion had earlier shown to improve gait variables after work. The aim of this study was to determine the effect of PYC on kinematic parameters of donkeys subjected to packing during the dry season. Ten male donkeys served as experimental subjects. Five donkeys were administered with PYC (10 mg/kg) in feed (test group) while another five donkeys were given feed only (control group) for one week pre-packing. Thermal environmental parameters of ambient temperature (AT), relative humidity (RH) and temperature humidity index (THI) were recorded. Kinematic parameters of speed (SP), stride length (SL), stride frequency (SF) and stride duration (SD) using videographic recordings 5 minutes post-packing with each animal walking at a self-selected speed were recorded. The THI values obtained pre- (95.4 ± 3.9) and during (85.3 ± 1.6) packing in the cold-dry season were significantly lower than the values of 148.8 ± 1.8 and 134.8 ± 1.1, respectively obtained during the hot-dry season. The speed recorded in test group (2.0 ± 0.3 m/s) was significantly (P < 0.05) higher when compared to the value (1.3 ± 0.2 m/s) obtained in control group during the hot-dry season while, during the cold-dry season, the test group recorded a significantly (P < 0.05) higher speed (1.3 ± 0.3 m/s) when compared with 1.0 ± 0.2 m/s recorded in control group. The increased speed recorded in the test group indicated that the group performed better as they were less fatigued when compared with the control group. Thus, PYC could be used as a potential agent in the management of fatigue in donkeys used for packing purposes in the region.

Keywords: Donkey, Humidity, Packing, Performance, Walking gait

Introduction

Donkeys (*Equus asinus*) are important economically as pack animals especially during the dry season in northern Nigeria where they are used for transporting harvested farm produce (Talla & Song, 2014; Gelaye & Fesseha, 2020). They are used for carrying or pulling heavy loads over long distances (Bennett & Pfuderer, 2020). They are also used for draught purposes such

as ploughing to make ridges in preparation for planting by subsistence farmers (Nengomasha et al., 1999) which may lead to stress in the donkeys because they are usually overstressed (Bale et al., 2003). The dry season is divided into the cold- and hot-dry seasons and spans from November to April (Dzenda et al., 2015). The cold-dry season, also known

as harmattan is characterized by low ambient temperature (AT) and low relative humidity (RH). It is also a cold-dusty period in the region and it has been reported to be stressful (Okeahialam, 2016; Balogun *et al.*, 2019) while the hot-dry season characterized by high AT and low RH, is a transition period between the dry and rainy seasons (Vathana *et al.*, 2002) and has also been reported to induce heat stress in donkeys (Olaifa *et al.*, 2013). Donkeys are also subjected to work in difficult terrain and mostly during adverse ambient conditions (Burden & Thiemann, 2015; Addis & Megra, 2017). These factors contribute to inducing oxidative stress (Olaifa *et al.*, 2012) which increases generation of reactive oxygen species and enhance lipid peroxidation which may cause tissue fatigue in working muscles, thereby leading to poor work performance and reduced walking speed (Serra Braganca *et al.*, 2020). Pycnogenol (PYC) is a potent antioxidant and anti-fatigue agent (Cerbo *et al.*, 2017) and may be useful in ameliorating these oxidative stress effects in donkeys subjected to packing during the dry season. Occurrence of muscle fatigue during physical exertion is usually manifested as a reduced ability to develop force and power (Vaz *et al.*, 2020) and may also result to muscular incoordination after physical exertion (Margolis & Pasiakos, 2013). Thus, evaluating changes in the locomotion of donkeys especially after subjecting them to packing is pertinent in order to detect fatigue. Kinematic analyses have been used to study speed in *Equidae*, especially horses (Michael *et al.*, 2007; Egenvall *et al.*, 2020) and it describes the geometry of animal movement including changes in the positions of the body parts (Volchenkov *et al.*, 2014; Vemula *et al.*, 2019). Quantitative analytical methods which include standard motion capture systems and a high-speed treadmill (Algheshyan *et al.*, 2014) have been used to study locomotion in *Equidae* under laboratory conditions. However, even though these systems are highly accurate, they are very expensive and require a dedicated facility for data collection and this makes them unsuitable for studying locomotion in animals especially under field conditions. This informed the use of videographic gait analysis in this study (Vemula *et al.*, 2019), in order to evaluate fatigue which may be caused by physical exertion. Kinematic analysis can be used to perform standard lameness assessment by observing walking gait in order to implement and monitor interventions in working donkeys (Serra Braganca *et al.*, 2020). The aim of the study was to use videographic gait analysis to determine the effects of PYC on kinematic parameters of working donkeys. The experimental

hypothesis is that administration of PYC prior to physical exertion will improve gait variables after work.

Materials and Methods

Study area

The study was performed at the donkey farm of the Equine and Camel Research Programme of the National Animal Production Research Institute (NAPRI), Shika-Zaria, located in the Northern Guinea Savannah zone of Nigeria (11° 12' N; 7° 33' E). The institute is located at an altitude of about 610 mm with average annual maximum and minimum AT of 31.0 ± 3.2 °C and 18.0 ± 3.7 °C, respectively. Data collection for this study was carried out during the cold- and hot-dry seasons, between November and June.

Measurement of thermal environment conditions

AT was recorded throughout the period of packing at the experimental site, using a wet- and dry-bulb thermometer (Brannan, England), and RH was obtained using Osmond's hygrometric table (Narindra Scientific industries, Haryana, India). Temperature-humidity index (THI) was calculated using the method of Bennett-Wimbush *et al.* (2014).

Experimental animals and management

Ten, apparently healthy, free-ranging non-neutered jacks, weighing 141 ± 1.2 kg, between 4.6 ± 0.2 years old, were used as experimental animals. The donkeys were pre-conditioned for two weeks, during which they were screened and treated against ectoparasites and endoparasites. They were kept under semi-intensive management system. They were subjected to packing during the day, after which they were allowed to graze on natural pasture, comprising mainly bermuda grass (*Cynodon dactylon*), gamba grass (*Andropogon gayanus*), Egyptian crowfoot grass (*Dactyloctenium aegyptium*) and supplemented with maize and sorghum bran. In addition, 1 kg of whole sorghum grain were fed to each donkey per experimental day. Salt licks were also provided and the donkeys had access to water throughout the experimental period except while they were being subjected to packing.

Experimental design

The experimental animals were divided into two groups consisting of 5 donkeys each. The groups were made up of the control (donkeys were loaded and performed packing work without PYC pre-supplementation) and the test (donkeys were loaded and performed packing work with seven days PYC

pre-supplementation) donkeys. A standard workload was performed that involved packing the donkeys over a distance of 10 km, for three days (at one day interval). Kinematic parameters were observed by videographic recordings about 5 minutes after packing.

Administration of pycnogenol

PYC (a registered trademark of Horphag Research Ltd., Geneva, Switzerland) was purchased from Viva Naturals Inc., New York, USA. The control group (n=5) received feed only while the test group (n = 5) received feed mixed with PYC at a dose of 10 mg/kg (Yang *et al.*, 2008) before subjecting them to packing. The administration of PYC was for 7 consecutive days, before subjecting all the donkeys to packing.

Packing procedure

Prior to packing, donkeys were saddled at 07:00 h, with a locally made saddle pack frame, filled with chopped dry grasses to provide a cushion effect on their back (Minka & Ayo, 2007). The donkeys were loaded with sand at a loading rate of 50% of their body weight (Pearson, 2003). The loads were balanced evenly with similar weight bulk on their left and right sides, and the paddings were arranged so that it was thickest along the sides of the vertebral spinous processes overlying the longissimus muscles (Oudman, 2002). All loaded donkeys were trekked over a distance of 10 km on each day of the experiment as described by Pal *et al.* (2002). The donkeys were subjected to packing every other day to and from NAPRI (11° 12' N; 7° 33' E) and its environs. They were allowed to rest on the intervening days.

Walking gait assessment

About 5 minutes post-packing, the donkeys were recorded while walking over a short linear straight line of about 50 metres, using a camcorder (Handycam DCR-SR42, Japan) according to the methods of Clayton (1991) and Catavittello *et al.* (2018). Briefly, the camera was fixed on a tripod stand to limit vibrations during recordings, and was oriented roughly orthogonal to the direction of the donkey walk. The distance between the camera and the donkey was about 5 m for all recordings. The donkeys were led in hand by an experienced handler throughout the study. From the videos, successful sequences of strides were identified when the gait occurred in a sagittal plane steadily and on a straight part roughly perpendicular to the optical axis of the camera to minimize errors in 2-D kinematic analysis. Only complete strides were analysed using hindlimb touchdown as the onset. Kinematics of the right side

was obtained by recording locomotion in right direction (relative to the camera). The videos were decomposed into images and analyzed frame by frame to determine quantitative parameters using a free proprietary software (Kinovea software, version 0.8.15). Stride length (SL), stride duration (SD), stride frequency (SF) and speed (SP) were measured and calculated using the formula below (Clayton, 1991):

- a) Stride length = Distance in the direction of movement between successive hoof prints of the same hoof.
- b) Stride duration = Time elapsing between successive footfalls of the same hoof.
- c) Stride frequency = Inverse of stride duration (1/SD) expressed as strides per second (S/s).
- d) Speed = The product of stride length and stride frequency (SL x SF).

Data analyses

Data were expressed as mean \pm standard deviation (Mean \pm SD). Statistical differences between and within groups were analysed by a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post-hoc* test. Pearson's correlation analysis was utilized to compare between thermal environmental parameters and kinematic parameters. GraphPad Prism 5.3 for Windows (GraphPad Software, San Diego, California, USA) was used for analyses. Values of $P < 0.05$ were considered significant.

Results

Table 1 shows the thermal environment conditions during the period of packing. The overall AT during the experiment was $20.1 \pm 0.7^\circ\text{C}$ and $32.1 \pm 0.9^\circ\text{C}$ during the cold and hot-dry seasons, respectively. While the overall RH was $18.9 \pm 2.2\%$ and $49.9 \pm 2.7\%$, during the cold and hot-dry seasons, respectively. During cold-dry season, the mean values of AT recorded pre- and during packing were $13.8 \pm 0.8^\circ\text{C}$ and $19.2 \pm 1.4^\circ\text{C}$. The values obtained increased significantly ($P < 0.05$) during the hot-dry season to $27.6 \pm 0.5^\circ\text{C}$ and $36.0 \pm 0.9^\circ\text{C}$ pre- and during packing, respectively. The values of RH recorded during the cold- and hot-dry seasons pre-packing were $35.8 \pm 4.0\%$ and $67.1 \pm 2.5\%$, respectively which decreased during packing period to $13.0 \pm 1.6\%$ and $38.0 \pm 2.4\%$, respectively. The THI obtained pre- and during packing in the cold-dry season were 95.4 ± 3.9 and 85.3 ± 1.6 , respectively, however, a significantly ($P < 0.05$) higher THI was recorded during the hot-dry season with values of 148.8 ± 1.8 and 134.8 ± 1.1 , respectively.

Table 2 shows the effect of pycnogenol and season on some kinematic variables of packed donkeys.

During the hot-dry season, there was no significant difference in SL between experimental groups. The SL recorded in the test group was 0.9 ± 0.1 m/s, which was the same with the value obtained in the control group during the cold-dry season. There was no significant ($P > 0.05$) difference between groups with regard to SD and SF. The SP recorded in the test group was significantly ($P < 0.05$) higher (2.0 ± 0.3 m/s) when compared to the value of 1.3 ± 0.2 m/s obtained in the control group during the hot-dry season. Furthermore, during the cold-dry season, the test group recorded a significantly ($P < 0.05$) higher SP of 1.3 ± 0.3 m/s when compared with 1.0 ± 0.2 m/s recorded in the control group. Overall, the control and the test groups recorded higher ($P < 0.05$) SP values during the hot-dry season when compared with the cold-dry season.

Table 3 shows the correlation coefficient between thermal environmental parameters and kinematic variables of packed donkeys. There was a significant and negative correlation between SP and AT in control ($r = -0.7068$, $P < 0.5$) and test ($r = -0.6963$, $P < 0.5$) donkeys during the hot-dry and cold-dry seasons, respectively. There was a negative and significant relationship between SF and AT ($r = -0.6885$, $P < 0.5$)

in the test donkeys during the cold-dry season. The relationship between SD and AT was positive and significant ($r = 0.7302$, $P < 0.5$) during the cold-dry season.

Discussion

The changes in SF, SL and SP are key parameters in animal locomotion research (Dewhurst *et al.*, 2017). There is a paucity of information about donkey kinematics in the literature, but one study of freely-walking, unloaded donkeys has reported SF of around 1.0 S/s at walking speed of around 1.0 m/s (Heglund & Taylor, 1988), which are similar to the values obtained in both groups of this study. The average SL of donkeys during packing is unknown; however, in this study SL was shorter than that of the horse reported by Galisteo *et al.* (1996).

On evaluating walking SP during the seasons, the donkeys walked faster during the walking gait assessment after packing during the hot-dry season than the cold-dry season. These differences may be due to seasonal variation. The finding establishes the fact that donkeys are cope better during the hot-dry season than during the cold-dry season, despite the harsh conditions they were subjected to (Proops *et al.*, 2019).

Table 1: Thermal environment conditions during the dry season

Season	Packing period	AT (°C)	RH (%)	THI
Cold-dry	Pre-packing	13.8 ± 0.8^a (8.0-16.5)	35.8 ± 4.0^a (22-70)	95.4 ± 3.9^a (82.0-130.8)
	During Packing	19.2 ± 1.4^a (8.0-26.0)	13.0 ± 1.6^b (5.0-39.0)	85.3 ± 1.6^b (74.0-112.4)
Hot-dry	Pre-packing	27.6 ± 0.5^b (24.9-29.4)	67.1 ± 2.5^c (56.0-84.0)	148.8 ± 1.8^c (140.9-160.8)
	During Packing	36.0 ± 0.9^b (29.8-42.1)	38.0 ± 2.3^d (26.0-58.0)	134.8 ± 1.1^d (127.8-143.6)

Values in parenthesis are minimum-maximum

^{a,b,c,d}= Values with different superscript letters are significantly ($P < 0.05$) different

AT = Ambient temperature

RH = Relative humidity

THI = Temperature humidity index

Table 2: Kinematic variables at walking gait after packing the donkeys during the dry season

Kinematic Parameters	Season	Test group (n=5)	Control group (n=5)
SL (m/s)	Cold-dry	0.9 ± 0.1	0.9 ± 0.1
	Hot-dry	1.6 ± 0.1	0.9 ± 0.1
SD (s)	Cold-dry	1.2 ± 0.1	0.9 ± 0.1
	Hot-dry	0.9 ± 0.2	0.6 ± 0.1
SF (S/s)	Cold-dry	1.0 ± 0.2	1.3 ± 0.2
	Hot-dry	1.4 ± 1.3	1.6 ± 0.3
SP (m/s)	Cold-dry	2.0 ± 0.3^a	1.3 ± 0.3^b
	Hot-dry	1.3 ± 0.2^c	1.0 ± 0.2^d

Values with different superscripts (^{a,b,c,d}) are significantly ($P < 0.05$) different between seasons and between groups.

SL = Stride length; SF = Stride frequency; SD = Stride duration; SP = Speed

During walking gait, the SP of an animal is self-selected, and it is determined by two major factors: SL and SF (Dewhirst *et al.*, 2017). In this study, the control donkeys increased their SP by increasing their SF while the pycnogenol-treated donkeys increased their speed by increasing their SL. The increased SL denotes that the test donkeys were more energetic and less fatigued after packing when compared to the control donkeys.

There are three important systems involved in rate of contraction of muscles during walking gait, in which when fatigue occurs, the functionality of these systems are compromised, and the rate of contraction of exercising muscles reduces, resulting in reduced SP. These systems include the muscular system, skeletal system and nervous system (Murphy *et al.*, 2018). The cardiovascular and respiratory systems are also included. The muscular system is directly involved with

contraction of muscles. These muscles are attached to bones and once the muscles contract the bones move concurrently (Walker, 2020). However, when there is shortage of oxygen supply to exercising muscles, there is decreased production of adenosine triphosphate (ATP), which is essential for muscle contraction (Hargreaves & Spriet, 2020). The deficiency of ATP reduces the contractile ability of the exercising muscles thereby leading to peripheral fatigue (Wan *et al.*, 2017). Furthermore, nervous signals are sent from the exercising limb to the spinal cord and brain in order to control locomotion (Grillner & El Manira, 2020), however in the case of central fatigue which is a general feeling of tiredness, the firing of nervous impulses reduces (De Russi de Lima *et al.*, 2018). The result of the present study demonstrates that the test group was less fatigued after being subjected to strenuous exercise and this may be attributed to the effect of PYC. Pycnogenol may have indirectly reduced fatigue by improving

muscle fitness and recovery (Vinciguerra *et al.*, 2013; Volpe, 2014). Study has shown that PYC improves fitness and muscle recovery by improving blood flow to muscles, alleviating cramping, muscle pain and soreness and controlling oxidative stress (Vinciguerra *et al.*, 2013).

The negative and significant relationship observed between AT with SF, SD and SP denotes that AT may strongly have an influence on locomotion of the donkeys. This study showed that as AT increased, SP and SF decreased and vice versa. This denotes that increased AT may reduce performance and vice versa. This finding agrees with that of Bryce & Halsey (2021), who reported that SP of movement deteriorated with hotter temperatures in the equine and this was attributed to their large body size which makes them have a lower surface area-to-volume ratio and greater thermal inertia thereby losing heat to a cooler environment slowly. The reduced SP observed during the hot-dry season in both groups suggests

Table 3: Correlation coefficient between thermal environmental parameters and kinematic variables at walk of packed donkeys

Donkey	Correlated parameters	Cold-dry season (<i>r</i>)	Hot-dry season (<i>r</i>)
Test	AT and SL	-0.6531	-0.2219
	RH and SL	0.4019	0.3845
	THI and SL	0.0235	0.3252
Control	AT and SL	-0.0546	-0.2798
	RH and SL	0.1908	-0.0544
	THI and SL	0.2577	0.0233
Test	AT and SD	0.7302*	-0.0043
	RH and SD	-0.5225	-0.5272
	THI and SD	-0.1388	-0.5178
Control	AT and SD	-0.3142	0.5347
	RH and SD	0.1062	-0.4794
	THI and SD	-0.1302	-0.5401
Test	AT and SF	-0.6885*	0.0124
	RH and SF	0.5729	0.5068
	THI and SF	0.2543	0.5216
Control	AT and SF	0.2584	-0.5998
	RH and SF	0.0263	0.5371
	THI and SF	0.2791	0.6052
Test	AT and SP	-0.6963*	0.2134
	RH and SP	0.5210	0.3469
	THI and SP	0.1672	0.3548
Control	AT and SP	0.1612	-0.7068*
	RH and SP	0.0995	0.3875
	THI and SP	0.3172	0.4518

*= Significant ($P < 0.05$)

AT = Ambient temperature; RH = Relative humidity; THI = Temperature humidity index;

SL = Stride length; SF = Stride frequency; SD = Stride duration; SP = Speed

that increased AT can affect locomotion. The donkeys while being exposed to high AT may have hyperthermia which may lead to exhaustion. Therefore, measures that will help in improving performance when donkeys are exposed to high AT should be researched on to improve the overall welfare of working donkeys.

In conclusion, packing the donkeys during the dry season induced fatigue; however, PYC may have reduced it. However, more studies are needed to evaluate PYC further as an ergogenic aid in work animals.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Addis B & Megra T (2017) Retroerspective studies of hyena bite in Ethiopia donkeys, Eastern Shoa, Central Ethiopia. *International Journal of Engineering and Research*, **5**(1): 500-508.

Algheshyan F, Eltoukhy M, Onar A & Asfour, S (2014) Development of a regression model for treadmill ground reaction force components. *Sport and Exercise Medicine Open Journal*, **1**(2): 35-41.

Bale OO, Lakpini CAM, Mohammed AK, Amodu JT, Chiezey UF, Ahmed HU, Achazie AA & Otchere EO (2003). An appraisal study of donkeys in three northern states of Nigeria. *Nigerian Journal of Animal Production*, **30**(2), 203-208.

Balogun IA, Ntekop AA & Daramola MT (2019). Assessment of the bioclimatic conditions over some selected stations in Nigeria. *SN Applied Sciences*, doi.10.1007/s42452-019-0589-y.

Bennett-Wimbush K, Amstutz PM & Willoughby D (2014) Characteristics and welfare of horses used for transportation in north east Ohio. *The Professional Animal Scientist*, **30**(1): 99-104.

Halsey LG & Bryce CM (2021). Proxy problems: Why calibration is essential for interpreting quantified changes in energy expenditure from biologging data. *Functional Ecology*, **35**(3): 627-634.

Bennett R & Pfuderer S (2020). The potential for new donkey farming systems to supply the growing demand for hides. *Animals*, doi.10.3390/ani10040718.

Burden F & Thiemann A (2015). Donkeys are different. *Journal of Equine Veterinary Science*, doi.10.1016/j.jevs.2015.03.00.

Catavittello G, Ivanenko, Y & Lacquaniti F (2018). A kinematic synergy for terrestrial locomotion shared by mammals and birds. *Computational and Systems Biology, Neuroscience*, doi.10.7554/elife.38190.

Cerbo DA, Morales-medina JC, Palmieri B, Pezzuto F, Cocco R, Flores G & Lannilti T (2017). Functional foods in pet nutrition: Focus on dogs and cats. *Research in Veterinary Science*, doi.10.1016/j.rvc.2017.03.020.

Clayton HM (1991) Gait evaluation: Making the most of the home video system. *Proceedings of the Annual Convention of the American Association of Equine Practitioners (USA)*. Pp 448-455.

De Russi de Lima F, Brietzke C, Franco-Alvarenga PE, Asano RY, Viana BF, Santos TM & Pires FO (2018) Traditional models of fatigue and physical performance. *Journal of Physical Education*, doi.10.4025/jphyseduc.v29i1.2915.

Dewhurst OP, Roskilly K, Hubel TY, Jordan NR, Golabek KA, Mcnutt J & Wilson AM (2017). An exploratory clustering approach for extracting stride parameters from tracking collars on free-ranging wild animals. *Journal of Experimental Biology*, **220**(3): 341-346.

Dzenda T, Ayo JO, Sinkalu VO & Yaqub LS (2015). Diurnal, seasonal and sex patterns of heart rate in grip-restrained African giant rats (*Cricetomys gambianus*, Waterhouse). *Physiological Reports*, **3**(10): e12581.

Egenvall A, Engstrom H & Bystrom A (2020) Kinematic effects of the circle with or without rider in walking horses. *Peer Journal*, doi.10.7717/peerj.10354.

Galisteo AM, Cano MR, Miro F, Vivo J, Morales JL & Aguera E (1996). Angular for joint parameters in the Andalusian horse at walk obtained by normal videography. *Journal of Equine Veterinary Science*, **16**(2): 73-77.

Gelaye A & Fesseha H (2020). Assessment of socio-economic importance and major constraints of working equines in and around Debre Berhan Town, Central Ethiopia. *Veterinary Medicine Open Journal*, doi.10.17140/VMOJ-5-14.

Grillner S & El Manira A (2020). Current principles of motor control with special reference to

- vertebrate locomotion. *Physiological Reviews*, doi.10.1152/physrev.00015.2019.
- Hargreaves M & Spriet LL (2020). Skeletal muscle energy metabolism during exercise. *Nature Metabolism*, doi.10.1038/s42255-020-0251-4.
- Heglund NC & Taylor RC (1988). Speed, stride frequency and energy cost per stride: How do they change with body size and gait? *Journal of Experimental Biology*, doi.10.1242/jeb.138.1.301.
- Margolis LM & Pasiakos SM (2013). Optimizing intramuscular adaptations to aerobic exercise: Effects of carbohydrate restrictions and protein supplementation on mitochondrial biogenesis. *Advances in Nutrition*, **4**(6): 657-664.
- Michael AW, Wiestner T, Hogg HP, Jordan P & Aver JA (2007). Compensatory load redistribution of horses with induced weight-bearing forelimb lameness. *Equine Veterinary Journal*, **36**(8): 727-733.
- Minka NS & Ayo JO (2007). Effect of shade provision on some physiological parameters, behavior and performance of pack donkeys (*Equus asinus*) during the hot-dry season. *Journal of Equine Science*, **18**(2): 39-46.
- Murphy AC, Muldoon SF, Baker D, Lastowka A, Bennett B, Yang M & Bassett DS (2018). Structure, function and control of human musculoskeletal network, *PloS Biology*, doi.10.1371/journal.pbio.2002811.
- Nengomasha EM, Pearson RA & World GA (1999). Empowering people through donkey power into the next millennium. In: *Empowering Farmers with Animal Traction* (PG Kaumbutho, RA Pearson, TE Simalenga, editors): *Proceedings of Workshop of the Animal Traction Network for Eastern and Southern Africa, held 20-24 September, 1999*, Mpumalanga, South Africa. Pp 22-31.
- Okeahialam BN (2016). The cold dusty harmattan: A season of anguish for cardiologists and patients. *Environmental Health Insight*, doi.10.4137/EHI.S38350.
- Olaifa F, Ayo JO, Ambali SF & Rekwot PI (2012). Effect of packing on erythrocyte osmotic fragility and malondialdehyde concentration in donkeys administered with ascorbic acid. *Onderstepoort Journal of Veterinary Research*, doi.10.4102/ojvr.v79i1.413.
- Olaifa F, Ayo JO, Ambali SF, Rekwot PI & Minka NS (2013). Rectal temperature responses of donkeys administered with ascorbic acid and subjected to load carrying (packing) during the harmattan season in Nigeria. *Tropical Animal Health and Production*, doi.10.1007/s11250-012-0242-x.
- Oudman L (2002). *Donkeys for Traction and Tillage*, De Goede Press, Wageningen, Netherlands Pp 1-25.
- Pal Y, Kumar S & Gupta AK (2002). Blood gases, acid-base and physiological indices in donkeys as pack animals. *Draught Animal News*, **37**(3): 27-33.
- Pearson RA (2003). Working Animals in Agriculture and Transport: A Collection of Some Current Research and Developmental Observations. *Wangenian Academic Publications, EAPP Technical Series*, doi.10.3920/978-90-8686-518-5.
- Proops L, Osthaus B, Bell N, Long S, Hayday K & Burden F (2019). Shelter-seeking behavior of donkeys and horses in a temperate climate. *Journal of Veterinary Behavior*, doi.10.1016/j.jveb.2019.03.008.
- Serra Braganca FM, Broome S, Rhodin M, Bjornsdottir S, Gunnarsson V, Voskamp JP, Perrson-Sjodin E, Back W, Lindgren G, Novoa-Bravo M, Gmel AI, Roepstorff C, van der Zwaag BJ, Van Weeren PR & Hernlund E (2020). Improving gait classification in horses by inertial measurement unit (IMU) generated data and machine learning. *Scientific Reports*, doi.10.1038/s41598-020-73215-9.
- Talla RT & Song IW (2014). Animal transport in early indigenous market economy of northern Nigeria. *International Journal of Humanities Social Sciences and Education*, **7**(7): 148-156.
- Vathana S, Kang K, Loan CP, Thingard G, Kabasa JD & Ter Meulen U (2002). Effect of vitamin C supplement on performance of broiler chickens in Cambodia. Conference on International and Agricultural Research for Development, Witzhausen: DeucherTropentag, October 9-11. <http://www.tropentag.de/2002/abstracts/full/211.pdf>, retrieved 21-08-2019.
- Vaz JR, Mendoca GV, Pezarat-Correia P, Rodrigues J, Alfaras M & Gamboa H (2020). Fatigue evaluation through machine learning and a global fatigue descriptor. *Journal of Health Care Engineering*, doi.10.1155/2020/6484129.
- Vemula MG, Deliagina TG & Zelenin PV (2019). Kinematics of forward and backward

- locomotion performed in different environmental conditions. *Journal of Neurophysiology*, **122**(5): 2142-2155.
- Vinciguerra G, Belcaro G, Bonnani E, Cesarore MR, Rotondi V, Ledda A, Hosoi M, Dugall M, Cacchio M & Cornelli U (2013). Evaluation of the effects of supplementation with Pycnogenol® on fitness in normal subjects with army physical fitness test and in performances of athletes in the 100-minute triathlon. *Journal of Sports Medicine and Physical Fitness*, **53**(6): 644-654.
- Volchenkov D, Blasing BE & Schack T (2014). Spatio-temporal Kinematic decomposition of movement. *Engineering*, **6**(8): 385-398.
- Volpe SL (2014). Pycnogenol®: What is it and can it help exercise performance? *ACSM's Health and Fitness Journal*, **18**(5): 40-41.
- Walker J (2020). Skeletal system 2: Structure and function of the musculoskeletal system. *Nursing Times*, **116**(2): 52-56.
- Wan J, Qin Z, Wang P, Sun Y & Liu X (2017). Muscle fatigue: general understanding and treatment. *Experimental and Molecular Medicine*, doi.10.1038/emm.2017.194.