—Note—

Effects of a joint supplement whose main components are resveratrol and hyaluronic acid on some biochemical parameters in aged lame horses

Mary U. EMEME[1]*, Usman S. ABDULLAHI[1], Anthony K. B. SACKEY[1], Joseph O. AYO[2], Wayuta P. MSHELIA[1] and Richard E. EDEH[1]

[1]Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria

This study aimed to evaluate the effects of a supplement that contains resveratrol and hyaluronic acid (RH supplement) in aged lame horses. A total of 16 horses of both sexes, aged between 15 and 22 years, weighing between 350–450 kg and showing lameness due to arthritis of the knee, hock, stifle, and fetlock joints and stiffness owing to ageing were used for the study. They comprised eight horses that were administered the RH supplement for three weeks and eight others that served as controls and were given only a Saccharomyces cerevisiae yeast strain used as carrier in the supplement. Blood samples were collected from each horse before supplementation (week 0) and at weekly intervals for the three weeks of the experiment. Biochemical parameters including creatine kinase, aspartate aminotransferase, urea, glucose, total cholesterol, sodium, potassium, chloride, and calcium were measured by standard methods. There was a significant ($P<0.05$) reduction in values of creatine kinase and glucose in the horses administered the RH supplement. It was concluded that the RH supplement may reduce the level of these biochemical parameters and their deleterious effects especially during ageing in horses.

Key words: *ageing, biochemical parameters, horse, hyaluronic acid, resveratrol*

J. Equine Sci.
Vol. 27, No. 1
pp. 19–22, 2016

In Nigeria, horses are kept for the purpose of traditional and religious festivals such as Durbar festival [28]. They are also used to pull agricultural implements on farms and for recreational purposes like polo and racing [35]. Studies have suggested that older horses from 15 to 20 years of age now comprise a much larger proportion of the overall horse population [18]. They are generally calmer than younger horses due to their experiences, which make them ideal teachers and wonderful companions [20]. Therefore it has become increasingly essential to preserve their good health and to extend their life spans. Serum biochemical assessments in horses are used as an aid to clinical diagnosis of a variety of diseases, in monitoring recovery during treatment, and to assess the health status of horses [19, 27]. They are also important in assessing the pathological changes of vital internal organs of the body such as the liver, kidney, pancreas, heart, and muscles [3, 31, 33]. Gurgoze and Icen [8] observed age-related alterations for most of the biochemical parameters. Previous studies have demonstrated that the phosphorus and calcium concentrations in serum decreased significantly with age in Chios sheep [29]. These studies suggested that age-related decrease probably reflects decreased bone metabolism as animals grow older. Studies indicate that blood glucose, cholesterol, creatine kinase (CK), and urea increase with age [15, 21]. Elevated blood glucose may indicate diabetes, stress, or Cushing's disease [8]. Changes in AST, CK and cholesterol may indicate increased enzymatic activity in horses with acute laminitis [26], and they may also suggest muscular and hepatic disorders that may occur in response to the endotoxemia [24]. Reactive oxygen species (ROS) are also implicated in skeletal muscle damage [16]. Resveratrol supplementation has been shown to attenuate many age-related diseases such as cardiovascular disease, various

Received: September 7, 2015
Accepted: December 22, 2015
*Corresponding author. e-mail: maryeneme@yahoo.com

types of cancers, neurodegeneration, and type II diabetes [4]. This biological activity is carried out by a wide variety of mechanisms, one of the most important of which is antioxidant activity due to free-radical scavenging [34]. Resveratrol is a polyphenol found naturally in a variety of sources, including grapes, Japanese knotweed, berries, peanuts, dark chocolate, and tea [30]. Resveratrol has antihyperglycemic effects by enhancing glucose transport in skeletal muscle and also has anti-catabolic effects in skeletal muscle via the inhibition of protein degradation [6]. Equithrive Joint (Biological Prospects) is a resveratrol supplement [11] for horses that contains sodium hyaluronic acid, which helps to relieve inflammation of the joint [14]. The aim of the study was to determine the effects of Equithrive Joint on some biochemical parameters in aged lame horse.

The experiment was carried out on a polo farm in Kaduna (10° 29′ N, 07° 28′ E), located in the Northern Guinea Savannah zone of Nigeria. The horses used for the study were 16 in number, of both sexes, of different breeds (Argentina polo ponies, Sudanese, Arewa, and South African), aged between 15 and 22 years, and between 350 and 450 kg in weight. They showed lameness due to arthritis of the knee, hock, stifle, and fetlock joints and stiffness owing to ageing. They were randomly assigned to experimental and control groups of eight animals each. The horses were housed in standard horse stables that were 10 × 12 m in size, comprised of a concrete floor, cement block wall, and asbestos roof and well ventilated. They were fed wheat bran, sorghum, hay, and fresh pasture. The horses were preconditioned for 2 weeks before the commencement of supplementation, and during this period, they were screened and treated for endoparasites and hemoparasites.

Equithrive Joint (Biological Prospects, Nicholasville, KY, U.S.A.) contains resveratrol (66.7 mg/g) and hyaluronic acid (6.7 mg/g). Experimental horses were fed 30 g of the supplement as loading dose for the first ten days of the experiment. Thereafter, the horses were administered 15 g of the supplement as a maintenance dose for the remaining 11 days of the study. The control group was given 30 g of *Saccharomyces cerevisiae* for the first ten days of the experiment and 15 g of S*accharomyces cerevisiae* for the remaining 11 days of the study. Horses were fed individually with the supplement mixed in 1 kg of feed (wheat bran and sorghum). They were fed twice daily and monitored during feed consumption to ensure that they consumed the entire supplement. The horses received an equal amount of their normal feed each day and were maintained on the same pasture and given access to water *ad libitum*. Blood sampling was carried out during the two-week preconditioning period to obtain baseline data and then at weekly intervals for 3 weeks of supplementation. A blood sample (5 m*l*) was collected from each animal in the morning before feeding by jugular venipuncture using disposable syringes and 18 gauge × 1.5 inch sterile needles. All 5 m*l* of the collected blood was dispensed into a sterile bottle without an anticoagulant, placed in ice, allowed to clot for 30 min, and then centrifuged for 15 min at approximately 1,000 g. The resultant serum was removed immediately, placed in plain tubes, and stored at −20°C for serum chemistry. Biochemical parameters, including creatine kinase, aspartate aminotransferase, urea nitrogen, glucose, total cholesterol, sodium, potassium, chloride, and calcium, were measured using a Hitachi 7700 Auto Analyzer (Hitachi High-Technologies Corp., Tokyo, Japan). GraphPad prism version 4.0 for windows (GraphPad Software, San Diego, CA, U.S.A.) was used for data analysis. Data obtained were expressed as the mean ± standard error of mean (mean ± SEM) and were subjected to Student's *t*-test to determine the difference between experimental and control horses at each period of sampling. Values of $P<0.05$ were considered significant.

The mean values of CK did not differ between the experimental and control horses in the first and second weeks of the experiment, but the value was lower ($P<0.05$) in the experimental horses compared with the control horses in the third week of the experiment (Table 1). The mean value for CK in the experimental horses also reduced ($P<0.05$) from the pre-administration value of 135.9 ± 3.06 to 116 ± 1.2 μ/*l* in the third week of treatment (Table 1). The value for glucose reduced ($P<0.05$) in the second and third weeks in the experimental horses compared with the controls. The values in the horses that received resveratrol and hyaluronic acid (RH supplement) in these periods were 4.55 ± 0.13 mmol/*l* and 4.29 ± 0.14 mmol/*l*. They were lower than the corresponding values of 5.05 ± 0.18 mmol/*l* and 5.03 ± 0.16 mmol/*l* observed in the control horses during these periods. Other biochemical parameters evaluated, including AST, cholesterol, urea, potassium, sodium, calcium, and chloride, did not differ between the experimental and control horses (Table 1).

The values for the biochemical parameters obtained during supplementation fell within the normal ranges observed by other researchers [7, 12, 13]. The significant reduction in values for CK in the treated horses showed that the RH supplement was able to reduce the activity of CK, which increases due to ageing. The supplement may also be used in cases of horses with polysaccharide storage myopathy (PSSM); serum CK is often abnormally high in horses with this condition [10]. The decrease in CK in the treated horses suggests that Equithrive Joint, acting as an antioxidant, may improve muscle leakage caused by oxidative stress [9]. In normal muscle function, CK is an enzyme involved in creating a high-energy molecule that is burned as a rapid energy source for muscle tissues, and elevated

Table 1. Biochemical changes in horses administered the resveratrol supplement (mean ± SEM)

Biochemical parameters	Baseline		First week of experiment		Second week of experiment		Third week of experiment	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
Creatine kinase (μ/l)	135.9 ± 3.06	135.4 ± 2.89	133 ± 2.6	126 ± 3.4	118.9 ± 3.9	126.8 ± 4.8	116 ± 1.2[a]	123 ± 2.0[b]
AST (μ/l)	70.5 ± 3.44	71.4 ± 3.44	72.3 ± 1.7	74 ± 2.3	67.6 ± 1.4	67.3 ± 2.3	55.3 ± 2.3	57.4 ± 3.3
Glucose (mmol/l)	4.65 ± 0.39	4.47 ± 0.17	4.28 ± 0.19	4.85 ± 0.21	4.55 ± 0.13[a]	5.05 ± 0.18[b]	4.29 ± 0.14[a]	5.03 ± 0.16[b]
Cholesterol (mmol/l)	2.19 ± 0.09	2.26 ± 0.10	2.40 ± 0.12	2.40 ± 0.08	2.14 ± 0.10	2.25 ± 0.12	2.04 ± 0.1	2.24 ± 0.1
Urea (mmol/l)	4.84 ± 0.42	4.23 ± 0.25	5.24 ± 0.34	5.31 ± 0.27	6.71 ± 0.17	7.14 ± 0.36	6.48 ± 0.26	7.09 ± 0.31
Potassium (mmol/l)	4.36 ± 0.13	4.29 ± 0.09	3.98 ± 0.13	4.18 ± 0.12	4.0 ± 0.12	4.26 ± 0.09	4.09 ± 0.09	4.15 ± 0.17
Sodium (mmol/l)	138 ± 0.53	140 ± 0.69	138 ± 0.73	140 ± 1.77	139 ± 1.5	140 ± 1.68	139 ± 0.61	140 ± 0.77
Calcium (mmol/l)	2.52 ± 0.04	2.46 ± 0.28	2.52 ± 0.06	2.52 ± 0.04	2.6 ± 0.03	2.63 ± 0.04	2.59 ± 0.09	2.63 ± 0.08
Chloride (mmol/l)	9.9 ± 0.99	101 ± 1.1	100 ± 2.6	102 ± 2.6	98.9 ± 1.3	99.9 ± 1.2	100 ± 0.08	9.5 ± 0.89

Values with different superscripts (a, b) are significantly ($P<0.05$) different.

CK in the blood stream is an indication that muscle damage has occurred [23]. Lameness is also a possible predisposing cause of increase in CK [17], and the reduction in activity of CK caused by this supplement shows that it could be beneficial in reducing lameness in aged horses. Despite the fact that the glucose values in the experimental and control horses fell within the normal range (4.16–6.38 mmol/l) recorded by other researchers [13], a reduction in glucose concentration in the second and third weeks of supplementation with the RH supplement shows that the supplement may reduce hyperglycemia due to the ageing process [1]. Insulin sensitivity has been reported to decrease with age [32]. This is associated with age-related decline in the serum dehydroepiandrosterone sulfate (DHEA) level [22], which is a hormone that can reduce visceral fat accumulation and improve insulin resistance. Sustained high levels of sugars in the blood ultimately cause proteins to stick together, thereby damaging the function of the proteins [25]. This leads to cross-linking of proteins called advanced glycation end products (AGEs). If the collagen in connective tissues becomes cross-linked as a result of AGEs, arthritis could occur [5]. Furthermore, glycation (sugar damaged proteins) accelerates the formation of damaging free radical molecules, which can accelerate ageing and increase risk of disease [2]. The values of other biochemical parameters evaluated, which were slightly lower in the experimental horses, indicated that the supplement may ameliorate their harmful effects during the ageing process. Further work needs to be done to prove whether these effects are durable and if higher doses and longer periods of treatment will produce greater effects.

References

1. Broughton, D.L., and Taylor, R. 1991. Review: deterioration of glucose tolerance with age: the role of insulin resistance. *Age Ageing* **20**: 221–225. [Medline] [CrossRef]
2. Camilo, A.L.S. 1997. The Glycation Hypothesis of Atherosclerosis. p. 219. Springer-Verlag, New York.
3. Coles, E.H. 1986. Veterinary Clinical Pathology, 4th ed. pp. 15–22, 53–54. W. B. Saunders, Philadelphia.
4. Cottart, C.H., Nivet-Antoine, V., Laguillier-Morizot, C., and Beaudeux, J.L. 2010. Resveratrol bioavailability and toxicity in humans. *Mol. Nutr. Food Res.* **54**: 7–16. [Medline] [CrossRef]
5. DeGroot, J., Verzijl, N., Wenting-van Wijk, M.J., Jacobs, K.M., Van El, B., Van Roermund, P.M., Bank, R.A., Bijlsma, J.W., TeKoppele, J.M., and Lafeber, F.P. 2004. Accumulation of advanced glycation end products as a molecular mechanism for aging as a risk factor in osteoarthritis. *Arthritis Rheum.* **50**: 1207–1215. [Medline] [CrossRef]
6. Dirks Naylor, A.J. 2009. Cellular effects of resveratrol in skeletal muscle. *Life Sci.* **84**: 637–640. [Medline] [CrossRef]
7. Ebge-Nwiyi, T.N., Kalu, N.A., and Naphtali, C. 2012. Preliminary studies on some haematological and serum biochemical parameters of apparently healthy adult horses in maiduguri, Nigeria. *Afr. J. Biomed. Res.* **15**: 49–53.
8. Gurgoze, S.Y., and Icen, H. 2010. The influence of age on clinical biochemical parameters in pure-bred Arabian mares. *Equine Vet. Sci.* **30**: 569–574. [CrossRef]
9. Hargreaves, B.J., Kronfeld, D.S., Waldron, J.N., Lopes, M.A., Gay, L.S., Saker, K.E., Cooper, W.L., Sklan, D.J., and Harris, P.A. 2002. Antioxidant status and muscle cell leakage during endurance exercise. *Equine Vet. J.* **34**(Suppl.): 116–121. [Medline] [CrossRef]
10. Hodgson, D.R., Davis, R.E., and McConaghy, F.F. 1994. Thermoregulation in the horse in response to exercise. *Br. Vet. J.* **150**: 219–235. [Medline] [CrossRef]
11. Lawless, P. 2010. Two New Research Projects Study Resveratrol's Effects on EMS. BiologicalProspects/Equithrive. http://www.equithrive.com/wpcontent/themes/equithrive/documents/EquiThrive.pdf (Accessed on March 5, 2013).
12. Ihedioha, J.I., and Agina, O.A. 2013. Serum biochemistry

profile of Nigerian horses. *Anim. Res. Int.* **10**: 1826–1833.
13. Kaneko, J.J., Harvey, J.W., and Bruss, M.L. 2008. Clinical Biochemistry of Domestic Animals, 6th ed. p. 904. Academic Press, San Diego.
14. Kawcak, C.E., Frisbie, D.D., Trotter, G.W., McIlwraith, C.W., Gillette, S.M., Powers, B.E., and Walton, R.M. 1997. Effects of intravenous administration of sodium hyaluronate on carpal joints in exercising horses after arthroscopic surgery and osteochondral fragmentation. *Am. J. Vet. Res.* **58**: 1132–1140. [Medline]
15. Lawan, A., Noraniza, M.A., Rasedee, A., and Bashir, A. 2013. Investigation on performance of different age and gender using biochemical profile of horses during 120 km endurance race. *MJVR* **4**: 17–22.
16. Matsuo, M., and Kaneko, T. 2000. The Chemistry of reactive oxygen Species and related free radicals. p. 25. *In*: Free Radicals in Exercise and Aging (Radak, Z. ed.), Human Kinetics Publishers, Champaign.
17. McEwen, S.A., and Hulland, T.J. 1986. Histochemical and morphometric evaluation of skeletal muscle from horses with exertional rhabdomyolysis (tying-up). *Vet. Pathol.* **23**: 400–410. [Medline] [CrossRef]
18. McFarlane, D., Sellon, D.C., Gaffney, D., Hedgpeth, V., Papich, M., and Gibbs, S. 1998. Hematologic and serum biochemical variables and plasma corticotropin concentration in healthy aged horses. *Am. J. Vet. Res.* **59**: 1247–1251. [Medline]
19. Messer, N.T. IV. 1995. The use of laboratory tests in equine practice. *Vet. Clin. North Am. Equine Pract.* **11**: 345–350. [Medline]
20. Moores, T. 2010. Horse Health Caring for Older Horses. http://www.easyequetrian.com (Accessed on September 12, 2003).
21. Nazifi, S., Saeb, M., Rategh, S., and Khojandi, A. 2005. Serum lipids and lipoproteins in clinically healthy Caspian Minatare horses. *Vet. Arh.* **75**: 175–182.
22. Perrini, S., Laviola, L., Natalicchio, A., and Giorgino, F. 2005. Associated hormonal declines in aging: DHEAS. *J. Endocrinol. Invest.* **28**(Suppl): 85–93. [Medline]
23. Piccione, G., Vazzana, I., Giannetto, C., Gianesella, M., and Ferrantelli, V. 2008. Modification of some hematological and hematochemical parameters in horse during long distance rides. *Res. J. Vet. Sci.* **1**: 37–43. [CrossRef]
24. Pollitt, C.C. 1996. Basement membrane pathology: a feature of acute equine laminitis. *Equine Vet. J.* **28**: 38–46. [Medline] [CrossRef]
25. Pratt, J.P. 2003. A book review of the antioxidant miracle by Lester Packer and Carol Colman. http://www.johnpratt.com/items/docs/health/packer/antioxidants.html (Accessed on June 20, 2015).
26. Riber, C., Rubio, M.D., Marquez, F., Pinedo, M., Muñoz, A., and Castejon, F. 1995. Hematological changes observed in Andalusian horses with laminitis. *J. Vet. Med. Sci.* **57**: 981–984. [Medline] [CrossRef]
27. Ricketts, S.W. 2004. Hematologic and biochemical abnormalities in athletic horses. pp. 950–966. *In*: Equine Sports Medicine and Surgery, 1st ed. (Hinchliff, K.W., Kaneps, A.J. and Geor, R.J. eds.), Saunders, Edinburgh.
28. Robin, L. 1982. The horse in West African history: the role of the horse in the societies of pre-colonial West Africa. *Afr. Aff. (Lond.)* **81**: 135.
29. Roubies, N., Panousis, N., Fytianou, A., Katsoulos, P.D., Giadinis, N., and Karatzias, H. 2006. Effects of age and reproductive stage on certain serum biochemical parameters of chios sheep under greek rearing conditions. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **53**: 277–281. [Medline] [CrossRef]
30. Shishodia, S., and Aggarwal, B.B. 2006. New York: Resveratrol in health and disease. pp. 1–16. *In*: Resveratrol: A Polyphenol for All Seasons (Aggarwal, B.B. and Shishodia, S. eds.), CRC Press, Stockholm.
31. Stockham, S. L., and Scott, M. A. 2008. Fundamentals of Veterinary Clinical Pathology, 2nd ed., Blackwell Publishing, Iowa.
32. Stout, R.W. 1994. Glucose tolerance and ageing. *J. R. Soc. Med.* **87**: 608–609. [Medline]
33. Tyson, A., and Sawhney, D.S. 1985. Organ Function Tests *In*: Toxicology Evaluation. p. 237. Noyes Publications, New Jersey.
34. Xia, E.Q., Deng, G.F., Guo, Y.J., and Li, H.B. 2010. Biological activities of polyphenols from grapes. *Int. J. Mol. Sci.* **11**: 622–646. [Medline] [CrossRef]
35. Zettl, W. 2007. The Circle of Trust: Reflection on the Essence of Horses and Horsemanship. p. 176. Half Halt Press, Boonsboro.