Journal of Equine Veterinary Science 35 (2015) 518–523

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

# Ameliorative Effects of Resveratrol on Oxidative Stress Biomarkers in Horses

Mary Ucheagha Ememe [a,*], Wayuta Philip Mshelia [a], Joseph Olusegun Ayo [b]

[a] Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
[b] Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria

ARTICLE INFO

*Article history:*
Received 15 December 2014
Received in revised form
4 February 2015
Accepted 20 February 2015
Available online 25 February 2015

*Keywords:*
Horse
Resveratrol
Lameness
Aging
Antioxidant

ABSTRACT

The aim of the present study was to evaluate the influence of age and lameness on antioxidant status in horses administered resveratrol supplement (Equithrive Joint). A total of 16 horses of both sexes, aged between 15 and 22 years, showing lameness score of 3 and weighing 350–450 kg were used, comprising eight horses which were administered resveratrol supplement for 4 weeks and eight others which served as control and given only *Saccharomyces cerevisiae* yeast strain used as carrier in the supplement. Blood samples were collected from each horse before supplementation (week 0) and at first, second, third, and fourth weeks of the experiment. Serum antioxidant marker of malondialdehyde (MDA) concentration, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase activities were determined by standard methods. Resveratrol supplement administration reduced significantly ($P < .05$) the concentration of MDA and activity of GPx but increased that of SOD and catalase. The result showed that aging and lameness increased oxidative damage in horses, and resveratrol supplement exerted some protective effects on the aged and lame horses by increasing the antioxidant capacity of the animals.


## 1. Introduction

Oxidative stress is a harmful imbalance in the oxidative–antioxidative system of cells [1]. Studies have shown that one or more antioxidant enzymes decrease as a consequence of aging [2–4]. Thus, superoxide dismutase (SOD) decreases, whereas catalase and glutathione peroxidase (GPx) increase during aging [5]. Chronic rheumatic disease and degenerative bone and joint diseases are linked to excessive reactive oxygen species (ROS) production [6], which are capable of degrading components of the joint as indicated in the pathogenesis of equine joint disease [7]. Superoxide dismutase, catalase, and GPx are antioxidant enzymes in mammalian cells which are necessary for oxygen metabolism [7]. The SODs convert superoxide radical into hydrogen peroxide and molecular oxygen ($O_2$), whereas the GPx and catalase convert hydrogen peroxide into oxygen and water [8]. However, the activities of these enzymes may decrease during oxidative stress [9,10]. Lipid peroxidation generates a variety of relatively stable decomposition end products, mainly α, β-unsaturated reactive aldehydes, such as malondialdehyde (MDA), and isoprostanes [11–13], which can then be measured as an indirect index of oxidative stress. Reported results on oxidative stress in horses in the literature were mainly based on the exercise physiology [14–16]. Antioxidant therapies are now designed to prevent oxidative stress which could potentially have significant impact on age-related diseases [17]. Older horses move more slowly and their joint, particularly limbs, are stiffer [18]. Arthritis is common condition and major factor in reducing an older horse's mobility and enthusiasm for exercise and can lead to the early retirement of otherwise healthy animals [19]. Hence, older exercising horses need more antioxidants

* Corresponding author at: Mary Ucheagha Ememe, Department of Veterinary Medicine, Ahmadu Bello University, Umudike, Zaria, Nigeria.
*E-mail address:* maryeneme@yahoo.com (M.U. Ememe).

0737-0806/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2015.02.006

because exercise can intensify their vulnerability to ROS damage [20]. Consequently, there is great interest in evaluating antioxidant agents that could protect aging horses from ROS. One possible agent, resveratrol, is a natural phytoalexin with antioxidant properties [21].

Resveratrol (3,4,5-trihydroxystilbene) is from a class of polyphenolic compounds called stilbenes [22]. It is found in the skins of certain red grapes, peanuts, blueberries, some pines, and the roots and stalks of Japanese knotweed [23]. Resveratrol has a lot of health benefits, including the improvement of cardiovascular and anti-inflammatory properties, prevention of joint diseases, and improvement of athletic endurance [24]. It exerts anticancer, antimicrobial, antiaging, and antioxidant effects [25,26]. This biological activity is carried out by a wide variety of mechanisms, one of the most important of which is antioxidant activity due to its free radical scavenging [27]. Equithrive Joint, a resveratrol preparation for horses, contains a high-quality source of resveratrol which is easily administered PO [28]. It also contains sodium hyaluronic which is used as a viscosupplement and increases the viscosity of the synovial fluid [29] which helps to lubricate, cushion, and reduce pain in the joint [30].

This study was carried out to evaluate the effects of resveratrol on some oxidative biomarkers in aging and lame horses.

## 2. Materials and Methods

The experiment was carried out in a private polo farm in Kaduna (10°29′N, 07°28′E), located in the Northern Guinea Savannah zone of Nigeria. The study involved 16 horses aged 18.5 ± 0.65 years and showing grade 3 lameness (lameness consistently observed at a trot in all circumstances) [31]. The horses were of different sexes, weighing between 350 and 450 kg. Estimation of their ages was carried out as described by Wayne and Melvin [32] and then confirmed by farm records. They were randomly assigned to treated and untreated (control) groups of eight animals each. They were housed in standard horse stables measuring 10 × 12 m made of concrete floor, cement block wall, and asbestos roof and well ventilated. The horses were fed with wheat bran, sorghum, hay, and fresh pasture. They were preconditioned for 2 weeks before the commencement of the supplementation; and during this period, they were screened and treated for endoparasites and hemoparasites.

The resveratrol supplement was purchased from Hagyard Pharmacy, Lexington, KY. Treated horses were fed four scoops (30 g) of Equithrive Joint powder containing 2,000 mg of resveratrol and 200 mg of sodium hyaluronic acid and the carrier *Saccharomyces cerevisiae* as the loading dose for the first 10 days of the experiment, and then two scoops (15 g) of Equithrive Joint powder containing 1,000 mg of resveratrol and 100 mg of sodium hyaluronic acid and the carrier *S. cerevisiae* as maintenance dose for the remaining 18 days of the study. Untreated horses were fed 30 g of the carrier *S. cerevisiae* as loading dose for the first 10 days of the experiment and then 15 g of the carrier *S. cerevisiae* as maintenance dose for the remaining 18 days of the study. The supplement was mixed in their daily feed during the period of the study [28]. The two groups received equal amount of their normal feed each day of the study period. All horses were fed twice daily and monitored during feed consumption and also maintained on the same pasture, and water provided ad libitum.

### 2.1. Blood Sample Collection

This was carried out during the 2-week preconditioning period to obtain baseline data and then during the 4-week treatment. Blood samples were collected from each animal in the morning before feeding at weekly intervals. At each blood sampling of each horse, 5 mL of blood was collected by jugular venipuncture using disposable syringes and 18-ga × 1.5-inch sterile needles. The collected blood was poured into a sterile sample bottle without anticoagulant and placed in ice. The samples were allowed to clot for 30 minutes and then centrifuged for 15 minutes at approximately 1,000*g*. The resultant serum was removed immediately and placed in plain sterile tubes and then stored at −80°C for determination of MDA concentration and GPx, SOD, and catalase activities.

### 2.2. Analysis of Oxidative Stress Biomarkers

#### 2.2.1. SOD Activity

Standard protocol of the Northwest Life Science Specialist, Vancouver, Canada, was used to measure the SOD activity. The method was based on monitoring the autoxidation rate of hematoxylin as originally described by Martin et al [33] with modification to increase robustness and reliability. Briefly, 230 μL of assay buffer was added to wells of the microplate. Then, 10 μL of assay buffer (for blank) and 10 μL of sample were added. The wells were properly shaken, mixed, and incubated for 2 minutes. A multichannel pipette was used to add 10 μL of hematoxylin reagent to begin the reaction. The content of each well was quickly mixed using the instrument's shaker function, and immediately, the absorbance at 560 nm was recorded. The SOD activity was calculated as: SOD U/mL = 1.25 × % inhibition.

#### 2.2.2. GPx Activity

Standard protocol of the Northwest Life Science Specialist, Vancouver, Canada, was used to measure the GPx activity. For standard procedure for microplate assay, all reagents were brought to room temperature (25°C). Diluted sample (50 μL) was added to wells and then 50 μL of working nicotinamide adenine dinucleotide phosphate (NADPH) added to each well. Working $H_2O_2$ (50 μL) was also added to each well. After waiting for 1 minute, microplate was placed in plate reader and read at 340 nm measurements [34].

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

$$[GPx] = \frac{2(mRate_s - mRate_b) \cdot V_{R_{xm}}}{2.74 \cdot V_s} \cdot df$$