Oxidative Stress, Oxidative Damage and Antioxidants – A Beginners Guide

David J Marlin[1] and Catherine E Dunnett[2]
David Marlin Consulting Ltd, Newmarket, Suffolk, UK[1]
Independent Equine Nutrition Ltd, Newmarket, UK[2]

Since 1995 there have been around over 65,000 papers published in relation to this topic in scientific journals – that equates to around 18 papers each day for the last 10 years. So in science and in the commercial world antioxidants are a popular topic. There are increasing numbers of articles and adverts in the media talking about the importance of antioxidants for our own health. In human healthcare there are a bewildering array of products advertised as having antioxidants in them and suggesting all kinds of benefits. Just take a look on the shelves in Boots the chemist. So are antioxidants the answer to all our health problems? It's unlikely. But it is true that many diseases and other processes (such as ageing) involve the antioxidant systems within the body and there is evidence that improving antioxidant defences can help reduce the risk of getting disease in the first place or help recovery following disease.

This review will explain in lay terms what antioxidants are, what they do and how they may be important for your horse.

The Free Radical

Each and every day, your horse's body is under constant attack from particles called "free radicals". These potentially dangerous molecules are formed as the result of normal processes taking place within the body, especially as a result of producing energy using oxygen and fighting infection. Therefore their production is increased with exercise and infection, but they may also be created following injury, disease or exposure to certain environmental factors, such as allergens (e.g. moulds, pollens), pollutants (e.g. ozone, sulphur dioxide) or radiation (e.g. from the sun or radioactive compounds).

What are Free Radicals?

Free radicals are independent yet unstable molecules that contain one or more unpaired electrons. The preferred state is for these electrons to be paired and therefore free radicals actively search for a partner for their unpaired electron. But in order to become stable they have to attract electrons from surrounding molecules, which usually do not have 'spare' electrons to give – the original free radical, therefore, effectively 'steals' the electron, which causes the 'donating' molecule to become a free radical itself. This can start what is known as a chain reaction as the new unstable free radical tries to attract an electron from the molecules surrounding it and so on. Under certain circumstances this can be harnessed and be of value; for example such a process is used by our white blood cells (the cells of our immune system) to help destroy potentially harmful bacteria and viruses.

The phrase 'reactive oxygen species' (or ROS) is often used to describe all those free radicals and other molecules, which contain one or more molecules of oxygen, that are capable of causing the reactions described above. These have the potential to oxidise essential phospholipids and proteins within cell membranes leading to cell damage and death. Such 'oxidative' damage can lead to alterations in the structure of cell membranes, cell DNA and other cellular components such as proteins, and cause disruption of normal physiological processes (some of which may be irreversible). To combat such damage an antioxidant defence system has evolved.

What is the Antioxidant Defence System?

Antioxidants, otherwise known as 'free- radical scavengers', (Not all antioxidants are free-radical scavengers) are the body's natural defence against oxidative damage. Antioxidants effectively neutralise free radicals, for example by giving them the electron that they so desperately seek, BUT without becoming an active free radical themselves – so preventing the chain reaction from continuing.

Therefore antioxidants play an important role in maintaining the health and integrity of all the different types of cells within the body. In fact the balance between free radical production and antioxidants is thought to be strongly related to lifespan.

The elaborate antioxidant defence system as a whole depends on dietary intake of antioxidant vitamins and minerals and the production within the body of antioxidant compounds such as glutathione and vitamin C. Some nutrients such as Vitamin E act as antioxidants in their own right. There are also antioxidant enzymes (proteins), including superoxide dismutase, glutathione peroxidase and catalase. Nutrients, including zinc, manganese, iron, selenium and copper, form an integral part of these antioxidant enzyme systems.

What do we mean by Oxidative Stress and Oxidative Damage?

Oxidative Stress

As the antioxidants effectively neutralise the free radicals they tend to become changed themselves. Most commonly they become oxidised and can no longer act as antioxidants until they are restored to their 'un-oxidised' or reduced state. 'Oxidative stress' occurs when the antioxidant defence system cannot cope with the rate of free radical production and a large proportion of the antioxidants have been oxidised thereby reducing the ability of the body to deal with the free radical threat.

Oxidative stress, therefore, may occur because of :

- Increased free radical production due to:
    - increased exposure to oxidants from the environment
    - increased production within the body e.g. an increase in processes that use oxygen such as exercise
- Decreased antioxidant capacities
- Imbalance in antioxidants e.g. from diet

Diet itself can increase the oxidative load and potentially lead to oxidative stress. For example, diets rich in polyunsaturated oils increase the requirement for antioxidants especially vitamin E. More importantly poor nutrition may reduce the horse's ability to mount a sufficient antioxidant defence. Non-dietary sources of oxidative stress include UV light, radiation, pollution, anaesthesia, inflammation, physical injury as well as exercise.

Oxidative Damage

If the free radical production continues the situation can then potentially lead to cell or tissue damage. This is termed 'Oxidative damage'. In recent years, there has been a growing awareness of the role of oxidative damage, at least in humans, in both the ageing process and the development of a number of diseases or conditions such as:

Cardio-vascular disease	ARDS & NRDS
Skin disease	Oxygen toxicity
Arthritis	Idiopathic pulmonary fibrosis
Muscular atrophy	COPD (human)
Liver disease	Cystic fibrosis
Auto-immune disease	Emphysema
Neurological disease	Pulmonary haemorrhage
Neuro-psychiatric disorders	
Ischaemia-reperfusion injury	
Renal failure	
Hyperthyroidism	

It is thought that oxidative damage may also be implicated in similar diseases in animals. Diseases or conditions in which oxidative stress and or damage may be involved include:

- Grass sickness
- Cushing's disease
- Exercise-induced pulmonary haemorrhage (EIPH)
- Joint disease
- Tying-up
- Motorneurone disease
- Recurrent airway obstruction (RAO) – also known as equine COPD or "heaves"

Which Dietary Antioxidants Are Most Important?

It is increasingly thought that nutrition may play an important part in helping to protect against oxidative stress and damage. Certain nutrients and dietary components have antioxidant properties that are important for the normal functioning of the body. Furthermore, some of these when fed in the optimum amounts and proportions may also offer a protective role against a variety of diseases which may be at least in part be associated with oxidative stress or damage. Work has suggested that a mixture of antioxidants, especially arising from natural sources, may be more beneficial than a single antioxidant in helping to support antioxidant defences.

Vitamin C

Vitamin C (also known as ascorbic acid) is believed to be one of the most important antioxidants in the fluids found outside the cells themselves. It is important also for the formation of cartilage and bone as well as for the optimal functioning of the immune system and wound healing. Vitamin C has been found to be the most important antioxidant in the fluid lining the lungs of horses and that levels are reduced in horses suffering from recurrent Airway Obstruction (RAO – previously known as COPD, "broken wind" or "heaves") and other types of airway inflammation (e.g. bacterial infection).

Unlike humans, primates (i.e. monkeys) and guinea pigs the horse is able to synthesise its own supply of Vitamin C. It has therefore been thought that the Vitamin C requirements of the healthy horse will be met by tissue synthesis. However, horses that are under certain types of stress e.g. exercise, transport, disease, trauma or surgery may require extra supplies. Unfortunately not all dietary sources of Vitamin C are very available for the horse and therefore it is important that if supplementation is provided it is via a source which has been shown to be both available and stable.

Vitamin E

Vitamin E is a collective name for a number of biologically similar compounds which share the same function. Another term that may be used for vitamin E is tocopherol. Whereas Vitamin C is water soluble, Vitamin E is fat soluble and is the major antioxidant involved in maintaining cell membrane integrity. Vitamin E has been suggested to also play an important role in the functioning of the immune system and is believed to be important for normal growth and muscle function.

A dietary source of Vitamin E is essential as, unlike Vitamin C, it cannot be synthesised in the body. Dietary sources of Vitamin E in equine diets include fresh green forage and the oils of some grain seeds. Cereals and preserved hays contain some but limited amounts of Vitamin E. Many horse diets, that are not supplemented, will not provide the amounts of Vitamin E that we now believe to be optimal.

Trace elements

Selenium is an essential part of an antioxidant enzyme, glutathione peroxidase, and as such plays an essential role in cellular antioxidant defences. Pasture content of selenium can be very variable as some plants may contain very high levels but most pastures, especially in the UK, tend to be low to very moderate sources of selenium. Dietary sources or selenium in feed supplements can be both inorganic (e.g Sodium Selenite) and organic (for example, seleno-yeasts – here selenium is provided mainly in the form of seleno methionine as yeasts are grown in a selenium rich growth media forming these organic selenium compounds).

Copper, zinc and manganese are also important antioxidant trace minerals as they form integral parts of a number of antioxidant enzymes, such as superoxide dismutase, which are part of the first line defence against free radicals. Hays, pastures and cereals tend to provide a variable intake of these trace elements and therefore for many horses additional dietary supplementation is needed either via the compound feed or via a broad spectrum vitamin and mineral supplement.

Carotenoids

Carotenoids are responsible for the red, yellow and orange pigments found in plant foods especially fruits and vegetables and in the tissues of animals which eat the plants. Some carotenoids are precursors of Vitamin A, but this property is unrelated to their antioxidant activity. Carotenoids such as beta-carotene (found in green grass etc.), lutein and lycopene (found in tomatoes) act as powerful antioxidants. Very mature hays, especially stemmy hays, tend to be very low in B-carotene as do most cereals (apart from yellow maize). In addition, hay that has been stored for a prolonged period will have lost much of its natural beta carotene content.

Are there any interactions between Antioxidants?

In addition, to their individual effects, dietary antioxidants may assist each other, for example Vitamin C may assist Vitamin E by recycling Vitamin E once it has been used up in neutralising certain free radicals. Vitamin E can help protect ß-carotene from oxidation, and may therefore have a sparing effect on this antioxidant. Vitamin E and the mineral selenium appear to act to work together as parts of a multi-component antioxidant defence system.

What about herbs ?

Herbs and spices can be very valuable sources of antioxidants, however they may also have a number of other properties which need to be taken into consideration when adding them to your horse's diet. At least 30 food spices and herbs have been suggested to possess antioxidant properties. For example eugenol is the active antioxidant compound in garlic and flavenoids are found in rosemary. Both of these antioxidants may be important to help maintain health in particular of the respiratory system which will be discussed in our next article on the lung. Grapeseed extract also contains flavenoids known as proanthocyanadins, which are reputed to have antioxidant properties.

Oxidative stress and diseases in horses

Grass sickness
As with other neurological diseases, oxidative stress may play a role in equine grass sickness. The antioxidant-pro-oxidant balance of plants collected from fields where a grass sickness episode has occurred has been shown to be significantly in favour of pro-oxidants. Grass sickness itself does not appear to result in marked systemic oxidative stress or oxidative damage, although there may be oxidative stress at the site of neuronal degeneration.

Exercise-induced pulmonary haemorrhage (EIPH) or "bleeding"
Oxidant injury has been suggested to contribute to the pathogenesis of EIPH, although to date there are no published experimental data investigating this hypothesis. ROS may enhance EIPH by damaging the delicate blood vessel walls within the lung. However, some supplement products on the market sold for use in bleeders do contain vitamin C.

Joint disease
The induction of synovial inflammation in horses by intra-articular administration of carrageenin resulted in an increase in free radical oxidation products in synovial fluid (joint fluid). Synovial fluid from horses with acute joint disease demonstrated evidence of protein oxidation indicating a role for oxidative stress in equine joint disease. Oxidative stress may increase joint friction, by reducing the viscosity of hyaluronan, and degrade components of the extracellular matrix, such as collagen.

Rhabdomyolysis (tying-up)
Severe rhabdomyolysis in foals has been reported to be caused by selenium deficiency. A low level of dietary selenium will reduce the activity of selenium-dependent glutathione peroxidase and as a consequence render muscle fibres more susceptible to oxidative muscle damage. In contrast to these findings, increases in glutathione peroxidase and glutathione reductase have been demonstrated in equine skeletal muscle with pathological changes after the development of rhabdomyolysis, which may be an adaptive response to an increased oxidant burden. An episode of rhabdomyolysis may result in oxidative stress with the consumption of antioxidants such as vitamin E, but it is unlikely that a deficiency in vitamin E or selenium is the primary cause for the development of the disease. Dietary supplementation with vitamin E and selenium has been used for prophylaxis (prevention), despite the absence of investigations identifying a protective role for these antioxidants in rhabdomyolysis.

Equine motorneurone disease (EMND)
Horses with EMND have higher copper concentrations in the spinal cord compared to healthy control horses, which may result in increased ROS production by the formation of highly reactive hydroxyl radicals from hydrogen peroxide. Low plasma vitamin E concentrations have been reported in horses with EMND, which gives further evidence of oxidative stress in EMND and low plasma vitamin E concentrations are a significant risk factor for EMND. It has been suggested that a diet deficient in antioxidants will render motor neurons (nerves that control muscle contraction) with the highest oxidative activity susceptible to oxidative damage and if a sufficient number of motor neurons are affected then EMND will occur. However, vitamin E deficiency is not likely to be the sole cause of EMND

Equine recurrent airway obstruction (RAO) (also known as equine COPD or "heaves" or "broken wind")
The oxygen rich environment in the lungs leaves this tissue particularly susceptible to damage from ROS and strong antioxidant defences led by vitamin C and glutathione are vital. The marked influx of inflammatory cells in the airways of RAO-affected horses in response to organic dust (moulds, pollens, dust mites, etc) inhalation results in a considerable oxidative burden. Horses with clinical signs of RAO following exposure to dusty hay and straw demonstrate significant oxidation of glutathione and an increase in lipid peroxidation in the fluid that lines the airways. Chronic airway inflammation in

RAO-affected horses also results in glutathione oxidation, as well as ascorbic acid oxidation. RAO-affected horses also have evidence of increased oxidative DNA damage in the white blood cells in the circulation. Therefore dietary supplementation of RAO horses with antioxidants has the potential to prevent oxidative damage and speed the resolution of airway inflammation. Lung airway ling fluid ascorbic acid concentration can also be elevated in RAO-affected horses by dietary supplementation and dietary antioxidant supplementation has also been demonstrated to decrease airway inflammation in RAO-affected horses.

Other Diseases/Conditions

Horses with equine Cushing's disease have been demonstrated to have oxidative stress in blood samples. Oxidative stress, as evidenced by lower plasma ascorbate concentrations, has also been reported in during weaning by confinement in stalls. Vitamin C supplementation (20g per day) has also been reported to improve antibody response to vaccine in aged horses, particularly those with Cushing's disease and may benefit those with chronic infections (Ralston 1999). However, other studies have shown no effect.

Conclusion

A number of conditions such as ageing, exercise, stress and poor diets may lead to a decreased antioxidant defence, oxidative stress and oxidative damage. There is also evidence that this may also occur as a consequence of certain diseases in horses. There are fewer scientific studies of the role of antioxidants in horses compared with in humans, but there is increasing evidence that the supply of additional antioxidants in the diet may help in a number of different conditions or diseases. This has led to a feed companies promoting various antioxidant products. The choice of which products to use is difficult for the horse owner. The success of an antioxidant supplement will depend on which antioxidants are present, in what proportions and if they are present in forms that the horse can readily absorb. Certain dietary antioxidants are not particularly stable and other may be expensive, for example, vitamin E. It is likely that the choice of products will increase. In general terms, a cheaper supplement may not necessarily be good value as this may indicate lower grade ingredients at lower concentrations, which may not produce the desired effect. Owners should get used to looking at labels and asking suppliers or companies to convince them that the products are worth investing in.