Impact of Nutrition and Feeding practices on equine health and performance

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Summary: For the horse, what and when they are able to eat is now predominantly determined by man and we therefore have to take responsibility for the effects that our choice of managemental practices have on their health and welfare. Nutrition can also have profound effects on their performance and, while good nutrition cannot improve the intrinsic ability of the horse (and rider), inappropriate or inbalanced nutrition may impose limitations. Especially in developed countries we therefore should no longer consider the feeding of horses purely as a means to provide the basic nutrients that they require. Instead we should be looking at how the type and amount of nutrients provided can help optimise performance, reduce the risk of disease, minimise any adverse welfare effects and, where necessary, provide the best possible support for the ill or convalescing horse. This paper will highlight the impact that diet, and the way we feed can have both positively and negatively on the performance and health. It will highlight recent work on the role of antioxidants in respiratory health as well as energy sources in the growing and exercising horse.

Introduction

Nutritional support perhaps can be considered to start with the dam, during pregnancy and lactation. Nutrition of the mare has perhaps traditionally been thought to be much less important than that of the foal. However, in the horse as well as in humans, the important role that nutrition, via the mother, may have in the subsequent health of any offspring is increasingly being recognised (Tauson et al 2006). One area of particular interest is currently whether the metabolic status of the pregnant dam may cause imprinting or programming of the offspring. Which in turn may result in a life-long metabolic adaptation to conditions experienced during foetal or early neonatal life. Many findings suggest that metabolic programming effects are imposed during certain time periods during gestation, *critical windows*, and that an inadequate nutrient supply in each of these periods may cause a different response. An example of this in the horse is the potential role of copper supplementation during gestation and the incidence of DOD (Harris et al 2005). Optimal nutrition and management of the foal and weanling is also important to help ensure the foundations for sound skeletal and muscular systems, in particular, are established. How energy is supplied (i.e. from which sources) may influence the somatotrophic axis in youngstock (Harris et al 2005) as well as the level of insulin resistance (Trieber et al 2005). It is important that the nutrition supplied is in line with the growth patterns of the individual, given that foals growing at a more rapid rate deposit greater quantities of bone, muscle and fat than their slower growing counterparts and therefore need more calcium, phosphorus and lysine for example. There is some limited work to suggest that management as a foal may influence gait as an adult (see Back et al 2002). Diet may also influence the behaviour of the horse especially around weaning and other periods of 'stress', which may influence their willingness to undertake 'novel' tasks (Nicol et al 2005). Later on, obviously, nutrition has a significant role to play in the provision of energy to fuel the horse during its training as well as providing the required amino acids to support the increase in muscle mass that is wanted in response to this training. Mineral nutrition is important to support bone development especially during training as well as during periods of inactivity. The appropriate provision of vitamins, minerals and antioxidants all have a role to play in supporting the health of the horse as do feeding practices and the nature of the feed ingredients used. One of the most important potential, and perhaps undervalued, roles for nutrition in the adult horse, is in the maintenance of health and reduction of the risk of disease –colic, laminitis, gastric health and tying are perhaps some of the major conditions linked closely with nutrition and feeding practices.

Whilst the extent of the influence that feeding and managemental practices can exert will vary during the life time of a horse, these are factors within our control and therefore they should be areas that we try to optimise in order to give the individual the best chance to be healthy and to provide the level of performance required. Especially in developed countries we therefore should no longer consider the feeding of horses purely as a means to provide the basic nutrients that they require. Instead we should be looking at how the type and amount of nutrients provided can help optimise performance, reduce the risk of disease, minimise any adverse welfare effects and, where necessary, provide the best possible support for the ill or convalescing horse.

This paper highlights the impact that diet and the way we feed can have, both positively and negatively, on the performance and health of horses. It will concentrate on the work published by WALTHAM and its collaborators since 1995 and highlight in particular the role of antioxidants in respiratory health plus the importance of energy sources in the growing and exercising animal. Nutrition can also have a significant impact on the behaviour and welfare of the horse (Davidson & Harris 2002) but these aspects will not be mentioned here.

Nutrition -Health and the growing animal

Modern feeding and managemental practices as mentioned above have considerable influence on the health, performance and welfare of horses. Perhaps this is most noticeable in the growing horse, especially when under commercial conditions where the goal may be 'production' for early sale. In particular, this often results in mares and foals either being kept on artificially enhanced pastures or being fed one to two large meals a day and the foals being weaned early (3-5months compared with ~9months in the wild). In such cases the diet typically consist of feedstuffs with a greatly reduced water content and often a radically different nutritional profile from the diet that they would be able or would choose to select in the wild as a non-ruminant herbivore. The term 'production disease' was applied to man-made diseases of livestock derived from breeding; feeding and management for high production e.g. milk fever, mastitis and laminitis. Certain conditions in the horse especially Developmental Orthopaedic Diseases have been considered to be production diseases (Kronfeld 1997).

Insulin-like growth factor I (IGF-I) is a key hormonal regulator of cartilage and bone development. A number of studies have suggested that rapid growth of foals, often in combination with a high starch diets, is associated with increased circulating concentrations of IGF-I, which in turn may be a factor associated with developmental orthopaedic disease (Staniar et al 2001, 2002, Trieber et al 2004).

The term developmental orthopaedic disease (DOD) was first coined in 1986 to encompass all orthopaedic problems seen in the growing horse and therefore encompasses all general growth disturbances of horses. It is non-specific and the definitions are not uniformly agreed. However, as defined by McIlwraith 2001, DOD may be taken to include Osteochondrosis (OC: 'a defect in endochondral ossification that can result in a number of different manifestations, depending on the site of the endochondral ossification defect' - one manifestation of which is osteochondritis dissecans (OCD) of cartilaginous origin); Subchondral cystic lesions (SBC); Physitis; Acquired Angular Limb deformities; Flexural deformities; Cuboidal bone malformation; Juvenile arthritis or juvenile degenerative joint disease and bony fragments of the palmer/plantar surface of the first phalanx of standardbred horses (believed traumatic lesions). It has been suggested that that the clinical signs of OC occur only after a progression of events that begin with a disturbance in the normal development of the cartilage (sometimes referred to as dyschondroplasia: DCP) leading to OC. At this point physical stresses are superimposed, leading to clinical signs. It is also thought possible that the initial defects/ lesions may heal or develop into OCD or into SBC. Due to the multifactorial nature of DOD, no single cause is likely to result in expression. Factors that may contribute include a genetic disposition, biomechanical trauma, and mechanical stress through inappropriate exercise, obesity, rapid growth and inappropriate or imbalanced nutrition. Different combinations may be involved in different cases. Environmental or management factors most likely determine if expression occurs (i.e. provide the final triggering factor(s) see Harris et al 2005.

Importance of Energy sources.

A number of studies have suggested that a high intake of energy may result in an increased incidence of DOD (Harris et al. 2005), including a study by Savage et al. (1993) in which feeding 129% NRC digestible energy (DE) requirements to foals from 130 days of age resulted in an increased incidence of lesions compared with the control group (fed 100% DE) or those fed 126% of the NRC recommendations (Anon 1989) for protein. Multiple DCP lesions were found on gross post mortem examination in 11 foals fed the high DE diet, one the high protein diet and one the control diet. However, it should be noted that there has been concern that the lesions produced by some of these studies are not directly comparable to those found in the field, and many field studies have reported foals being fed much higher energy intakes without an apparent increase in clinical incidence (Kronfeld 1990). This may perhaps be linked to the background level of predisposition within individuals, nature of the energy being provided, concomitant exercise level and the overall balance of the diet. Considerable research has been carried out to explore the suggestion that diets which intrinsically produce high glycaemic peaks, or individual horses that respond to certain diets to produce, high glycaemic peaks (and subsequent effects on insulin and other hormones) may have an increased risk of developing DOD (Glade and Belling 1986, Ralston 1995, Pagan 2001). Such diets have the potential to establish a feeding fasting cycle, which is a perturbation from hormonal patterns likely seen in grazing animals (Kronfeld et al 1996, Kronfeld & Harris 2003, Harris & Kronfeld 2003). This in turn may adversely influence bone development as the cyclical changes in glucose and or insulin may influence bone maturation via effects on the somatotropic axis including:

- Growth hormone (Freud et al., 1939)
- Thyroxine and Triiodothyronine (Glade and Reimers, 1985)
- Insulin-like Growth Factor I and equine chondrocytes (Cymbaluk and Smart 1993, Staniar et al 2001, Staniar et al 2002).

This has led to the development of alternative rations for the growing horse which rely less on starch and sugar (SS) as the main supplementary energy sources and more on oil and particular fibres (a mixture of rapidly, moderately and slowly fermentable fibres - FF diets: Kronfeld & Harris 2003, Kronfeld & Harris 2003, Harris & Kronfeld 2003). Initial reports suggested that the fat and fibre rich concentrate might afford some protection against the high sugar and low fibre content of spring pasture (Hoffman et al 1996) and enable some weanlings to cope better with seasonal changes in pasture contents of soluble carbohydrates and fibres (Kronfeld et al 1996). Although a study reported in 1999 suggested that whilst these fat and fibre based diets may have metabolic advantages, the bone mineral content was found to be lower in those fed a FF supplement than in those fed the more traditional SS diet (Hoffman et al 1999). It was later found that such FF diets with 0.9% or 1.35% calcium resulted in similar bone densities as previously found with SS diets (Hoffman et al 2001).

Diet - Insulin Sensitivity

Metabolic changes, most notably in insulin sensitivity, are associated with several equine diseases including some forms of exertional rhabdomyolysis, osteochondrosis, hyperadrenocorticism, hyperlipaemia and laminitis. Insulin sensitivity is also a key determinant of energy regulation during pregnancy and lactation, exercise, ageing and obesity, affecting the efficiency of metabolism and growth rate as well as susceptibility to certain diseases. Interestingly recent work has suggested that insulin sensitivity may vary during the oestrus cycle (Cubbitt et al 2007) although the clinical relevance of this is currently unknown.

Glucose metabolism in mares (Williams et al 2001b) is adapted during pregnancy and lactation to enable the mare to provide for the energy demands of the developing foetus and suckling foal. There has been relatively little published work on the effect of diet and energy source in particular during pregnancy but one study looked at the influence of different supplementary energy sources on glucose metabolism. (Hoffman et al 2003) Metabolic adaptation of the mares to pregnancy was reflected in larger areas under time-concentration curves for plasma glucose and insulin following an oral glucose load, and by slower glucose clearance, during late gestation compared with early lactation see Figure 1 below. With respect to the diet, it was evident that mares fed a supplementary concentrate rich in sugar and starch showed a more rapid glucose peak than those fed the fat/fibre supplement, during both late gestation and early lactation perhaps reflecting the adaptation to meals rich in hydrolysable carbohydrate and the feeding-fasting meal cycle. The more sluggish glucose clearance in mares fed the fat and fibre rich supplement more closely mimicked the natural state of horses grazing pasture and not acclimated to feeding-fasting cycles. It was suggested that potentially this more effectively allowed for the adaptation of glucose metabolism required during pregnancy and lactation in order to make energy available to the foetus or suckling foal.

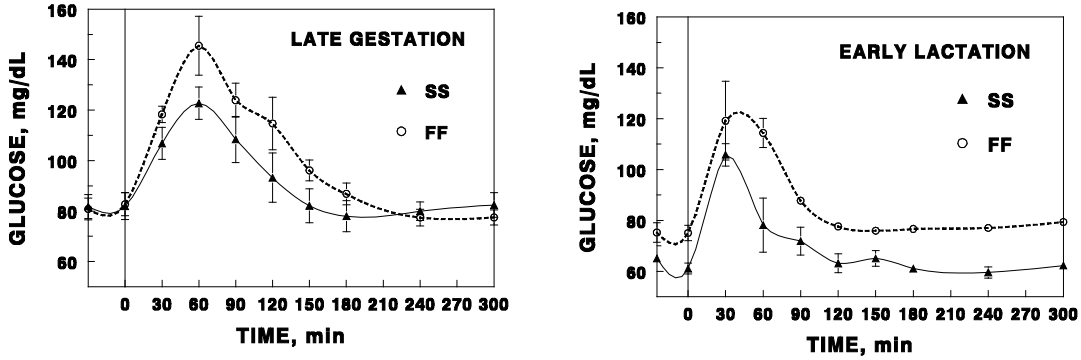


Figure 1 Plasma changes in glucose concentrations in response to an oral glucose dose (0.2 g/kg BW) in mares during (a) the third trimester of pregnancy, and (b) early lactation. Data are summarised as means ± SE. Dietary treatments were forage (grass or hay) plus a supplement rich in either starch and sugar (SS, solid line) or fat and fibre (FF, dashed line).

A series of studies have been undertaken to look at how insulin sensitivity varies from birth to weaning and to test for any influence of feed energy source (Cubitt et al 2005, George et al 2007). Blood samples were collected from 40 Thoroughbred foals born to mares fed pasture supplements rich in fat and fibre or starch and sugar. The foals also had free access to these feeds. Plasma glucose and insulin concentrations were measured and proxies for insulin sensitivity and pancreatic β-cell response calculated. Glucose and insulin concentrations decreased linearly with age, and this was associated with a linear increase in insulin sensitivity but not pancreatic β cell response as assessed using proxies based on the minimal model (Trieber et al 2005a). The decline in glucose concentration and increase in insulin sensitivity were greater in foals on the fat and fibre supplement, whereas pancreatic β-cell response was unaffected by the diet. The results showed that insulin sensitivity increases from birth to weaning in Thoroughbreds and that this

increase is blunted in foals fed a diet with a high glycaemic response as represented by the starch and sugar pasture supplement. Thus, there may be a chronic adaptation to high glycaemic meals in these young foals, as seen previously in weanling and mature Thoroughbreds, and which is regarded as a risk factor for dychondroplasia and laminitis. This effect of diet was explored with more detailed measures of insulin sensitivity, using the minimal model technique, in 12 Thoroughbred weanlings fed either the fat/fibre or starch/sugar diets (Trieber et al 2005b). This confirmed that insulin sensitivity was reduced by nearly 40% in the foals fed the starch and sugar supplement, and that they compensated for this by increasing insulin secretion. There was no effect of diet on plasma cortisol or triglyceride concentrations, but basal IGF-I levels were higher in foals fed starch and sugar. The starch/sugar ration comprised 49% non-structural carbohydrates (starch, and sugar) compared with only 12% in the fat and fibre ration. Such, chronic adaptation to high glycaemic diets that occurs in young foals may have important consequences for the rest of a horse's life although further work is needed to explore this further. Recently we have explored the potential that mechanisms that control glucose metabolism in the horse may be affected by maternal diet during gestation, as seen by the difference in basal plasma glucose in foals born from mares fed differently during late gestation in a recent study (George et al 2007).

The somatropic axis links nutrition to growth through the interaction of hormones and metabolites, including glucose, insulin, growth hormone and IGF-I. It has been proposed that the large fluctuations in glucose and insulin that follow grain meals rich in hydrolysable carbohydrate influence growth hormone and IGF-I. These latter hormones regulate chondrocyte metabolism and this could explain the link between high glycaemic diets, insulin insensitivity and dyschondroplasia. This hypothesis was tested by comparing plasma growth hormone and IGF-I concentrations during intravenous glucose tolerance tests from weanlings fed either fat and fibre or starch and sugar concentrates (Treiber et al 2005c & d). While there was no effect of diet on growth hormone responses, basal IGF-I concentrations were higher in foals fed starch and sugar and increased in response to the glucose challenge. In contrast, IGF-I remained constant following glucose administration in the foals fed fibre and fat. These studies suggest that the chronic adaptation to a high glycaemic diet in early life can be associated with increased circulating levels of IGF-I, which may explain the link between developmental bone disease and diets that stimulate an exaggerated glycaemic response. These findings are consistent with *in vitro* demonstrations of the effects of IGF-I on cultured equine chondrocytes and are further proof of the importance of correct dietary management for optimal growth and development.

Diet and immunity in foals

One of the other major factors influencing the health and welfare of young foals is their ability to combat infection. The young prenatal foal is particularly vulnerable, as it has to rely on immunity gained from the mare rather than its own innate ability, especially for the first few months of life. The failure of passive transfer of colostral immunoglobulins to the newborn foal increases the risk of neonatal septicaemia etc. A study looked at the effects of feeding 10 mares a corn and molasses based concentrate (SS) compared to feeding 10 mares an isocaloric and isonitrogenous corn oil and fibre supplement (FF) during gestation and lactation. In the colostrum samples at 6 to 12 hrs post foaling there was a higher percentage of milk protein and mild solids with a lower percentage of lactose in the FF mares. Most interestingly there was a higher IgG content in the mares fed the FF which provided a higher Vitamin E content (Hoffman et al 1998). Vitamin E had been implicated in the stimulation of serum antibody synthesis particularly IgG in calves and therefore this study was followed by one in which pregnant mares were fed either Vitamin E at levels that met or were double the current NRC recommendations. This showed that by two weeks prior to foaling the serum IgG concentrations were greater in the Vitamin E supplemented mares. The IgG levels were significantly greater in the presuckled colostrum of the Vit. E supplemented mares, and, after suckling the serum IgG concentrations were significantly greater in the foals of Vitamin E supplemented mares (Hoffman et al 1999). This work confirmed that dietary supplementation of Vitamin E, above that currently recommended, during the periparturient period of the mare may positively influence colostrum and passive transfer of immunoglobulins which may be of benefit for the health and welfare of foals.

Nutrition - Health and the competing animal

The majority of clinical problems, especially in the upper level of competition horses, other than lameness *per se*, tend to be metabolic conditions such as tying up, loss of appetite due to stress and long term training, dehydration during heavy training with sweat and fluid loss and poor recovery after hard competition. Appropriate nutritional management may not only help to ensure that horses have sufficient nutrients to be healthy and active throughout their training and the competition but help to reduce the incidence of elimination, or failure to perform, especially for metabolic reasons. This is especially true for the endurance horse. In an overview of ~7000 starts on international endurance races only 50% completed the ride with 30% being eliminated – 63% because of lameness, 24% for metabolic reasons and 13% for other causes. This review will not discuss the effect of nutrition on metabolic conditions such as tying-up and laminitis but will look at energy provision, and the role of antioxidants.

Energy provision

The supply of energy is crucial for life and movement. In general terms if a horse is fed too little energy for its needs it will tend to become dull and lethargic and/or lose weight and/or become clinically ill. If a horse is fed too much energy or inappropriate energy it may become hyperactive and/or gain weight and/or become ill. Critical then to the feeding of any horse for health and vitality is the appropriate and adequate supply of energy. However, increasingly we are learning that the sources of energy and when and how they are fed may be important factors in optimal performance. In addition, horses like us are individuals and what might be the ideal diet for one horse may not be optimal for its fellow stable mate and we may increasingly need in the future to consider individual feeding and management practices.

Energy is supplied to the horse via its diet but fundamentally energy is not a nutrient (Harris 1997a). The chemical energy or gross energy contained within feeds needs to be converted into a form of energy that the cells can use for mechanical work or movement (useable or **net energy**). Ultimately the 'currency' used to fuel this movement is Adenosine Triphosphate (ATP). Muscles only have a small store of ATP – only enough for 1-2 secs of exercise. Creatine phosphate stores may help support ATP production and utilisation for a few more seconds – but then the muscles have to resynthesise ATP. Stored energy, primarily in the form of muscle and liver glycogen, as well as intramuscular and adipose triglycerides, are primarily used to provide this ATP. These stored energy sources in turn are replenished ultimately by the diet (Harris 1997a).

Our increasing demand for horses to perform repeatedly has resulted in energy requirements that, for some horses, are above those able to be provided by their more 'natural' diet of fresh forage. Cereals provide more net energy than hay, which in turn provides more than twice the net or useable energy than straw. However, the upper part of the gastrointestinal tract has a relatively small capacity and the horse has digestive and metabolic limitations to high grain, starch and sugar based diets (Harris 1999). Large grain meals may overwhelm the digestive capacity of the stomach and small intestine leading to the rapid fermentation of the grain carbohydrate in the hindgut. The rapid digestion of a concentrated meal has been shown to cause distinct physiologic disturbances when compared with grazing or steady state feeding conditions. This includes (Clarke et al., 1990, Pagan et al., 1999, Williard et al., 1977):

- Fluctuations of plasma glucose and metabolic hormones
- Post-feeding increases of plasma proteins and osmolarity
- Activation of the rennin angiotensin – aldosterone system
- Periods of intense colonic fermentation with induction of transmural fluid secretion and reduced colonic pH.

Such episodic processes have been suggested to contribute to the incidence of disorders in the stabled horse including colic, diarrhoea and laminitis (Clarke et al., 1990, Ralston 1992, Harris 1999, Harris & Kronfeld 2003, Kronfeld & Harris 2003, Harris & Arkell 2005, Harris & Geor 2007). There has therefore been increasing interest in the use of alternative energy sources for horses, especially alternative fibre sources which do not cause such marked disturbances in the hindgut and yet provide more energy than typical forages. In addition, because vegetable oils provide proportionally more net energy than the cereals, yet contain no starch or sugar and may provide other advantages, there is an increasing use of supplementary vegetable oils ***(Harris 1997b and Kronfeld & Harris 1997b, Harris 1999).***

Use of alternative energy sources.

Increased interest in oil/fat supplementation developed in the 1980s following work by Slade et al 1975. This suggested that horses fed a diet containing 12% fat (9% added corn oil) and ridden 67km over mountainous terrain for 8-10 hrs performed better and had higher blood glucose levels at the end of the ride than horses fed the control diet (3% fat). In another study, (Hambleton et al 1980) diets containing 4, 8, 12 or 16% fat (soybean) were fed to horses trotting 67km in 6 hrs. It was found that horses fed the highest percentage fat had the highest blood glucose concentrations after exercise. It was therefore suggested that fat might have a glucose sparing effect and that horses fed fat might show increased mobilisation of free fatty acids. Since then numerous studies have been carried out to investigate the potential of feeding fat supplemented diets to 'performance' horses (see Potter et al 1992, Harris 1997b). A range of effects on a variety of physiological, metabolic parameters as well as on performance has been reported. These variations may result from the variances in the study protocols and horses used in these trials. It is also difficult in some studies to differentiate effects of the oil supplementation itself versus the reduction of the starch intake.

Long term fat supplementation in combination with appropriate training, however, has been proposed to result in the following metabolic adaptations, which could result in improved performance (see also Harris 1997a; Potter et al 1992) – note that not all studies in this area have shown these effects:

- increased mobilisation of free fatty acids (FFA) and increased speed of mobilisation
- increased speed of uptake of FFA into muscle (Orme et al 1997) - often considered to be rate limiting
- a glycogen sparing effect so that fatigue is delayed and performance improved - could be especially important in endurance activities (Griewe et al 1989)
- increased high intensity exercise capacity (Eaton et al 1995)
- increased pre-exercise muscle glycogen levels (Kronfeld et al 1994, Meyers et al 1987, Scott et al 1992, Hughes et al 1995).

There are also potential health benefits by providing energy from fat and fibre rather than traditional starch and sugar sources potential health benefits by providing energy from fat and fibre rather than traditional starch and sugar sources (, Kronfeld & Harris 2003, Harris & Kronfeld 2003)

Some WALTHAM Studies

From some of our own early work (Pagan et al 1993) beneficial responses were reported in standardised exercise tests (up to I mile at ~ 11m/s min) of moderately oil supplemented diets (such as higher VLA_4, lower lactates, higher free fatty acids, lower blood ammonias). At the same time, greater blood lactates were found during studies which used a much higher oil supplemented diet (10% oil total diet) during incremental exercise tests at higher speeds (Taylor et al 1993). It was suggested that this could reflect enhanced glycolysis in fat adapted horses during anaerobic work – which would enable sprinting but suppressed glycolysis during aerobic work, which would confer stamina (Kronfeld et al 1998a & b). In Crandell et al's study (1999) where a proportion of the grain was replaced by Soya oil or (molassed) sugar beet pulp over a 5week period, blood glucose was significantly lower in the oil supplemented horses during the 3 hours post feeding. Cortisol was also lower during exercise and the oil-supplemented horses drank more than those fed the control or fibre substituted diet. In 2002 a study confirmed that, compared to a control diet, a fat supplemented diet (oil provided 29% of the total DE) for 5 – 10 weeks (Pagan et al 2002) was associated with an altered metabolic response to low intensity exercise (more than 30% reduction in the production and utilisation of glucose; a decrease in RE, a decrease in the estimated rate of whole body carbohydrate utilisation and an increase in lipid utilisation). Work also provided further evidence that the Arabian horse might be better adapted for endurance exercise as suggested by the possible greater use of fat as an energy source during lower intensity work (Prince et al 2002). A review (Kronfeld et al 1998) also suggested that oil supplementation beneficially moderates acid- base responses, enhances metabolic regulation, reduces heat production and water losses, substantially decreases bowel ballast as well as diminishing spontaneous activity and reactivity.

Many of the studies that were initially reported by all groups working in this area were short term in nature but in 1995 the results of feeding thoroughbreds either a control or an oil supplemented diet for 7 months were reported (Pagan et al 1995). This confirmed that when using training regimens and exercise tests similar to those used for sport horses, feeding a level of oil at around 12% of the daily DE intake caused no detrimental effects on either health or exercise responses. In fact adding the oil in these studies reduced the drop in blood glucose seen in the control group during a standardised step-wise exercise test (Pagan et al 1995). Due to higher intakes of oil being recommended by some this study was followed up by one that monitored the effects of feeding ~19% of the daily digestible energy from oil for 10 months followed by feeding ~27% of the DE from oil for a further 6months (Harris et al 1999b). Two sources of oil were used: a highly unsaturated form (soy oil) or a more saturated vegetable oil (coconut oil). Again this study showed no apparent adverse effects of feeding either of these oils. However, as a non-oil supplemented dietary group was not included effects on performance were not evaluated.

Which, when and how much to add?

Our initial research suggested that corn oil might be one of the most palatable oils (Holland et al 1998). However, horses will vary in their likes and dislikes and providing the vegetable oil is fresh, not rancid, of a good quality (preferably human grade), palatable and digestible to that individual, it may be acceptable as many different oils have been used over the years in trials. The optimal desired fatty acid composition of any supplemental oil is not yet known. However, we do know that in order to obtain metabolic benefits from the feeding of oil, in addition to those associated with its high energy density and lack of starch content, the oil needs to be fed for several weeks/months (Harris 1999a, Kronfeld and Harris 1997, Kronfeld et al 2000, Pagan et al 2002). The amount of oil that should be added however is also still open to some debate. Horses have been shown to be able to digest and utilise up to 20% or more of the diet as oil. Through a series of studies we have determined that the true absorption of fat is around 100% but the presence of endogenous faecal fat of around 55g/day accounts for the partial digestibility values of 95% (Kronfeld et al 2004). Fat digestibility was not reduced even when high levels were added (230g/kg) but in some studies a negative effect of added fat on crude protein was found. This led us to confirm that for practical purposes the DE for added feed fats is 9.0Mcal/Kg or ~37MJ/Kg and 5.2Mcal/kg for endogenous fat in typical horse feeds (Kronfeld et al 2001b, Kronfeld et al 2004).

A parabolic relationship between dietary fat and muscle glycogen concentration indicated a peak glycogen at 12% oil by weight (Kronfeld et al 1994). A variety of trials have confirmed the value of incorporating this much oil in a complete and balanced feed (Kronfeld & Harris 1997) although it has been suggested that especially in racehorse diets levels >9-10% by weight in the total diet may not be advisable with current managemental

practices as they may reduce muscle glycogen levels although this has not been proven. Adding oil to existing feed, however, has the potential to create multiple imbalances and therefore could be considered less safe than feeding a diet where the oil has been balanced in relation to all of the essential nutrients in the feed. Therefore, in regard to adding oil to diets it is likely to be prudent to use less than the 12% suggested above. Levels of 5 – 8 % in the total diet are more common in the competition horse. For example, it has been recommended to feed less than effectively 100g/100kg BW/day and the majority of animals (500kg BW) can be supplemented up to 400mls/day (~370g) in divided doses without any problems (Harris 1999b) – provided that as stated above the oil has been introduced gradually, is required and is not rancid (and the Vit. E levels are considered). It is very important to note that oil does not provide any additional protein, vitamins (Vitamin E content is variable) or minerals. If the horse is not receiving sufficient of these nutrients, for its workload, from its basal diet, then an appropriate additional vitamin and mineral mix may be needed and it is recommended that additional Vitamin E be fed in combination with supplemental oil. Exact recommendations are not known but an additional 100 -150iu Vitamin E/100mls added supplemental oil has been suggested (Harris & Arkell 2005). It is also important to realise that any supplemental oil should be introduced slowly since it might in turn result in GIT disturbances.

Role of FF diets

Whilst forage is considered essential to the horse for psychological and physiological reasons due to its high fibre content, it will provide less energy than cereals. In addition, there are higher energy costs associated with its ingestion and digestion. Fibre tends to hold water and contributes to the non-functional weight that a horse carries, and fibre is thermogenic, which will contribute to the thermal load that the horse has to dissipate. Whilst this can be useful in cold conditions, it is likely to be a disadvantage when working in hot and humid conditions (Kronfeld 1996). Fibre rich rations, however, help promote positive behaviors and sound gastrointestinal health (Nicol et al 2005, Harris & Arkell 2005). This led to the inclusion in many performance horse diets of 'highly digestible fibre' sources such as sugar beet pulp and soya hulls. Analysis would suggest that a major reason for why these are perhaps of more value than their traditional crude fibre, protein, etc. is that the traditional methods of describing the nutrient value of a feedstuff are inadequate. This highlights one of the fundamental problems still facing equine nutritionists today… 'how best to define feedstuffs for the horse in general and in particular fibre content and its 'value'. Much of the nomenclature used is better suited for the ruminant or the human and not for the single stomach, predominantly hindgut fermenting, horse (Cuddeford 1999, Hoffman et al 2001). Ideally we need to appreciate which method or methods for fibre analysis provide an accurate guide as to where the fibre is degraded, to what extent, over what time period and which end products are produced (Anon (NRC) 2007). It is currently thought that fibre, or more specifically the non-starch polysaccharide (NSP), in beet pulp is highly digestible over the total tract with a significant proportion being degraded (around 16.5% of unmolassed SBP NSP) in the small intestine during transit to the hind gut (Hyslop 1998, Hyslop et al 1998a & b, Moore-Colyer et al. 1997). The various digestibility studies suggest that not only is SBP well fermented in the horse but that this degradation occurs to a large extent within the time period that such a feedstuff would remain within the gut (Hyslop et al., 1998a, Stefansdottir et al., 1996). Recent studies have shown that the addition of sugar beet pulp to the diet may increase the nutrient value of concurrently fed hay, especially if this has a low protein content (Moore Colyer and Longland 2001), and also may have an effect on alfalfa based diets (Hastie and Longland 2001).

Recently the value of combining within a diet different fibre sources including the highly digestible fibres mentioned above and vegetable oil has been explored for the exercising horse as well as those prone to laminitis, tying up etc. (Harris & Geor 2007). In other species, it has been shown that replacing carbohydrates with fat as an energy source avoids insulin resistance and improves metabolic regulation during exercise. Whether this is also the case in horses was tested in two studies that investigated the effects of diet and exercise on insulin sensitivity. In the first study, 12 Arabian geldings were adapted to feeds high in starch and sugar or fat and fibre (Trieber et al 2005e). Horses were exercised during the 8-week of period adaptation and then blood sampled during moderate-intensity exercise on a treadmill. Whilst there were no differences at rest, the insulin and glucose concentrations after the 25min warm up were significantly lower in the FF group. Insulin sensitivity, as determined by basal proxies, increased during exercise in all 12 horses, whereas β-cell response was lower. Horses adapted to the fat and fibre diet had higher insulin sensitivity during exercise than those on starch and sugar, but diet had no effect on β-cell response. These findings indicate that horses adapted to high carbohydrate diets have lower insulin sensitivity and impaired metabolic regulation, involving insulin signalling rather than hormone production. Such effects appear to be avoided in horse adapted to diets where energy comes from fat, with potential benefits for their health and athletic performance. The metabolic effects of these two diets were examined further in a second study (Hess et al 2005), where 40 endurance horses participating in an 80-km race were split into three groups and fed either concentrates rich in starch (33%) and low in fat (8%), rich in fat (15%), or intermediate in starch (16%) and fat (11%). The horses were monitored before, during and after the race, and blood samples collected for assessment of insulin sensitivity using basal proxies. The endurance ride was associated with increases in insulin sensitivity and cortisol, and decreases in insulin and β-cell response. Horses fed the concentrate rich in starch and sugar, as well as the intermediate concentrate, had lower insulin sensitivity than those fed the fat and fibre diet. This was associated with higher insulin concentrations but there were no significant differences between the dietary groups in β-cell response. Horses on the starch and sugar diet had significantly higher plasma creatine kinase activities than those in the other two dietary groups. Fifteen horses were eliminated and these were found to have lower insulin sensitivity than the finishing horses, higher insulin and cortisol concentrations, and more marked rise in muscle enzymes. This postulates, for the first time, that the development of insulin resistance could be a factor in the elimination of horses during an endurance race. The results provide additional support for the role of diets, where energy comes from fat rather than hydrolysable carbohydrates, in avoiding insulin resistance, improving energy utilisation and enhancing athletic performance.

Thyroid hormones play an important role in the metabolism of carbohydrates, fats and proteins, forming another key link between nutrition and metabolism. These hormones also help with glucose transport, energy metabolism, neuromuscular activity and cardiac function during exercise and conditioning. Little is known about these relationships in horses and for this reason the effects of dietary energy sources and exercise on thyroid function were investigated in a group of mature Arabian geldings (Carter et al 2005). After a 15-week training period, during which time the horses were fed feeds high in starch and sugar or fat and fibre, the horses underwent a standard exercise test on a treadmill. Thyrotropin releasing hormone response tests were performed before and at the end of the training period, and the morning after the exercise test. Total and free T4 concentration increased over the first 4 weeks of training but then fell back to pre-training levels by weeks 13 and 5, respectively. There was no change in total or free T3 levels over this period and there were no differences in any of the hormone concentrations between the two dietary groups. During the exercise test, total and free T4, and total T3, increased progressively. There were large variations in free T3 between horses and this did not change significantly with exercise. Total and free T4 concentrations were similar in the two dietary groups during exercise, whereas total and free T3 were found to be higher for the horses fed the sugar and starch ration. There was an effect of training hormonal responses to TRH stimulation, with greater increase in total and free T4. There was also an influence of diet with horses fed starch and sugar showing greater changes in total and free T3 both before and after the training period. The results showed that thyroid hormone output is increased during athletic conditioning and exercise, presumably as a response to increased demand upon the thyroid gland. These effects were predominantly mediated through T4, whereas the dietary influence was mediated through T3. Increased T3 levels may indicate an increase in activity of the enzyme 5'-deiodinase due to higher glucose and insulin levels.

What about protein

Additional protein over maintenance may be needed with exercise and training because of the accompanying muscular development, the need for muscle repair and to replenish the nitrogen lost in sweat (~20- 25g/kg sweat loss). Higher protein diets may be undesirable because of the effects of excess dietary protein on heat production, acid-base balance, water requirements and, potentially, respiratory health (Graham-Thiers et al. 2002). There

is also evidence that dietary protein level alters urea metabolism in horses and it has been estimated that a change in dietary CP from 10 to 15% would increase water requirement by approximately 5% because of an obligate increase in urine production for clearance of endogenous urea loads (Kronfeld 1996). Moreover, the higher urinary urea load could adversely affect the respiratory health of confined horses because urea is converted to ammonia, a known respiratory irritant.

Oxidation of the phosphorus and sulphur in protein adds to the acid load on the body. In this context, Graham-Thiers et al. (2002) evaluated the effects of a restricted protein diet (7.5% CP with added lysine and threonine) on acid-base balance in horses in moderate work. When compared to a 14.5% CP diet, protein restriction resulted in a slight increase in resting blood pH and mitigation of exercise-associated acidaemia during repeated sprints. These effects may provide a performance advantage during exercise, although the actual effect of such changes on exercise performance has not been determined.

Antioxidant supplementation exercise and lung health

Oxidative damage to the body's cells is believed to underlie a number of chronic diseases, in particular those affecting the respiratory system, and has been implicated in processes such as ageing and cancer. The major cause of this damage is free radicals that attack key cellular components, principally DNA and membrane structures. Protection against oxidative damage is provided by sophisticated antioxidant defences, which work to prevent the formation of free radicals, trap and 'disarm' any free radicals produced, and to repair any cellular or DNA damage that does happen to occur. Oxidative stress occurs when these antioxidant defences are overwhelmed, either due to the inadequacy of the defences, increased free radical production, or a combination of both.

Antioxidant supplementation and exercise

Production of reactive oxygen species increases substantially with metabolism of oxygen during exercise, with the potential to overwhelm antioxidant defences and cause tissue, and in particular muscle, damage. This can have severe health and welfare consequences and over the past five years we have been looking at the effects of exercise in particular field endurance exercise on the levels of oxidative stress (Hargreaves et al 2001, Hargreaves et al 2002 a & b, Marlin et al 2001). Together with our colleagues in this area we have shown that whilst prolonged endurance exercise caused changes in blood and plasma antioxidants there was marked individual variability in the level of oxidative stress which might be related to factors such as the diet, fitness, severity of the course etc. (Marlin et al 2002).

In order to assess the impact of oxidative stress in endurance horses, our colleagues in Virginia together with ourselves have staged two 80 km ride over ground ranging in elevation from 100 to 400 m. The first ride was held in April 2001 (Hoffman et al 2002) and the second in 2002. Data from the second ride was compared with that from the first research ride as well as a competitive ride both of the same distance but run under differing conditions of ambient temperature, terrain and degree of competitiveness (Williams et al 2005a). In the second research ride, veterinary checks were carried out on the day prior to the race and after 27, 48, 72 and 80 km, and the horses were weighed, heart rate and rectal temperature were taken, and blood samples were collected at each check point, with the exception of that at 72 km, plus after 170-190 minutes of recovery. The blood samples were used to evaluate the level of oxidative stress (total plasma lipid peroxides), antioxidant status (total glutathione, glutathione peroxidase, α-tocopherol), as well as the extent of dehydration (albumin) and muscle involvement (creatine kinase, aspartate aminotransferase). The average time for completion of the race was 10 hours 8 minutes and horses lost an average of 5% of their body weight during the race. Distance was correlated with increases in lipid peroxides, total white blood cell glutathione and muscle enzyme activities, as well as decreases in red and white blood cell glutathione peroxidase and plasma α-tocopherol. This data is indicative of increasing oxidative stress and lowering of antioxidant status throughout the ride resulting in greater permeability of muscle cells membranes and leakage of enzymes (Williams et al 2005a). Of the 40 horses that started the ride, 16 did not finish for reasons that included lameness (n=6), metabolic complication (n=5), rider option (n=3) and miscellaneous (n=3). Comparison of results from the horses that did not finish with those that did showed no significant differences in antioxidant status, or degrees of oxidative stress or dehydration. Three non-finishers did, however, have higher plasma creatine kinase and aspartate aminotransferase activities suggesting greater muscle damage, which may have contributed to their elimination from the event (Williams et al 2005a)

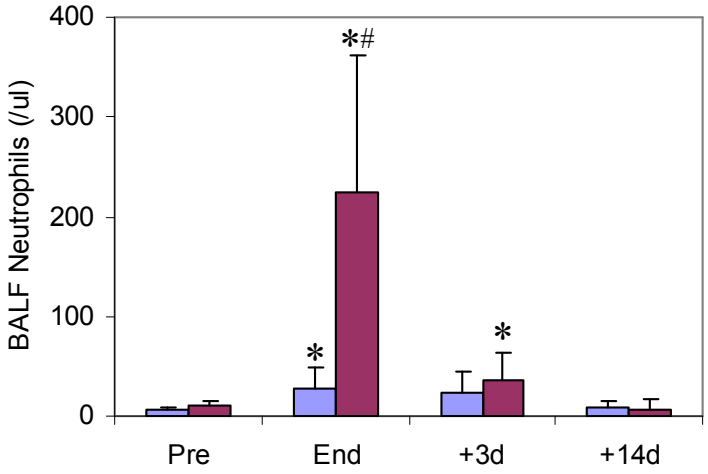
With respect to the potential beneficial role that antioxidant supplementation might play a number of our studies have looked at lipoic acid as well as vitamin E supplementation (Williams et al 2001, 2004a & b). In one study (Williams et al 2004a), 46 horses participating in an 80-km endurance race were supplemented with either vitamin E alone (DL-α-tocopherol acetate, 5,000 IU/day) or vitamin E and vitamin C (ascorbic acid, 7 g/d) for three weeks prior to the ride. Blood samples, rectal temperature and heart rate were taken the day before the race, at 21 and 56 km into the ride, and 20 minutes after finishing. Twelve horses, six in each treatment group, did not finish the race for reasons that included lameness, metabolic problems and rider option. Plasma ascorbate levels were significantly higher in horses supplemented with both vitamins E and C. There were increases with distance travelled in heart rate, temperature, as well as plasma creatine kinase (CK) and asparatate aminotransferase (AST) activities, and plasma lipid hydroperoxide concentrations consistent with the development of oxidative stress. There were concomitant decreases in the antioxidants glutathione peroxidase in red and white blood cells, and glutathione in white blood cells. These changes did not differ between horses that received vitamin E or E and C. The increases in muscle enzymes AST and CK were correlated with plasma lipid peroxides. This confirms previous reports of muscle involvement and membrane leakage during endurance exercise in horses and demonstrates a potential causative role for oxidative stress. Plasma AST and CK were also found to be higher in horses that did not finish the race because of metabolic reasons. The effects of oxidative stress may be immediate or cumulative, and highlight a potential role for antioxidant supplements in endurance horses. All of the participants in this race insisted on using vitamin E for this purpose, at five times the recommended minimum dose. Co-supplementation with vitamin C had no additional effect on antioxidant capacity and further work is required to identify which antioxidants and at what appropriate doses might provide optimum protection from oxidative stress. It was also noted that the higher placed horses in this ride tended to have higher vitamin E intakes. Together these findings indicate that supplementation of dietary vitamin E to levels in excess of those currently recommended holds the promise of improving health, welfare and performance of horses undergoing heavy or prolonged exercise. This was the objective of the second study, in which the antioxidant effects of lipoic acid were investigated (Williams et al 2004b) Lipoic acid interacts with vitamin C and glutathione and may also recycle vitamin E, and has been used to help alleviate oxidative stress during aging, disease and exercise in laboratory animals. Three groups of six Arabian horses received either lipoic acid (DL-α-lipoic acid, 10 mg/kg bodyweight/day), vitamin E (DL-α-tocopherol acetate, 5,000 IU/day) or no antioxidant supplement for 3 weeks prior to a treadmill simulated 55-km endurance exercise test (note no cross-over). Plasma vitamin E and blood glutathione concentrations were higher in both antioxidant-supplemented groups, and ascorbate levels were higher in those horses that were given lipoic acid. Plasma muscle enzyme activities increased during exercise but this response was significantly blunted in the antioxidant-supplemented horses. Apoptosis (programmed cell death) in white blood cells increased significantly with distance in the control horses, but did not change with exercise in those supplemented with vitamin E or lipoic acid where it was approximately 10-fold lower. This study suggested that both vitamin E and lipoic acid can augment antioxidant status, protect against oxidative stress-mediated muscle damage and minimise apoptosis. The latter effect is likely to be of most benefit in horses competing under extreme conditions, where lymphocytes and skeletal muscle are targets for exercise-induced apoptosis.

Antioxidants and lung health

Pulmonary function is aimed at achieving optimal gas exchange. This requires close apposition between the alveolar air space and the vascular endothelium, adequate alveolar ventilation, adequate perfusion and co-ordination or matching of pulmonary vascular flow to well-ventilated regions of the lung. Oxidant injury can potentially affect both the structural integrity of the alveolar blood-gas barrier and the regulation of vascular perfusion and therefore can affect both performance and health of the individual. The respiratory tract lining fluid forms an interface between the underlying respiratory tract epithelial cells and the external environment. It is exposed to relatively high levels of oxidative insult, which result from production of reactive oxygen species by activated inflammatory cells in response to inhaled allergens and from inhaled oxidants such as ozone and other pollutants. A normal healthy lung is equipped with structural and non- structural defence systems to counteract the effects of a variety of injurious agents present in the inspired air. The structural defence system includes specialised cilliary lining cells of large and small airways, secretory products of specialised airway cells providing a sticky mucus blanket to line the airways and phagocytic macrophages capable of ingesting and digesting most of the particulate materials. Pulmonary non-structural antioxidant defences are widely distributed within the cells and the respiratory tract lining fluids and include both enzymatic (superoxide dismutase, the glutathione system) and non-enzymatic systems (uric acid, Vitamin E and C). Impaired antioxidant capacity may play a role in the development of certain conditions affecting the respiratory tract such as human asthma, for instance, where there are thought to be decreased concentrations of key antioxidants (ascorbic acid, α-tocopherol, reduced glutathione) in the lung lining fluid (Olusi 1979, Smith et al 1993, Kelly et al 1999). Recent work has shown the importance of oxidative stress in the lung both in healthy exercising horses and in diseased horses. Work has indicated that animals, which suffer from recurrent airway obstruction, may have disturbed oxidant-antioxidant equilibrium (Art et al 1999, Kirschvink et al 1999). Recurrent Airway Obstruction (RAO – previously known as Chronic Obstructive Pulmonary Disease - COPD) is the most common non-infectious respiratory disease of horses and has many similarities with human asthma. It is characterised by periods of acute neutrophilic airway inflammation and allergen induced airway obstruction and periods of remission. Work by researchers at the Animal Health Trust, Newmarket together with WALTHAM has demonstrated that oxidative stress and damage also plays a key role in the pathogenesis of RAO (Deaton et al 2004). The role of ascorbic acid in RAO was evaluated by determining its concentration and degree of oxidation in horses affected by RAO in the presence and absence of neutrophilic airway inflammation. Concentrations of ascorbic acid in plasma and pulmonary epithelial lining fluid were significantly lower in six RAO-affected horses with airway inflammation, compared with the same horses following resolution of inflammation and eight healthy controls. Concentrations were also higher in RAO-affected horses without inflammation than in the control horses. Furthermore, the ascorbic acid redox ratio – a measure of oxidation of ascorbic acid – was higher in RAO-affected horses with inflammation than without. The number of neutrophils in bronchoalveolar lavage fluid was inversely related to pulmonary ascorbic acid concentrations and passively correlated with ascorbic acid redox ratio. These results confirmed that neutrophilic inflammation was associated with a reduction in the major pulmonary antioxidant and its plasma concentrations. This reduction was not completely reversed with resolution of airway inflammation, giving therefore, justification for nutritional supplementation with ascorbic acid derivatives in horses with RAO, when clinically affected as well as when in remission.

Activated inflammatory cells produce hydrogen peroxide through a respiratory burst. Elevated hydrogen peroxide concentrations are detected in expired breath from humans with a variety of inflammatory lung disorders, including adult respiratory distress syndrome and asthma. To determine whether this is also true of horses with RAO, expired breath was collected, condensed and analysed for hydrogen peroxide concentrations. (Deaton et al 2004b). Horses with RAO and marked airway inflammation had significantly higher concentrations of breath condensate hydrogen peroxide than control horses and horses with RAO that were in remission. Hydrogen peroxide concentrations were positively correlated with bronchoalveolar neutrophil counts and inflammation scores, and inversely related to pulmonary ascorbic acid concentrations. .Measurement of breath hydrogen peroxide thus shows promise as a non-invasive marker and diagnostic indicator of lower airway inflammation, and for application in research of RAO (Deaton et al 2004b). We also investigated whether similar changes in pulmonary ascorbic acid and hydrogen peroxide concentrations occur in horses with acute, as opposed to chronic, airway inflammation and evaluated the potential for dietary antioxidant supplementation to increase pulmonary and systemic antioxidant capacities in horses with RAO (Deaton et al 2003a). This work has real welfare implications for all stabled horses. In this study the relationships between acute airway inflammation and pulmonary concentrations of ascorbic acid were examined in horses with RAO that were in remission. They were subsequently challenged with allergens by stabling for 24 hours on a straw bed and were provided with hay. Bronchoalveolar fluid (BALF, fluid lining the lungs) was collected for measurement of ascorbic acid concentration as well as the number of white blood cells, in particular the neutrophils, 1 week prior to exposure and then immediately, 3 and 14 days after stabling, and the results compared with those from healthy control horses. Stabling for 24 hrs caused mild signs of airway obstruction in the RAO affected horses (which was not clinically obvious) and this was associated with an immediate increase in the numbers of neutrophils in the bronchoalveolar lavage fluid, which then returned to pre-stabling values at 3 and 14 days (Fig2). There was also an immediate decline in ascorbic acid concentrations in these horses (Fig 3), which recovered to pre-stabling values at 3 and 14 days. The control horses showed a much smaller, although still significant, change in neutrophil counts but only a slight decrease in the ascorbic acid concentrations following stabling which was not significant.

*Figure 2 Number of neutrophils (mean ± SD) in bronchoalveolar lavage fluid (BALF) before (Pre) and immediately (End), 3 days and 14 days after exposure to hay and straw in control horses (blue n = 6) and horses affected by recurrent airway obstruction (red: n = 6). # Denotes significantly different from control horses (P = 0.004). *Denotes significantly different from prior to exposure (P < 0.05).*



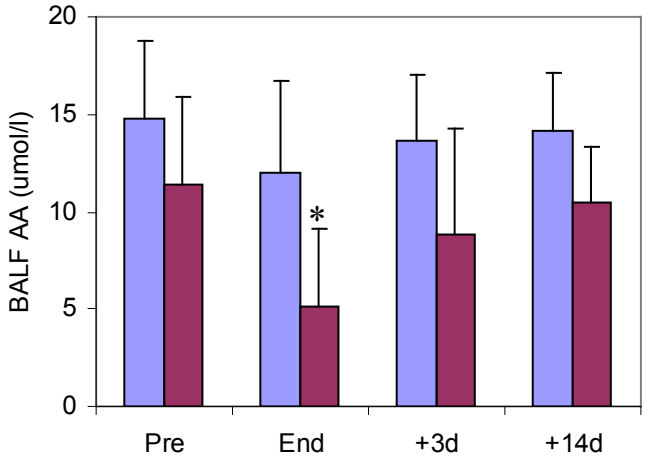


*Figure 3: Concentrations (mean ± SD) of ascorbic acid (AA in bronchoalveolar lavage fluid (BALF) before (Pre) and immediately (End), 3 days and 14 days after end exposure to hay and straw in control horses (blue; n = 6) and horses affected by recurrent airway obstruction (red; n = 6). * Post-exposure time point significantly different from Pre (P < 0.05).*

In the same study the effects of acute exposure to hay and straw on airway resistance and hydrogen peroxide were also investigated (Deaton et al 2003b). Four of the RAO-affected horses demonstrated an increase in airway resistance after 24 hours of stabling, whilst there was no change in any of the control horses. The decrease in ascorbic acid concentrations was correlated with both the increase in the number of neutrophils and the increase in airway resistance. There was, however, no change in concentrations of hydrogen peroxide in exhaled breath concentrate in either group – suggesting that such changes only occur when the antioxidant capacity is overwhelmed. This provided further evidence that ascorbic acid is the primary defence against neutrophil-derived reactive oxygen species during acute, as well as chronic, neutrophilic inflammation in horses with RAO. This process is associated with reduction in pulmonary ascorbic acid concentrations, which are then rapidly replenished with the resolution of inflammation.

The prospect of enhancing pulmonary ascorbic acid concentrations through dietary supplementation, and thus helping to protect against oxidative stress associated with neutrophilic inflammation, was investigated in a number of other studies. Whilst no- one had looked at the pulmonary bioavailability of ascorbic acid (Vitamin C) workers at the Animal Health Trust had previously shown that some forms of ascorbic acid were not readily bio-available (Snow et al 1987). Therefore, in order to first assess the feasibility of increasing pulmonary concentrations of ascorbic acid, the effects of oral supplementation with two forms of ascorbic acid on pulmonary and systemic antioxidant status were assessed in six healthy ponies (Deaton et al 2003c). Plasma ascorbic acid concentrations were significantly increased after two weeks of oral supplementation with ascorbyl palmitate, at a daily dose equivalent to 20 mg ascorbic acid per kg body weight. A more stable form of ascorbic acid, calcium ascorbyl-2-monophosphate, at the same dose also increased systemic concentrations but not significantly. Both supplements did, however, increase concentrations of ascorbic acid in bronchoalveolar lavage fluid in 5 out of the 6 ponies – one pony did not respond to either supplement (Fig4). Neither supplement altered plasma or pulmonary concentrations of the other major antioxidants, namely glutathione, uric acid and α-tocopherol.

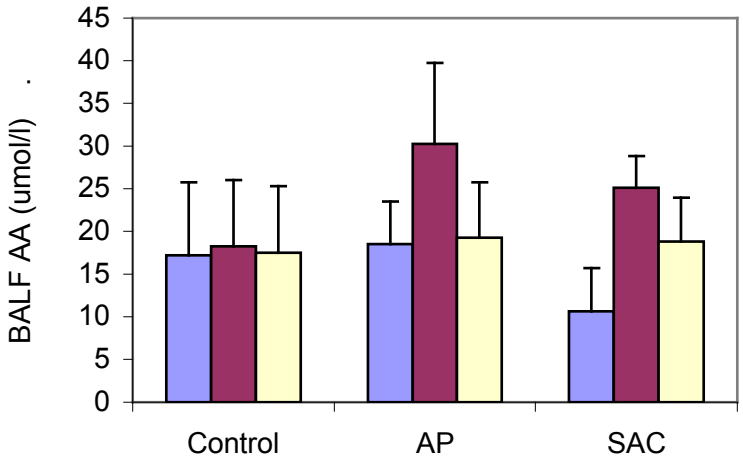


Figure 4 *BALF ascorbic acid (AA;* mean ± SD*) concentrations (υmol/l) after a 2-week lead in period (blue), 2 weeks of supplementation (red) and a 2 week washout period (Cream). A: Control, no supplement; B: Ascorbyl palmitate; C: Stabilized form of Ascorbic acid SAC; D: Mean ± S.D. of the six ponies for each supplement.*

In a study that looked at the effect of antioxidant supplementation in RAO horses during exercise it was shown that the antioxidant supplementation could significantly increase concentrations of ascorbic acid in plasma in all the horses (Deaton et al 2004c) and both bronchoalveolar cell-free fluid and cellular components in four of the five horses (Fig5).

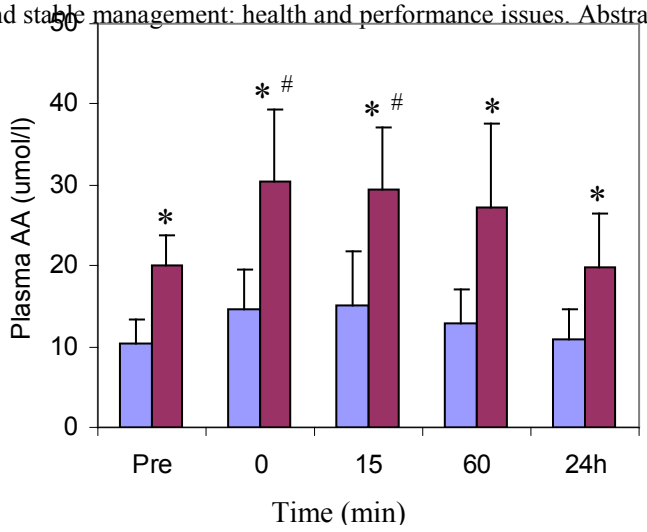


*Fig 5 Plasma concentrations (mean ± SD) of ascorbic acid (AA) before and after an exercise test in 5 horses affected by recurrent airway obstruction following 4 weeks supplementation with an antioxidant cocktail (blue) or a placebo (red). Statistical analysis was performed with the Kruskal-Wallis test and the Wilcoxon Signed Rank Test. * significantly different from placebo (P<0.05). # Significantly different from Rest (P<0.05).*

There was also a trend for bronchoalveolar lavage fluid concentrations of oxidised glutathione, a marker of oxidative stress, to be decreased when the antioxidant supplement was fed (see Fig6).

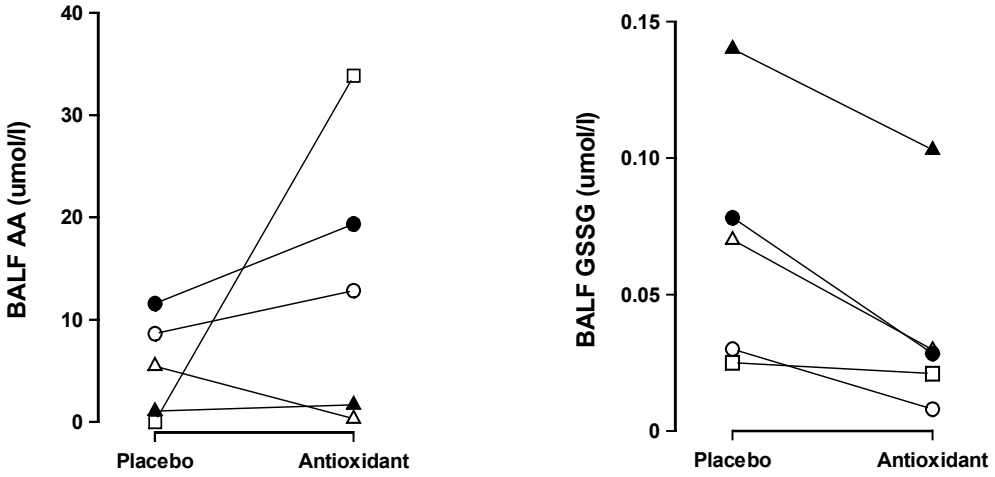


Fig6 shows the effect of supplementation on the individual animals BALF Ascorbic acid and Oxidised Glutathione (GSSG – indicator of oxidative stress).

Exercise was found not to induce the oxidation of ascorbic acid or glutathione, or increase lipid peroxidation at any stage, indicating that, even in RAO-affected horses with reduced antioxidant capacity, the exercise intensity was insufficient to cause oxidative stress.

These results confirm that dietary supplementation can increase antioxidant capacity, both pulmonary and systemic, in horses with RAO and has the potential to reduce oxidative stress. Supplementation may also be beneficial in other circumstances where there is significant oxidative stress such as strenuous exercise. This said, it is not clear from previous work to what extent decreased pulmonary antioxidant capacity in horses affected by RAO renders them more susceptible to oxidative challenge. Data linking acute oxidative stress with the development of airway inflammation is also lacking and this was addressed in a series of studies. First, the prevalence of oxidative damage in horses with RAO was examined using the Comet assay (also known as single-cell gel electrophoresis). In preliminary work, the assay was adapted and validated for use with equine peripheral blood mononuclear cells. Using this, it was found that eight RAO-affected horses in remission had significantly greater endogenous oxidative DNA damage than eight healthy controls. In two subsequent studies, seven healthy and seven RAO-affected horses, albeit in remission, were exposed to the pro-oxidant ozone for 2 hours and samples of tracheal mucus and bronchoalveolar fluid collected prior to exposure and 6 and 72 hours after exposure. None of the horse coughed or showed changes in respiratory character after exposure to ozone. The RAO-affected horses had higher inflammation scores but exposure to ozone was not associated with significant alterations in tracheal wash or bronchoalveolar cytology after exposure in either group. There was no effect of ozone exposure on circulatory antioxidant status. As found previously, bronchoalveolar ascorbic acid concentrations were lower in RAO-

affected horses. Ozone appeared to preferentially oxidise glutathione rather than ascorbic acid, with both groups demonstrating glutathione oxidation following exposure. Overall, there was no difference in degree of pulmonary oxidative stress experienced by the healthy and RAO-affected horses, and ozone did not induce significant airway inflammation in either group. There was a strong inverse correlation between changes in bronchoaleveolar neutrophils and changes in ascorbic acid concentrations after ozone exposure and the concentration of ascorbic acid in cells increased after exposure. This suggests that while ozone exposure does not deplete pulmonary ascorbic acid, ozone-induced neutrophilia does result in oxidation and intracellular accumulation of ascorbic acid. These studies show that RAO-affected horses that are in remission are not more sensitive to ozone despite having a decreased pulmonary antioxidant status. The absence of an inflammatory response to ozone suggests that, at the exposure level used, antioxidant defences were adequate in both groups of horses. This may be cause ozone preferentially oxidised glutathione, which in fact did not differ in pulmonary concentration between the RAO and normal horses.

Airway inflammation was more reliably induced in the next study by exposing six RAO-affected horses and 6 control horses to organic dust from straw for 24 hours. This resulted in significant increases in bronchoalveolar neutrophils, which was associated with a decrease in pulmonary ascorbic acid and increase in elastase concentrations. The latter are a measure of neutrophil activity and degranulation. Mild clinical signs associated with increased respiratory effort were observed in one horse and the decrease in ascorbic acid was correlated with increased respiratory resistance. Markers of oxidative stress were, however, unaffected by exposure to dust and levels of hydrogen peroxide in exhaled breath were also unaffected. While these results provide further confirmation of the role of ascorbic acid in acute neutrophilic inflammation, either the period or level of dust exposure was inadequate to result in significant pulmonary oxidative stress.

Given that the roles of ascorbic acid and oxidative stress in the development of neutrophilic inflammation and RAO, there is a strong rationale for dietary supplementation to improve antioxidant status and protect oxidative damage. The question of whether nutritional antioxidant supplementation might reduce oxidative damage by enhancement of the antioxidant defence, thereby modulating inflammatory processes, was tested by another group in a placebo-controlled, blind study (Kirshvink et al 2002) using RAO horses in clinical remission following a different exercise protocol. This study evaluated whether a dietary antioxidant cocktail supplementation (based on WINERGY Ventil-ate®→ – UK patent granted) for four weeks would improve lung function and reduce airway inflammation in heaves-affected horses. Eight horses in clinical remission of heaves were investigated at rest and after a standardised exercise test before and after antioxidant cocktail or placebo treatment. Pulmonary function and exercise tolerance were monitored; systemic and pulmonary lining fluid uric acid, glutathione and 8-epi-$PGF_{2\alpha}$ were analysed, and bronchoalveolar lavage (BAL) cytology and inflammatory scoring of the airways were performed. The antioxidant treatment significantly improved exercise tolerance and significantly reduced the endoscopic inflammatory score. Plasma uric acid concentrations were significantly reduced, suggesting down regulation of the xanthine-dehydrogenase and xanthine-oxydase pathway. Haemolysate glutathione showed a non-significant trend to increase, whilst plasma 8-epi-$PGF_{2\alpha}$ remained unchanged. Pulmonary markers and BAL cytology were not significantly affected by antioxidant cocktail. This study suggested that the antioxidant cocktail tested improves lung function of heaves-affected horses by modulating oxidant/antioxidant balance and airway inflammation (Kirschvink et al 2002). In another study where 5 RAO-affected horses in remission received an oral antioxidant supplement and pulmonary and systemic parameters were compared with those when the horses were fed a placebo. The supplement contained vitamins E and C, and selenium providing 6 mg/kg, 10 mg/kg and 5.1 µg/kg bodyweight/day, respectively. There was no significant effect of supplementation on bronchoalveolar fluid cytology. Plasma ascorbic acid and α-tocopherol concentrations were increased on the supplement, with the former increasing to levels found in healthy control horses. Bronchoalveolar ascorbic acid concentrations increased with antioxidant supplementation but this did not attain statistical significance. At the end of each treatment period, the horses underwent a standardised exercise test. This failed to induce either pulmonary or systemic oxidative stress, although it should be borne in mind that the RAO-affected horses were in remission at the time of the study. The effects of exercise in RAO-affected horses was further investigated in a companion study.[9] Human asthmatics have diminished basal plasma cortisol concentrations and a blunted cortisol response to exercise. Furthermore, in healthy humans, supplementation with ascorbic acid has been shown to attenuate the exercise-induced increase in plasma cortisol following prolonged, submaximal exercise. The purpose of this study was to ascertain if there are similar relationships between cortisol and ascorbic acid in horses. Five RAO-affected and six healthy horses performed a standard exercise test after receiving 10 mg ascorbic acid per kg per day or a placebo. Exercise was associated with significant increases in plasma cortisol concentrations in both groups, and basal and post-exercise values were similar in the RAO and control groups.

Antioxidant supplementation increased basal and post-exercise plasma ascorbic acid concentrations in both groups, but had no effect on plasma cortisol concentration in either group before or after exercise. These findings show that RAO-affected horses, when in remission, demonstrate the same cortisol response to exercise as healthy controls. Although antioxidant supplementation had no impact upon these responses, it remains possible that it would be beneficial in RAO-affected horses that are not completely in remission.

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Conclusion

Reviewing some of our work over the last 10 years has highlighted how influential diet and managemental practices can be on the health welfare and performance of the horse. Whilst our understanding and knowledge have obviously grown, there is still a lot to learn about how to feed and manage our horses optimally so that both their and our needs and requirements are met.

References and some additional reading

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