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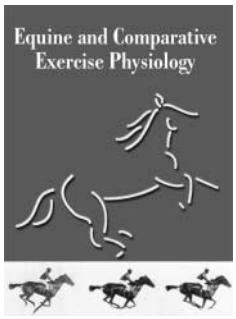

The role of nutritional supplements and feeding strategies in equine athletic performance

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Abstract

In human and animal nutrition, much interest has been focused on the potential role of dietary supplements in promoting health, athletic performance and disease mitigation. Supplements may include essential nutrients provided in amounts greater than required to prevent a deficiency state, or substances purported to have a role in metabolism or tissue function but that are not recognized as an essential nutrient. This review aims to provide the rationale and scientific evidence for use (or not) of some of the supplements marketed for use in horses, with emphasis on supplements purported to directly boost performance, such as creatine, carnitine and branched-chain amino acids. It also discusses the so-called 'joint supplements' (or slow-acting, disease-modifying osteoarthritis agents), such as glucosamine and chondroitin sulphate. The effects of selected feeding strategies on performance, including fat supplementation, are also examined. It is concluded that although the use of nutritional supplements is commonly alleged to boost performance or health in horses, for most, if not all, of these supplements there is little or no scientific evidence of efficacy.

Keywords: horse; exercise; supplements; dietary fat; ergogenic aids

Introduction

The marketplace is overflowing with 'special' supplements that promise to provide the equine athlete with a performance advantage, either by boosting athletic capacity or by mitigating a problem that may impair performance (e.g. osteoarthritis, OA), and use of this type of dietary additive is very common[1], perhaps reflecting the desire of owners, trainers and riders to gain a competitive edge and/or assure the general health and well-being of horses under their care. Supplements may include essential nutrients or substances purported to have a role in metabolism or tissue function but that are not recognized as an essential nutrient (e.g. chromium, coenzyme Q10, herbal products and glucosamine). It is the use of this second category of supplements that has escalated in recent years even though, for most, there is a lack of scientific data on safety and efficacy. The purpose of this review is to provide the rationale and scientific evidence for use (or not) of some of these supplements, with emphasis on supplements purported to directly boost performance (e.g. creatine, carnitine and branched-chain amino acids) as well as the so-called 'joint supplements', also termed slow-acting, disease-modifying OA (SADMOAs) agents, e.g. glucosamine and chondroitin sulphate (CS). The effects of selected feeding strategies on performance are also discussed.

Performance enhancement

The term ergogenic means 'work generating'[2]. In a general sense, the term ergogenic aid has been used in reference to manipulations that are purported to increase athletic performance, such as an increase in speed, endurance or strength. Ergogenic aids have been classified into several different categories, including[3]:

- mechanical factors (e.g. lighter shoes or tack, use of external nasal strips);
- pharmacological agents (e.g. drugs, such as anabolic steroids and erythropoietin);
- physiological improvements (e.g. the effects of physical conditioning, pre-exercise administration of sodium bicarbonate); and

- nutritional supplements or altered feeding strategy (e.g. supplements containing creatine or carnitine, adaptation to a fat-supplemented diet).

The control of energy transduction and utilization in skeletal muscle is thought to be one of the key determinants of athletic performance. Regardless of discipline (e.g. a 1000 m flat race *versus* endurance exercise lasting several hours), muscular performance is dependent on maintenance of a continual supply of adenosine triphosphate (ATP). In this context, many nutritional supplements are purported to enhance the performance by fine-tuning the control of ATP synthesis, thereby deferring the onset of fatigue associated with impaired energy transduction in muscle. Specific mechanisms of an ergogenic effect might include[3]:

- A decrease in the energetic cost of locomotion (or an increase in the power-to-weight ratio), for example, due to decreased bodyweight without loss of lean mass.
- Increased levels of available stored energy, e.g. muscle glycogen loading.
- Provision of a supplementary fuel source, e.g. administration of glucose before and/or during exercise.
- More efficient fuel utilization in muscle, e.g. increased oxidative metabolism of glycogen with a concomitant decrease in anaerobic glycogenolysis.
- Decreased accumulation of substances that impair cellular function, e.g. improved intracellular acid–base regulation.
- Improved ATP/adenosine monophosphate (ADP) homeostasis in contracting muscle fibres.
- Increased power or strength due to muscle anabolism.
- Mitigation of the effects of free radicals on tissues (e.g. skeletal muscle) during exercise.

Evaluating the safety and efficacy of nutritional ergogenics

In keeping with the practice of evidence-based medicine (or nutrition), it is the role of the veterinarian and/or nutritionist to critically evaluate marketing claims regarding a given supplement so they can educate their clients regarding potential benefits and risks. This is not a small task given the number of new products that emerge onto the market each year, and the persuasive nature of claims, such as 'prolonged endurance', 'faster recovery', 'increase in muscle mass and strength' and 'improved joint health', all of which are extremely attractive to horse trainers and owners seeking an extra edge for their horse. Horse owners and trainers also may not be aware that, at least in some countries and jurisdictions, the use of these supplements is subject to little regulation. Despite claims often made in advertising, many supplements have not been subjected to the scientific scrutiny required for registration of an animal health remedy. Furthermore, given the size and continued growth of the supplement industry, it will always be very difficult for regulatory agencies to monitor these products effectively. This can lead to unscrupulous advertising and lack of quality control in manufacturing.

It has been argued that there are four basic steps in the development of an effective nutritional ergogenic aid[3,4]. First, to identify a physiological factor that limits some type of athletic performance. Secondly, to identify a nutritional substance or strategy that will positively affect this limiting factor, i.e. what is the potential mechanism of action? Thirdly and most importantly, there must be proof that the supplement or feeding strategy actually works, preferably including data from the target species. Specifically: (1) Is the supplement absorbed from the gastrointestinal tract? (2) Is there any evidence of uptake of the compound into the target tissue (e.g. skeletal muscle)? (3) Does the compound improve tissue function and overall athletic performance? Ideally, the scientific data will have been obtained from both laboratory and field studies. Lastly, there must be evidence that the compound, supplement or feeding strategy is safe to be fed and without harmful side effects.

Unfortunately, a large proportion of so-called performance-enhancing supplements on the market fail to meet the criteria outlined above with respect to the proof of efficacy. In particular, claims regarding mechanism of action are often unfounded and frequently there are no data on the efficacy of the supplement. When scientific data are available, it is rarely from studies performed in horses. On the other hand, it should be acknowledged that it is difficult to confirm the efficacy of an ergogenic aid[3]. Studies in horses are very expensive to perform and often lack the statistical power to detect small differences in performance that, in actual competitions, may make the difference between winning and losing. For example, when only a small number of horses are studied (e.g. $n = 6$ or less, which is the rule rather than the exception) a 1 s decrease in mean run-time in the treatment group may not be a statistically significant finding even though such an effect would be huge in terms of actual race performance. The laboratory assessment of athletic performance is also challenging, particularly when the onset of 'volitional fatigue' is used as the end point because there tends to be large within-horse variation in this measure. In addition, it can be questioned whether the results of treadmill exercise tests are applicable to the field. This includes the measurement of physiological or biochemical variables that have little or no bearing on athletic performance.

Another important consideration is whether use of a given supplement is in contravention of the rules of competition (e.g. racing authorities, FEI (Federation

Equestre Internationale)). For the most part, nutritional supplements for oral administration or consumption are not banned substances. However, positive drug tests have occurred in association with the use of some supplements (e.g. herbal preparations) used as calming agents.

Ergogenic feeding strategies

Feeding supplemental fat

Both animal fats and vegetable fats or oils have been fed to horses, although use of vegetable sources is more prevalent in part due to superior palatability. Fats or oils are generally used in equine diets to increase the energy density of the ration or to substitute for hydrolysable carbohydrates in the form of cereal grains, but fat supplementation may have other benefits such as enhanced athletic performance. Several mechanisms have been put forth as potential explanations for improved performance with adaptation to a fat-supplemented diet, including:

- An improved power-to-weight ratio due to a reduction in dry matter intake and bowel ballast[5].
- Decreased metabolic heat production associated with feeding and exercise[5].
- Enhanced stamina as a result of increased lipid oxidation and muscle glycogen sparing[6–8].
- Improved sprint performance as a result of increased energy transduction from anaerobic glycolysis, which may be due to higher muscle glycogen stores and/or altered regulation of glycogenolysis[6,9].
- Mitigation of acidaemia during high-intensity exercise[6].
- A calmer demeanour[10,11] that may be beneficial in certain disciplines, e.g. dressage.

Fat supplementation is characterized by a dose-dependent increase in the activity of lipoprotein lipase and, in some studies, an increase in the activity of skeletal muscle citrate synthase and β-hydroxy acyl-CoA dehydrogenase[8,12]. Furthermore, horses fed a diet providing *c.* 25% digestible energy from fat (as soya oil) have lower respiratory exchange ratio[7,8] and decreased glucose utilization[7] during low-intensity (~25–35% VO_{2max}) exercise when compared with the control diet. These adaptations suggest that horses adapted to a fat-supplemented diet have increased capacity for the uptake and oxidation of fatty acids in muscle during low-intensity exercise, with a concomitant decrease in use of endogenous carbohydrate stores. In human athletes adapted to a low-carbohydrate, high-fat (~65–70% energy) diet, a similar up-regulation in the capacity for oxidation of fat with decreased utilization of muscle glycogen has been observed during exercise.[13,14] Theoretically, such a glycogen-sparing effect could enhance the performance, particularly during endurance exercise in which depletion of muscle glycogen stores is one factor that can limit exercise capacity. However, it is noteworthy that 'fat adaptation' does not result in a clear enhancement of exercise capacity or performance in humans. In fact, there is evidence of an increase in the perceived effort of training and an impairment of the response to training when the high-fat, low-carbohydrate diet is maintained for periods longer than 4 weeks[14–16]. What was once viewed as a 'glycogen-sparing' effect after adaptations to a high-fat diet may actually be a reflection of a down-regulation of carbohydrate metabolism ('glycogen impairment').[16] One study of human athletes has reported that fat adaptation is associated with a reduction in the activity of skeletal muscle pyruvate dehydrogenase.[17] It was suggested that this decrease in pyruvate dehydrogenase activity could impair muscle glycogenolysis at a time when requirements for carbohydrate are high.[16,17]

Several studies have examined the effects of fat supplementation on exercise capacity or performance of horses. Some of these studies have been conducted under field conditions (e.g. a simulated race), while others have employed treadmill exercise protocols (e.g. run-time to 'volitional fatigue' during low- or high-intensity treadmill exercise)[9,18–26]. Some authors have reported improved performance[9,18,19,24–26], while others have found no change[21–23]. A number of factors could account for the variability in results between studies, including the type (e.g. animal *versus* vegetable sources) and the amount of fat supplemented, the duration of fat feeding, variation in the intensity and duration of exercise tests, the small number of horses per treatment (most often <6) and differences in the physical conditioning status of the horses.

Harkins et al.[19] reported that 14 of 15 horses ran a 1600 m simulated race faster when fed a corn oil-supplemented diet (3% DM for 3 weeks) compared with a control ration. Mean race time improved by 2.5 s (2.1%) after consuming the fat-added diet, mainly due to increased speed over the first 200 m. This study by Harkins *et al.*[19] is often cited as evidence for the ergogenic effects of fat supplementation. However, the design of this experiment was not ideal and its limitations warrant mention. First, the experiment was run in a longitudinal fashion with all horses completing the control-diet race first, with the fat-diet race undertaken after a further 3 weeks of training. It is possible, therefore, that the observed decrease in simulated race time was due to training rather than to fat (corn oil) supplementation. Secondly, the horses were fed hay in the control period but not when the oil-supplemented diet was fed. As a result, the horses received a larger percentage of digestible energy from starch, which could explain the higher muscle glycogen concentrations

reported after the period of oil supplementation. Alternatively, it is possible that the faster race time was associated with a reduction in gut weight (bowel ballast) due to decreased roughage intake.

In another study, 4 weeks of fat supplementation (12% digestible energy from corn oil) was associated with a small but statistically significant increase in run-time to fatigue in Thoroughbred horses undertaking treadmill exercise at an intensity equivalent to 120% maximum oxygen consumption (VO_{2max})[18]. Earlier studies of the effects of fat supplementation found enhanced performance during exercise protocols consisting of repeated sprints[9,24,25] or efforts during protocols that simulated exercise undertaken by cutting horses[26]. Improved work performance during high-intensity exercise was attributed to increase in resting muscle glycogen content and the rate of glycogen utilization. However, in the study by Eaton *et al.*[18], fat supplementation did not alter muscle glycogen content or glycogen utilization rate during exercise.

In summary, although there is an inconsistency in results there is some evidence that fat supplementation of horses in training may enhance the performance during exercise requiring single or repeated, high-intensity efforts. Enhancement in the capacity for fat oxidation following 'fat adaptation' may also be beneficial for endurance-type exercise. However, the adverse effects of high-fat diets on human endurance performance point to the need for well-designed studies that directly assess the effects of fat supplementation on the endurance capacity of horses.

Manipulation of carbohydrate supply

In humans, it is universally accepted that carbohydrate availability to skeletal muscle is an important determinant of exercise performance, particularly during moderate-intensity exercise lasting 1 h or more[27,28]. Low muscle glycogen content before exercise is associated with decreased performance, whereas high muscle glycogen content enhances endurance performance[27,29]. Similarly, an increase in blood glucose availability by ingestion of glucose or glucose polymers before and/or during exercise enhances the performance during prolonged moderate-intensity exercise[28]. Therefore, feeding strategies that increase pre-exercise muscle glycogen content and enhance blood glucose supply to skeletal muscle during exercise are ergogenic in human athletes. This is especially true for events lasting more than 60–90 min.

The effect of carbohydrate supply on exercise performance in horses is not well studied, but similar the situation to humans, there is evidence that glucose availability and muscle glycogen content are important determinants of performance during moderate and intense exercise. Time to exhaustion in horses running at 6–7 mph was decreased by 35% when pre-exercise muscle glycogen content was 70% lower than normal[20], while anaerobic work capacity in horses, as assessed by the run-time until fatigue during a 'supramaximal' treadmill exercise test, was decreased by *c.* 28% when muscle glycogen content was 60–70% lower relative to a control treatment[30]. Thus, similar to the human situation, low pre-exercise muscle glycogen content is associated with decreased exercise performance in horses. On the other hand, an increase in blood glucose availability has been demonstrated to enhance the performance in horses undertaking moderate-intensity exercise. In two studies of horses running on a treadmill at 50–60% VO_{2max}, the time to fatigue was increased by 14–20% when glucose availability was increased by intravenous administration of glucose (2–3 g min^{-1})[31,32]. These data have generated interest in the development of nutritional strategies for horses that optimize pre-exercise muscle glycogen content or glucose availability during exercise.

Muscle glycogen loading

In humans, the term 'glycogen loading' refers to practices that aim to maximize muscle glycogen stores prior to a competitive event in which performance is limited by the depletion of muscle glycogen stores. The original carbohydrate loading protocols, pioneered by Bergström and Hultman[29], involved a 3–4-day depletion phase of hard training and a low-carbohydrate diet, followed by a 3–4-day loading phase of high carbohydrate intake and exercise taper. This protocol resulted in more than 50% increase in glycogen content, hence the term 'muscle glycogen supercompensation'. More recent studies have shown that well-trained athletes are able to achieve similar muscle glycogen supercompensation without the need to undertake a glycogen-depletion phase (see Kiens[33] for review). Nowadays, the more practiced method for glycogen loading in human athletes involves 3 days of exercise taper combined with a high carbohydrate intake (7–10 g kg^{-1} bwt day^{-1})[34].

Research in horses has indicated that only modest (~10%) increase in muscle glycogen content can be achieved through dietary manipulation[20–22]. For example, Essén-Gustavsson *et al.*[21] reported an *c.* 12% increase in the resting muscle glycogen content of Standardbred horses fed a diet that provided *c.* 2 kg day^{-1} starch and sugar when compared to an isocaloric diet that provided about 1.3 kg day^{-1} hydrolysable carbohydrate. An increase in muscle glycogen content of this magnitude was not associated with improved performance during moderate-intensity exercise[20–22]. These observations notwithstanding, a number of glycogen or 'carbo' loader products are marketed for use in athletic horses.

The rate of post-exercise muscle glycogen replenishment is much slower in horses when compared to

humans and other species. In humans, the ingestion of carbohydrate at a rate of 0.7–1.0 g kg^{-1} bwt every 2 h results in muscle glycogen synthetic rates of 5–8 mmol kg^{-1} h^{-1} during the 6–12-h period after glycogen-depleting exercise, and complete glycogen replenishment is achieved within 24 h[34]. By comparison, when carbohydrate is administered orally the maximum rate of muscle glycogen synthesis in horses is *c.* 1–2 mmol kg^{-1} h^{-1}, and as much as 48–72 h are required for complete replenishment[35–37]. Following exercise that depletes muscle glycogen content (middle gluteal muscle) by greater than 50–60%, complete glycogen replenishment is achieved by 24 h when glucose (6 g kg^{-1}) is administered intravenously[30,38]. In contrast, oral administration of a glucose polymer (3 g kg^{-1} bwt) within 60 min of the completion of glycogen-depleting exercise does not accelerate glycogen replenishment[39]. However, more frequent administration of an oral glucose polymer or the feeding of meals with high hydrolysable carbohydrate content (grain) every 6–8 h results in a modest acceleration in muscle glycogen replenishment[36]. The horse appears to have a limited capacity for the digestion of hydrolysable carbohydrates, and this may limit systemic glucose availability, thereby restraining the rate of muscle glycogen resynthesis. It is noteworthy that in humans a daily carbohydrate intake of 7–10 g kg^{-1} bwt is required for a substantial increase in muscle glycogen content[34]. For a 500 kg horse, an equivalent carbohydrate dose would require the consumption of 7–10 kg oats (~50% starch) per day. Such a high grain (starch) intake is not realistic for horses, nor recommended given the possible risk of gastrointestinal dysfunction associated with high starch intake.

The performance implications of the slow rate of glycogen replenishment in horses are probably dependent on the type of athletic activity. In a study of Thoroughbreds fed a hay and grain concentrate diet, the muscle glycogen loss sustained during training gallops (19–25% decrease) was fully restored within 2–3 days regardless of whether hay alone or hay and grain was fed[40]. Therefore, in situations where intense exercise bouts occur at 3-day intervals, as is typical in the conventional training of Thoroughbred racehorses, muscle glycogen content can be well maintained. However, for horses competing in multi-day events (e.g. 3-day event) or multiple heats on a single day (e.g. Standardbred racehorses), it is possible that inadequate glycogen replenishment adversely affects subsequent exercise performance. For these horses, it has been suggested that small grain meals (e.g. 1–1.5 kg for a 500 kg horse) be fed at frequent intervals (e.g. every 3 h) during the first 12 h recovery[36]. However, the efficacy of this strategy has not been determined.

Enhancement of blood glucose supply during exercise

Blood glucose availability during exercise may be increased by the pre-exercise feeding of a high glycaemic meal or the intragastric administration of a glucose or glucose polymer solution. For endurance horses, similar strategies could be applied at rest stops during races. However, there is some controversy regarding the merits of these pre- (or mid-) feeding strategies. It has been argued that the suppression in lipid oxidation associated with pre-exercise carbohydrate ingestion may be detrimental to performance because an accelerated rate of carbohydrate oxidation will result in premature depletion of endogenous carbohydrate stores. Certainly, there is evidence that the hyperglycaemia and hyperinsulinaemia consequent to grain ingestion or intragastric glucose administration alter substrate selection during moderate–intensity exercise. The consumption of a grain meal (~2 kg corn) 2 h before exercise[41] or the oral administration of glucose (2 g kg^{-1} bwt) 1 h pre-exercise[42] increases the rate of blood-borne glucose utilization and the rate of whole-body carbohydrate oxidation in horses during treadmill exercise at 50–55% VO_{2max}. Conversely, the rate of whole body lipid oxidation is suppressed under these conditions, most probably the result of an insulin-induced suppression in lipolysis[41,42].

It must be emphasized that the effects of pre-exercise carbohydrate ingestion (grain meals or glucose solutions) on exercise performance have not been determined in horses. Although speculative, it is possible that the pre-exercise ingestion of hydrolysable carbohydrate is beneficial for events requiring moderate and intense exercise (e.g. cross-country of a 3-day event, show jumping). However, for more prolonged, lower-intensity exercise, such as that required of the endurance horse, the suppression in lipid oxidation associated with pre-exercise carbohydrate ingestion may be detrimental to performance. Therefore, for endurance events it is recommended that no grain be fed in the 3-h period before competition exercise. Further research is required to determine the metabolic and performance effects of various pre-event (and mid-event) feeding strategies.

Reduction in bodyweight

The amount of energy required for running is dependent on running speed and the weight being moved (e.g. horse and rider). Therefore, a change in bodyweight will alter the energy cost of locomotion, which could affect athletic performance. The horse's large intestine contains a large mass of fluid and ingesta (8–12% bodyweight), the size of which is influenced by dietary fibre (roughage) intake. High-fibre diets increase the mass of ingesta in the large

intestine due to increased water consumption (*c.* 3–4 l water kg^{-1} fibre) and the ability of fibre to bind water. Anecdotally, many racehorse trainers limit hay intake in the day leading up to races or, alternatively, eliminate hay from the diet and instead feed a 'complete' ration that provides a limited amount of fibre. There is some evidence that a short-term reduction in forage intake alters bodyweight and metabolism during high-intensity exercise. When compared to *ad libitum* hay consumption, restricting hay intake to ~1% bodyweight for a 3-day period before a treadmill exercise test (2 min at 115% VO_{2max}) resulted in a 2% decrease in the bodyweight and a reduction in anaerobic energy expenditure during exercise, as evidenced by reduced oxygen deficit and plasma lactate concentrations[43]. Notably, the observed reduction in bodyweight, which was attributed to a decrease in gut fill[43], was similar to that seen after administration of the diuretic furosemide (1 mg kg^{-1} bodyweight IV)[3]. Given that weight reduction is thought to underlie the ergogenic effect of furosemide during racing[3], it is possible that a decrease in bodyweight via short-term reduction in dietary fibre intake could also confer a performance advantage. However, this hypothesis has not been tested. Furthermore, the practical implications of the findings of Rice *et al.*[43] are uncertain given that many racehorses do not consume more than 1% bodyweight as roughage. A more drastic reduction in roughage intake (e.g. <0.75% bodyweight) is not recommended because low-fibre diets may predispose horses to gastrointestinal dysfunction (e.g. gastric ulcers, colic).

Nutritional Ergogenic Supplements

Creatine

Creatine (methylguanidine-acetic acid) is a compound derived from amino acids that is stored primarily in skeletal muscle at typical concentrations of 100–150 mmol kg^{-1} dry weight (dw) of muscle. About 60–65% of this creatine is phosphorylated. Creatine phosphate (CP) provides a rapid but brief source of phosphate for the resynthesis of ATP during intense exercise and, therefore, helps to maintain normal ATP/ADP homeostasis. Other functions of CP metabolism include the buffering of hydrogen ions produced during anaerobic glycolysis[44]. As both ADP and hydrogen ion accumulation are factors that may contribute to the development of fatigue during sprint exercise, the size of the skeletal muscle CP store may be an important determinant of performance during high-intensity exercise. Therefore, nutritional manipulations leading to increase in total muscle creatine and CP might be expected to have an ergogenic effect during intense exercise. The use of creatine supplements by human athletes is widespread; according to one estimate, 80% of athletes competing in the 1996 Olympic Games were using a creatine supplement[45]. There is support for an ergogenic effect of creatine supplementation in human athletes engaged in repeated sprints, probably related to an increase in the rate of CP resynthesis during recovery between bouts of exercise. However, oral creatine supplementation is not considered ergogenic for single- or first-bout sprints, or for prolonged, submaximal exercise. The evidence is inconclusive for the effects on muscle strength, although creatine supplements are widely used by bodybuilders and weightlifters[44].

Studies in man have demonstrated that a daily creatine dose of 20–25 g (~250 mg kg^{-1} bwt day^{-1}), divided into four doses, results in increased muscle creatine concentrations, with an apparent upper threshold of 150–160 mmol kg^{-1} dw. About 20% of the increased muscle creatine content is stored as CP, and saturation occurs 2–3 days after the start of supplementation[46]. The increase in muscle creatine content is greatest in those subjects with a low initial concentration. Lower daily doses of 3 g day^{-1} (~40–44 mg kg^{-1} bwt) resulted in a slower increase in muscle creatine content over a 14–28-day period, and elevated muscle creatine stores can be maintained by continued daily supplementation of 2–3 g creatine[44]. Creatine is transported into muscle against a high concentration gradient, via saturable transport processes that are stimulated by exercise and by insulin[47].

There have been two published reports of oral creatine supplementation in horses[48,49]. Sewell and Harris[48] demonstrated that, in contrast to man and dog, creatine is poorly absorbed in the horse. The intragastric administration of 50 mg creatine per kg bwt resulted in an increase in plasma creatine concentration from 40 to 100 fmol l^{-1} after 4–6 h. By comparison, the same dose in humans results in plasma concentrations of 800–1000 fmol l^{-2}. Furthermore, the administration of creatine at 150 mg kg^{-1} day^{-1} (divided into three doses) for 13 days had no effect on muscle creatine content[48]. In a randomized, crossover design, Schuback *et al.*[49] fed Standardbred trotters 25 g creatine monohydrate twice daily (total daily dose of ~100–120 mg kg^{-1} bwt) for 14 days. Before and after the period of supplementation, horses completed an incremental treadmill exercise test until exhaustion. There was no significant effect of supplementation on plasma or muscle creatine concentrations, or an effect on treadmill run-time and the muscle metabolic response to exercise[49]. Thus, creatine supplementation in the horse at dosages shown to be effective in man has failed to result in an increase in muscle creatine content. Without a change in muscle creatine content, creatine supplementation is unlikely to exert an ergogenic effect in horses. The reason for the apparent low bioavailability of orally administered creatine in horses has not been

determined. It is possible that the horse's gastrointestinal tract is not well adapted to the absorption of creatine.

L-Carnitine

L-Carnitine is a component of the enzymes carnitine-palmityltransferase I, carnitine-palmityltransferase II and carnitine-acylcarnitine translocase that are involved in the transport of long-chain fatty acids across the inner mitochondrial membrane[50]. As such, long-chain fatty acid oxidation is carnitine dependent, and, therefore, it has been proposed that increased availability of L-carnitine by supplementary ingestion might up-regulate the capacity to transport fatty acids into the mitochondria and increase fatty acid oxidation[2]. This augmentation in fat oxidation could be of benefit during endurance exercise. Another role of carnitine is to act as a 'sink' for acetyl-CoA units produced during high-intensity exercise. The conversion of acetyl-CoA to acetyl-carnitine maintains CoA availability and decreases the ratio of acetyl-CoA:CoA. As such, an increase in carnitine availability could enhance substrate flux through the citric acid cycle and increase the activity of pyruvate dehydrogenase, which is otherwise inhibited by high levels of acetyl-CoA. These mechanisms would serve to increase oxidative metabolism of glucose, decrease lactate production and perhaps enhance the performance during exercise tasks that might be limited by excess hydrogen ion and lactate accumulation[2]. However, the weight of evidence from human studies indicates that oral carnitine supplementation has no effect on muscle carnitine concentration. In addition, there is no evidence that muscle carnitine content limits fat oxidation other than in patients with inborn errors in metabolism that result in inadequate muscle carnitine. This is also likely to be the case in horses as the carnitine content of equine skeletal muscle is two- to threefold higher when compared with human muscle[51]. Supplementation studies in humans have failed to demonstrate an effect of carnitine on measures of fat oxidation or muscle metabolism during exercise[2,50].

Studies in man[52] and horses[53] have demonstrated that the oral bioavailability of L-carnitine is poor. In horses, large oral doses of L-carnitine (10–60 g) are required to effect an approximate doubling in plasma carnitine concentration[51]. Importantly, supplementation at these levels for 58 days had no effect on muscle carnitine content. In a subsequent study, intravenous doses of 10 g L-carnitine were administered daily for 26 days[54]. These infusions resulted in peak plasma carnitine concentrations 30-fold higher when compared to pre-injection values, and concentrations remained threefold higher after 6 h. Yet, there was no change in muscle carnitine content. Thus from the available data, there is no compelling evidence that muscle carnitine content is enhanced as a result of oral or IV L-carnitine supplementation.

Although the weight of evidence suggests that L-carnitine is unlikely to be ergogenic in horses, a recent study has provided evidence that supplementation during conditioning may augment training-associated skeletal muscle adaptations[55]. In a group of 2-year-old Standardbred horses subjected to a 10-week conditioning programme, supplementation with L-carnitine ($10\,g\,day^{-1}\,os^{-1}$) was associated with significant increases in the percentage of type IIA muscle fibres and the intensity of periodic acid Schiff staining (an indicator of intrafibre glycogen content) when compared with untreated control horses[55]. Further studies are required to evaluate the performance effects of these apparent carnitine-induced muscular adaptations. However, a recent study by the same group[56] demonstrated that the identical L-carnitine supplementation protocol ($10\,g\,day^{-1}$) did not affect heart rate and blood lactate responses to submaximal exercise in Standardbred horses.

Amino Acids

Amino acid or 'refined' protein supplements are often touted for their ability to 'build' muscle mass or, in the case of the branched-chain amino acids (leucine, isoleucine and valine), enhance endurance performance by modifying factors that contribute to central fatigue[57,58]. In addition, it has been proposed that branched-chain amino acid (BCAA) supplementation during exercise may provide carbon intermediates for the citric acid cycle at a time when endogenous carbohydrate reserves are depleted, thereby delaying the onset of fatigue[2]. The 'central fatigue' hypothesis proposes that increased brain serotonin contributes to fatigue development during prolonged moderate-intensity exercise[57]. The increase in brain serotonin synthesis occurs as a result of increased transport of free (unbound) tryptophan transfer across the blood–brain barrier. Key to this increase in tryptophan uptake is an increase in the plasma ratio of free tryptophan to BCAA. This ratio may increase for two reasons. First, as blood-free fatty acid (FFA) concentration rises during exercise, the FFA compete with tryptophan for binding sites on albumin, and the FFA displace some of the tryptophan molecules from albumin; therefore, free tryptophan concentration increases. Secondly, an increase in the oxidation of BCAA in muscle results in a decrease in blood BCAA concentration. As BCAA and tryptophan compete for carrier-mediated entry into the central nervous system, an increase in the free tryptophan to BCAA ratio leads to increased tryptophan transport. Therefore, it has been theorized that BCAA supplementation could reduce the exercise-induced increase in brain tryptophan uptake and thus delay fatigue[57,58]. Interestingly, when horses were

infused with tryptophan (100 mg kg^{-1}, IV) during submaximal exercise, run-time to fatigue was decreased by ~15% relative to the placebo treatment, providing evidence that an increase in circulating tryptophan adversely affects endurance performance in horses[32]. However, the oral ingestion of a tryptophan solution that markedly increased plasma-free tryptophan concentration and estimated brain tryptophan uptake had no effect on time to exhaustion in exercising humans[59]. Furthermore, in this and other studies, the ingestion of large quantities of BCAA also had no effect on endurance performance[2,59]. These data cast some doubt on the 'central fatigue' hypothesis and suggest that BCAA supplementation is not ergogenic during dynamic exercise in humans.

BCAA supplements are marketed for use in horses, but there are no published data regarding the effects of BCAA supplementation on exercise performance *per se*. Glade[60] reported that BCAA supplementation mitigated the increase in blood lactate during exercise. However, the exercise test involved treadmill walking and the applicability of these results to equine athletic activities is questionable. More recent studies have failed to demonstrate a beneficial effect of BCAA supplementation in horses. The administration of a mixture of L-leucine (9 g), isoleucine (4.5 g) and L-valine (9 g) to Standardbreds 1 h before training had no measurable effect on energy metabolism during intense exercise[61]. Similarly, there were no changes in plasma biochemical variables during and after exercise in horses fed BCAA three times per week for 5 weeks[62].

Several studies in man have demonstrated that increased amino acid availability early in the post-exercise period modifies protein metabolism in skeletal muscle[63]. Specifically, hyperaminoacidaemia resulting from ingestion or intravenous infusion of amino acids increases post-exercise muscle protein synthetic rate and prevents the exercise-induced increase in protein degradation. Thus, post-exercise amino acid or protein supplementation may promote anabolism in skeletal muscle during conditioning. Neither the effects of exercise in muscle protein metabolism nor the effect of post-exercise amino acid or protein supplementation on these processes has been investigated in horses.

Miscellaneous

A number of other substances are marketed on the basis of a performance-enhancing effect. These include Q10, *N*,*N*-dimethylglycine (DMG), trimethylglycine (TMG) (the precursor of DMG), γ-oryzanol and β-hydroxy-β-methylbutyrate (HMB). There is also an interest in the effects of antioxidant supplementation, particularly for horses undertaking endurance exercise.[Q2]

Q10 is touted as enhancing oxidative metabolism in skeletal muscle because of its known role in mitochondrial electron transport. However, in any species there is no evidence that Q10 availability is limiting for oxidative metabolism, and certainly no studies in horses demonstrating uptake from the gut, accumulation in muscle or an effect on athletic performance. Both DMG and TMG are purported to decrease the accumulation of lactate in muscle via activation of pyruvate dehydrogenase. Such an effect could be beneficial during high-intensity exercise because the accumulation of lactate and associated protons (intracellular acidosis) is one potential mechanism of fatigue. However, studies in horses have shown that neither DMG[64] nor TMG[65] alters lactate accumulation or performance during exercise. Γ-Oryzanol (GO) is a mixture of ferulic acid esters and triterpene alcohols that is extracted from rice bran. For a time, supplementation with GO was popular among bodybuilders and similar 'muscle building' claims have been made in literature marketing equine GO supplements. To the author's knowledge, there is no evidence in any species that supplementation with GO increases muscle mass or strength, or any other aspect of physical performance. HMB is a metabolite of the amino acid leucine that has been implicated in the regulation of protein synthesis and/or breakdown in muscle. Thus, similar to GO, there are claims that supplementation with HMB exerts a positive effect on lean muscle mass and/or muscular performance[2]. Presently, there are no published studies on the effects of HMB supplementation in horses.

Slow-acting, disease-modifying osteoarthritis agents

In human and veterinary medicine, there is widespread use of nutritional supplements for treatment and/or prevention of OA, the so-called 'joint supplements'. Their popularity is understandable given that lameness is the most significant cause for the loss of use in horses, with joint disease (particularly OA) accounting for up to 60% of lameness problems[66]. Most oral joint supplements (SADMOAs) contain CS and/or glucosamine HCl along with other ingredients, such as manganese ascorbate, green-lipped mussel (*Perna canaliculus*), methyl sulphonylmethane, hyaluronan or pentosan polysulphate. Glucosamine is an amino monosaccharide that is a precursor of glycosaminoglycan (GAG) components, including hyaluronan and keratan sulphate, while CS is a GAG consisting of alternating disaccharide subunits of glucuronic acid and N-acetylgalactosamine.

The main premise for use of these supplements is that they are 'chondroprotective', supplying 'building blocks' for articular cartilage that are effective in delaying, stabilizing or even repairing OA lesions[67]. A number of *in vitro* studies have examined the effect of glucosamine and/or CS on the catabolic response

of cartilage explants[67–71], and the reader is referred elsewhere for a comprehensive review of these data[72]. In brief, there is some evidence that glucosamine and CS limit GAG degradation and enhance GAG synthesis in cartilage explants incubated with lipopolysaccharide (LPS) or preconditioned with interleukin-1, the end result being an increase in total GAG content when compared to placebo-treated explants[72]. Glucosamine also inhibited the release of nitric oxide and prostaglandin E2 from explants incubated with LPS, suggesting it may exert anti-inflammatory effects in this model. Collectively, the results of these studies suggest that glucosamine and CS could be beneficial to articular cartilage metabolism by preventing GAG degradation and/or enhancing GAG synthesis. However, these data cannot be taken as proof of efficacy in the treatment or prevention of OA in horses.

Questions remain regarding the bioavailability of orally administered glucosamine and CS in horses, as well as the concentrations achieved in target tissues (i.e. synovial fluid or articular cartilage) after oral dosing. The oral bioavailability of low-molecular weight CS products has been examined in horses. The mean bioavailability of 8.0 and 16.9 kDa forms of CS was 32 and 22%, respectively[73]. However, the oral bioavailability of glucosamine HCl was lower at 2.5%, perhaps due to poor intestinal absorption and/or extensive first-pass metabolism[72]. After oral administration of 20 mg kg^{-1} glucosamine HCl, maximum serum and synovial fluid concentrations were 5.8 ± 1.7 and 0.3–0.7 μM, with glucosamine still detectable in synovial fluid up to 12 h after dosing. Corresponding peak serum and synovial fluid concentrations after IV administration of the same dose were 288 ± 53 and 250 μM. The glucosamine concentration achieved in synovial fluid after oral administration was markedly lower when compared to concentrations used in the aforementioned *in vitro* studies, casting doubt on the relevance of these data[72].

A number of *in vivo* studies have been performed in horses to examine the efficacy of glucosamine and/or CS for the treatment of joint disease (see McIlraith, 2004[72] and Keil et al., 2005[71] for review). Although some studies have reported positive effects of supplementation on, for example, lameness, these data must be viewed with caution due to flaws in experimental design, for example, lack of controls, no blinding of investigators. Thus, there is a need for appropriately designed clinical trials. Long-term studies are also needed to address the efficacy of these agents for the prevention of OA, which appears to be the basis for widespread use of oral joint supplements in horses. Variability in product quality, a potential problem with all nutritional supplements, is another concern. Studies of oral SADMOA products intended for human or animal use have demonstrated that few consistently meet label claims of guaranteed analysis. In one study of equine products, actual composition in comparison to label claims ranged from 63 to 112% for five glucosamine products and 22 to 155% for five CS products[74].

Conclusion

The use of nutritional supplements alleged to boost performance or health in horses is widespread. However, for most, if not all, of these supplements there is little or no scientific evidence of efficacy in horses. Also, there are only limited data on the effects of manipulations in the macronutrient composition of the diet on exercise capacity and performance. The key word in 'nutrition supplement' is 'nutrition'; the addition of a supplement to a horse's ration may be justifiable on nutritional grounds, but rarely on the basis of improved athletic performance.

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