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SPECIAL ISSUE*: Critical issues for equine performance*

Nutraceuticals: a goldmine but for whom?

K.H. McKeever

Equine Science Center, Department of Animal Sciences, Rutgers-The State University of New Jersey, 84 Lipman Drive, New Brunswick, NJ 08901, USA; mckeever@aesop.rutgers.edu

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OPINION ARTICLE

Abstract

A whole new industry has grown up around dietary supplements that purportedly can, enhance exercise performance or enhance the recovery from exercise. Many of these new supplements are 'functional foods' or nutraceuticals that have active molecules or ingredients that purportedly can reduce inflammation, prevent oxidative stress or have other benefits for whatever may ails an equine athlete. The blitz of advertising that usually accompanies such miracle ergogenic (i.e. performance enhancing) products suggests that a great deal of scientific research has been published to support those claims. Unfortunately, the majority of new dietary supplements are being promoted with little or no scientific basis for the claims made on their labels or in the advertisements touting their benefits. In many cases, no research has been performed to demonstrate efficacy of these new expensive, avant-garde, dietary supplements in the horse. So how can we determine if a new supplement improves recovery from exercise or has potential to improve athletic capacity? The purpose of this review is to outline the important questions a horse owner, trainer, veterinarian, or regulator should ask before deciding it is ok (safety, legality) or even beneficial to feed a supplement to an athletic horse.

Keywords: dietary supplements, nutraceuticals, horses

In the United States dietary supplements are regulated by the Food and Drug Administration (FDA) under the Federal Food, Drug, and Cosmetic Act (US-FDA, 2017a). That act defines 'a dietary ingredient as a vitamin, mineral, herb or other botanical, amino acid, dietary substance for use by man to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent, extract, or combination of the preceding substances' (US-FDA, 2017b). The increased production and use of dietary supplements for humans has resulted in further regulation under the Dietary Supplement Health and Education Act (DSHEA) that was passed by the US Congress in 1994. According to the FDA, 'supplements are not intended to treat, diagnose, prevent, or cure diseases and manufacturers cannot market as such.' (US-FDA, 2017a). Under the current regulations the manufacturer is responsible for determining if a supplement is safe and properly labelled as far as its intended use (US-FDA, 2017a). The FDA only steps in if the supplement is adulterated or unsafe or if the manufacturer has mislabelled the product and made unsupported claims in the marketing of the supplement (US-FDA, 2017a). Similar rules and regulations, such as the 'Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements' (EC, 2002) have been promulgated in the European Union to guide the manufacture and distribution of dietary supplements.

Since the passage of the DSHEA the dietary supplement field has seen the research and development of functional foods that have the capacity to alter biochemical pathways and physiological function in ways that are similar to synthetic pharmaceutical agents (McKeever, 2005, 2011). In many cases those functional foods are refined further through the purifying of extracts from the original plant that contain the bioactive molecules. This purportedly enhances the bioavailability and efficacy of the final 'dietary supplement.' However, this has moved the supplement industry into a 'grey zone' where specific plant derived bioactive molecules are extracted and purified for their potent biochemical

and physiological actions (McKeever, 2011, 2014). These extracts, while still classified as dietary supplements, have biological effects more like pharmaceuticals, and thus some have termed them 'nutraceuticals' (McKeever, 2011, 2014). For example, resveratrol, quercetin, curcumin and other flavanols are the key active ingredients that researchers have identified in many functional foods (McKeever, 2011, 2014). They have been isolated and are administered alone or in combination with other potentially beneficial extracts or active molecules that purport to have beneficial effects (McKeever, 2011, 2014). They may be safe and they may work; however, testing requirements are minimal under the current regulations in the United States. The guidelines posted on the FDA Center for Veterinary Medicine website (https://www.fda.gov/animalveterinary) state that 'the ultimate responsibility for the production of safe and effective animal feed products lies with the manufacturers and distributors of the products'. Thus a horse owner or clinician can only use the word of the manufacturer/marketer as far as the reliability of statements of safety and efficacy.

The use of supplements in the horse world has paralleled the explosion of products being used by humans (McKeever, 2011, 2014). The list of dietary supplements, functional foods, and nutraceuticals that are on the market grows every day and one just has to open the latest lay publication to find a new nutraceutical that is being marketed to improve recovery, calm the fractious horse, or improve athletic performance. Manufacturers and distributors of supplements and nutraceuticals have a vested interest in selling product and they do not have to conduct extensive research to back up their claims. Some supplements for horses are marketed with claims derived from sound scientific studies. Unfortunately, many of the products that are currently being marketed in the United States have no sound science to back up the claims made on the label. In the United Kingdom there is another dilemma. Supplements are regulated by the Veterinary Medicines Directorate and if an experiment shows that a nutraceutical has efficacy, for example it reduces inflammation or reduces respiratory symptoms, then the marketers are (1) not permitted to show that data; and (2) are not allowed to make claims in relation to whether the supplement 'reduces, improves, treats, etc.' any clinical condition (David Marlin, personal communication). Thus, what is a horse owner, trainer, or veterinarian to do to sort through the multitude of dietary supplements on the market? We cannot review all of the scientific literature here; therefore, the purpose of the present editorial review is to present key concepts and questions to consider when one reviews a published study on a dietary supplement or nutraceutical.

Care must be taken by owners, trainers, and veterinarians when administering a supplement to a horse. One should rigorously question the claims made by supplement marketers and manufacturers as it is a buyer beware situation. In his article, Robertson (1991) suggested that when considering using a dietary supplement, human athletes should ask three important initial questions: 'Is the product safe? Is it legal? Is it effective?' Those same questions should be asked by all equine nutritionists, horse owners, veterinarians, and trainers before considering the use of a dietary supplement or nutraceuticals in equine athletes.

Unfortunately, most of the dietary supplements administered to horses have not been tested for safety in controlled trials. Minimally, acute safety studies should be as part of the initial pilot experiments conduct before full experiments. If there are no adverse reactions, then the next step is to determine if there are any toxicity problems associated with chronic use of the supplement. A similar lack of research exists with most of the supplements market for use by humans. For example, ephedra was used as a weight loss supplement and in various energy products for a number of years. A senior student of mine took some of the supplements containing ephedra to aid in his body building efforts and consequentially experienced heart palpitations. His experience led to a study we conducted using horses that documented that ephedra administration did not improve performance, impaired thermoregulation, and caused a dramatic increase in blood pressure (Norton *et al.*, 2010). About, that time there were a number documented cases of cardiovascular complications associated with ephedra supplementation and a number of well publicised deaths of athletes in the United States. This led to a ban on the production of ephedra as a dietary supplement in the United States in 1994 (Norton *et al.*, 2010). However, supplements containing ephedra alkaloids imported from outside the US are still available for human consumption through internet sales from other countries. I mention our study because the US-FDA ban was on ephedra and there are some supplements containing ephedrine that are being sold via the internet and through compounding pharmacies to treat airway problems in horses.

As far as the question of legality, even though some supplements are derived from 'natural' ingredients, they may contain active ingredients that have ergogenic properties similar to drugs. Thus, in the opinion of many regulatory agencies, any nutraceutical shown to enhance performance should be banned for use in athletic horses during competition. For example, 'EPO Equine' is a product on the market that is derived from the *Echinacea* plant. The manufacturer claims it is a 'natural way' to enhance the production of red blood cells through an increase in erythropoietin. However, in the study used as a basis for the marketing claims the investigators did not measure erythropoietin or absolute red blood cell volume (O'Neill

et al., 2002). Thus, there is a questionable marketing claim for the supplement. More importantly, the purported increase in EPO claimed by the manufacturer of this supplement places it in the same category as other blood doping agents, like recombinant human erythropoietin and cobalt salts. Using it could be considered a violation under 'ARCI-011-015 Prohibited Practices, section #3' in the model rules recommended by the Association of Racing Commissioners International (ARCI, 2017). The Federation Equestrian International also has very stringent guidelines and warnings regarding herbal supplements, as well as contamination of feeds with such ingredients (FEI, 2014). Both the ARCI and FEI have published threshold recommendations for plasma total carbon dioxide (tCO_2) concentration to prevent the administration of alkalinizing agents (ARCI, 2017; FEI, 2014). Unfortunately, many pelleted feeds and other supplements contain ingredients that can raise plasma [tCO_2]. The bottom line is that it is imperative that all trainers, owners, and veterinarians read the labels of all supplements and feeds and consult the lists of supplements that have the potential to cause a violation.

If a supplement is safe and legal, the question most horse owners want answered is whether it works. Does it enhance recovery? Does it prevent joint problems or inflammation? Does the supplement prevent oxidative stress and damage? Those are some of the 'million dollar' questions asked by horse owners, trainers, and veterinarians.

The rationale for giving a supplement to a horse requires researchers as well as horse owners to ask and answer a series of questions. The first question one needs to ask is if there is a sound biochemical or physiological basis for the claims made by the researchers or marketers of a supplement? There are many supplements and products on the market that have purported benefits that have no support in the scientific literature. It is an egregious error for the author of a scientific article or marketer of a supplement to make speculative claims that are counter to well established biochemical or physiological principles based upon scientific data published in peer reviewed research literature. Serendipity is not science and paradoxical findings that are not consistent with real physiological mechanisms should be viewed as theories based on unfounded speculation. Claims based on the latter, or excessive cure-all claims, should be red flags when interpreting the assertions made by unscrupulous marketers of wishful thinking. For example, there are a number of products on the market that ignore or erroneously market supplements to benefit anaerobic metabolism when the actions of the supplement are really affecting aerobic pathways.

So how do you design an experiment if there are no well-designed horse experiments used to establish the rationale for the experiment? First, one must look at the *in vitro* literature and sound peer reviewed studies from other species that suggest that the product may have an appropriate effect when used in an athletic horse. However, when reading papers from rodents, humans, cell culture, etc., one should play the 'devil's advocate' and consider whether the horse utilises the substance in the same way as other species. There are many similarities between all mammalian species. But there are also important species related differences that one should consider when formulating the rationale behind testing a nutraceutical in the horse. For example, the gastrointestinal system of the horse is different from other monogastrics, like humans, rodents, and pigs. The horse has a substantial caecum and colon, where the microbiome population utilises fibre and other nutrients to produce volatile fatty acids and other metabolic substrates (Janabi *et al.,* 2016, 2017). The microbiome in the hindgut of the horse can exert a significant effect on any supplement, a fact that must be considered when relying on studies of rodents and humans. If there is a sound rationale from *in vitro* work and from studies involving of other animals, then one should conduct pilot work to determine if a nutraceutical is safe to give to a horse.

If one is going to give a supplement to a horse one should also determine if the active molecules of the supplement actually get into the blood stream or into target tissues, such as muscle or synovial fluid. Too often, papers in the literature offer no evidence that a nutraceutical or its active ingredients are absorbed from the gastrointestinal into the blood stream. This important information should be obtained in pilot work, if there is no information in the literature on the uptake and distribution of the nutraceutical. That does not mean that pilot work cannot be done. For example, we were fortunate several years ago to obtain funding from United States Department of Defence to use the horse as an intermediate animal model to examine the effects of food extracts on performance- and inflammation markers (Baldassari *et al.*, 2012; Lehnhard *et al.*, 2011; Liburt *et al.*, 2009, 2010; Streltsova *et al.,* 2006). Previous efforts at the Center for Advanced Food Technology at Rutgers University had identified more than a dozen food extracts that reduced inflammatory cytokine production in cell culture and in a rodent ear inflammation model. We were tasked to determine whether four of the most promising extracts – black tea, orange peel, cranberry, and ginger – were safe and efficacious in an animal model that could perform intense exercise. The ultimate objective was to determine if these extracts could be used as an alternative to using non-steroidal anti-inflammatory agents to fight delayed onset muscle soreness (Baldassari *et al.*, 2012; Lehnhard *et al.*, 2011; Liburt *et al.*, 2009, 2010; Smarsh *et al.*, 2010; Streltsova *et al.,* 2006).

For our pilot work we asked key questions before we started our larger experiments; questions that should be asked by anyone interested administering any supplement to a horse (Liburt *et al.*, 2009; Streltsova *et al.,* 2006). Is the extract safe in the horse even though it is generally regarded as safe (GRAS) for administration to humans? Were the bioactive molecules or ingredient(s) in the extracts (polymethyflavones, phenolic compounds, gallic acid, etc.) bioavailable? When did their concentrations peak in the blood so we could time our exercise tests? Later studies of cranberry and quercetin required confirmation that the concentration of the flavanols, like quercetin, could be measured in the muscle as well as the blood. All of those experiments required pilot studies where we collected safety data and blood, urine, and muscle biopsy samples. Unfortunately, most *in vivo* studies of supplements and nutraceuticals administered to horses are not provided enough funds to conduct critical pilot studies or to make the measurements during the actual experiment and to confirm that the active molecules are absorbed into the bloodstream and actually reach the tissue of interest.

Even if the active molecules in a supplement are actually absorbed, one also must consider if it is altered in the liver in a way that enhances its bioactivity. For example, debate exists over which form of the quercetin molecule has the greatest effect as an anti-inflammatory agent. Some studies suggest the glycosylated molecule and others the non-glycosylated molecule. Nevertheless, one should confirm that a nutraceutical or its key components are absorbed and bioavailable.

The efficacy of a nutraceutical needs to be tested using properly designed studies that are the designed, analysed, and interpreted using a hypothesis driven approach. Proof that a nutraceutical has an effect requires a statistically testable hypothesis that guides the choice of treatment groups and overall design of the study. Too many of the papers I review do not state a testable hypothesis and one can see in the presentation that the design and statistical analyses have errors that planning could have prevented.

A properly planned experimental design should include proper controls, something far too many experiments involving horses fail to incorporate. A parallel control group costs more, but in my opinion it is essential, especially if there is an extended period of nutraceutical administration. Even though it costs more, when designing a robust experiment, one should include two pre-exercise time points to document that the parameters measured were stable prior to the test. When possible, a randomised crossover design with a proper washout period between crossovers should be used to enhance the statistical power of the experiment. It also is important that the investigators be blind to the treatment to prevent bias in the analysis of the data. Finally, it is imperative that all of the experimental design, the choice of statistics, and the setting of the alpha level for the rejection of the null hypothesis should be done *a priori.*

A well designed experiment should also have an *a priori* determination of how many animals are needed to have the statistical power to detect a difference in key variables. One can increase the statistical power by increasing animal numbers; however, funding limitations many times limits the number of horses that can be used in an experiment. The calculation of statistical power also uses the experimental error or variation and another way to enhance statistical power is to determine and control all of the possible sources of experimental error that can reasonably be controlled.

Reviewing an article to see if there is proof that a nutraceutical works goes beyond the above mentioned considerations. One should check to see if the methods acceptable, accurate, and repeatable. The method section of any nutraceutical paper should provide enough details to allow another investigator to repeat the study and get reasonably similar results. Upon review the paper should tell the reader details about the horses. Were they similar ages, breed, weight and body condition? Were the horses fit or unconditioned and what physiological markers were used to document that the horses were in the same state of fitness? Did the horses have the same ration, access to water and salt and mineral. If horses were given hay, was the hay from the same source and lot? Importantly, was a sample analysed for nutrient composition? One should confirm the composition of the supplement given to the horses. Housing is another important variable to control and should be detailed for the reader. An adaptation period of at least two weeks is needed if the housing of horses is to be changed prior to an experiment, especially if the horses go from outside to stalls or *vice versa*. One should not switch housing during an experiment or have the groups housed with one group in stalls and another on pasture or dry lot. That may sound like common sense, but I have seen experiments conducted by others with one group housed inside and the other outside. Seasonal effects should be also be consider if an experiment conducted over the course of multiple seasons, especially if the horses are housed on pasture where the quality and composition of the grass can vary. Ideally, outside housing should be in dry lot paddocks where the forage intake and other feed can be controlled. Horses should be reacclimatised to the experimental surroundings in the testing facility prior to an experiment, especially if they have had an extended time off between experiments. Were the horses in the same state of fitness and if so how was the state of fitness quantified? If assays are conducted, the paper should provide details of the methods not just refer to another paper. Furthermore, there needs to be data to show that the assay was accurate, precise

and linear in the range measured. At a minimum, the details on the performance of any assay should include within and between assay coefficients of variation. Equipment, such as calorimeters, blood gas chemistry analysers, etc. should also be validated performance details provided for the individual laboratory. This is particularly a problem with some of the new portable gas exchange systems used for measuring oxygen consumption where the investigators refer to validation, but nothing is documented as far as calibration and verification in their own laboratory.

If a supplement is intended for use in an athletic horse one should ask if the investigators measured physiological capacity or athletic performance? Were the markers of performance appropriate for testing the effects of the product on performance or recovery? Were the parameters measured as markers of aerobic or anaerobic capacity? Is there specificity regarding the tests used to evaluate the effect on performance? The concept of specificity dictates that the test used to evaluate performance and the training used to condition the horse should match the type of competition to be performed. This is important because some nutraceuticals affect biochemical pathways associated with aerobic metabolism whereas some affect anaerobic pathways. Thus, do the exercise tests used in the experiment simulate the specific type of competition the nutraceutical is going to be marketed to? Along the same line of thought, is the breed of horse used in the experiment appropriate (Thoroughbreds and Standardbreds vs Quarter Horses vs Arabians, etc.)? Are the subjects middle distance athletes? Sprinters? Endurance athletes?

Finally, have the results presented in a reference associated with a nutraceutical been interpreted properly? Be careful if there is excessive speculation not supported by the what was measured in the experiment. Be careful if a supplement contains a mixture of ingredients as there may be synergistic effects or one nutraceutical may counteract another. These are just a few of the questions that one should be asked when designing a study and/or interpreting scientific results used in an efficacy claim for any supplement or nutraceutical. One penultimate observation to note: researchers many times do not publish papers about negative results and marketers surely are not going to highlight the lack of an effect or negative effect of a supplement. Always be sure to check the claims made in the marketing of a nutraceutical and beware of claims that a product cures or treats a multitude of problems. Remember, an ounce of hope is worth a 'million bucks (or Euros) for those marketing the ever increasing list of nutraceuticals on the market for horses.

References

Association of Racing Commissioners International (ARCI), 2017. The Association of Racing Commissioners International's Model Rules for Racing, Version 8.1: 253-256. Available at: https://tinyurl.com/yayzlzml.

Baldassari, J., Franke, W.C, Horohov, D.W., Betancourt, A. and McKeever, K.H., 2012. Effects of quercetin on exercise potential and exercise-induced cytokines in the horse. Comparative Exercise Physiology 8: 131-142.

European Commission (EC), 2002. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. Official Journal of the European Union L 183: 51-57.

Federation Equestrian International (FEI), 2014. fei warning regarding the administration of supplements to horses. Available at: http://tinyurl.com/yagqudpn.

Janabi, A.H.D., Biddle, A.S., Klein, D. and McKeever, K.H., 2016. Exercise training-induced changes in the gut microbiota of Standardbred racehorses. Comparative Exercise Physiology 12: 119-130.

Janabi, A.H.D., Biddle, A.S., Klein, D.J. and McKeever, K.H., 2017. The effects of acute strenuous exercise on the faecal microbiota in Standardbred racehorses. Comparative Exercise Physiology 13: 13-24.

Lehnhard, R.A., Adams, A., Betancourt, A., Horohov, D.W., Liburt, N.R., Streltsova, J.M, Franke, W.C. and McKeever, K.H., 2011. Phenylbutazone blocks the cytokine response following a high intensity incremental exercise challenge in horses. Comparative Exercise Physiology 7: 103-108.

Liburt, N.R., Adams, A., Betancourt, A., Horohov, D.W. and McKeever, K.H., 2010. Exercise-induced increases in cytokine markers of inflammation in muscle and blood in horses. Equine Veterinary Journal, 42 (S38) 280-288.

Liburt, N.R., McKeever, K.H., Streltsova, J.M., Franke, W.C., Gordon, M.E., Manso-Filho, H.C., Horohov, D.W., Rosen, R.T., Ho, C.T., Singh, A.P. and Vorsa, N., 2009. Effects of cranberry and ginger on the physiological response to exercise and markers of inflammation following acute exercise in horses. Comparative Exercise Physiology 6: 157-169.

McKeever, K.H., 2005. Does it work? Testing the efficacy of feed supplements. In: Pagan, J.D. (ed.) Advances in equine nutrition Vol. III. Nottingham University Press, Nottingham, UK, pp. 65-68.

McKeever, K.H., 2011. Feed supplements to maintain performance and health. In: Lindner, A. (ed.) Applied equine nutrition and training – ENUTRACO 2011. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 179-191.

McKeever, K.H., 2014. Recent research testing the efficacy of equine feed supplements. Proceedings of the California Animal Nutrition Conference, Fresno, CA, USA, pp. 147-151.

Norton, R.P., Lehnhard, RA, Kearns, C.F. and McKeever, K.H., 2013. Ephedra-induced alterations in cardiovascular function and thermoregulation during acute exercise in horses. Comparative Exercise Physiology 9: 109-117.

O'Neill, W., McKee, S. and Clarke, A.F., 2002. Immunological and haematinic consequences of feeding a standardised *Echinacea* (*Echinacea angustifolia*) extract to healthy horses. Equine Veterinary Journal 34: 222-227.

Robertson, R.J., 1991. Introductory notes on validation and application of ergogenics. In: Lamb, D.R. and Williams, M.H. (eds.) Perspectives in exercise science and sports medicine Vol. 4. Ergogenics enhancement of performance in exercise and sport. Brown and Benchmark Press, Carmel, IN, USA, pp. xvii-xxii.

Smarsh, D., Williams, C.A., Liburt, N.R., Streltsova, J.M. and McKeever, K.H., 2010. Black tea, orange, ginger, and cranberry extract supplementation to horses undergoing an intense bout of exercise affects oxidative stress and antioxidant status. Equine Veterinary Journal 42 Suppl. 38: 317-322.

Streltsova, J.M., McKeever, K.H., Liburt, N.R., Manso, H.C., Gordon, M.E., Horohov, D.W., Rosen, R. and Franke, W.C., 2006. Effect of orange peel and black tea extracts on markers of performance and cytokine markers of inflammation in horses. Equine and Comparative Exercise Physiology 3: 121-130.

US Food and Drug Association (US-FDA), 2017a. Dietary supplements. Available at: http://tinyurl.com/ydxe3xkt.

US Food and Drug Association (US-FDA), 2017b. Animal food and feeds – ingredients and additives. Available at: http://tinyurl.com/ycqgwym2.