JOINT SUPPLEMENTS AND EFFECTS ON EQUINE PERFORMANCE

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INTRODUCTION

All horses perform in one way or another, whether contributing to the aesthetic quality of a scenic view, going on the occasional trail ride, or performing in high intensity athletic endeavors. No matter how you define performance, the measure of quality or success related to that performance is likely impacted by joint health. Average horses as well as the elite equine athlete face many of the same challenges that human, canine and other athletic species face when it comes to preserving or regaining optimal joint health and function. Exercise, transportation stress, injury, and disease are stressors that initiate an immune response that may evolve into a chronic condition under certain circumstances. Tissue micro-damage, oxidative damage, and inflammation resulting from these stressors can ultimately compromise tissue integrity, organ function, and overall health and performance of the animal if left unattenuated (Petersen et al., 2004). Post-exercise problems associated with increased expression of inflammatory mediators and oxidative stress can range from mild symptoms of delayed-onset muscle soreness to debilitating conditions related to soft tissue, joint and bone damage. Joint pain associated with tissue micro-damage and inflammation is the most common cause of lameness. This leads to diminished athletic performance, decline in quality of life, economic burden, and animal loss (Kidd et al., 2001), and therefore is of significant concern to the equine industry. In the early 1980's, it was reported that 67.6% of the lost training days for racehorses were attributable to lameness (Rossdale et al., 1985). Similar data indicated that over 80 % of total days lost from training in 2 and 3 yr old Thoroughbred race horses were attributable to lameness, and 14 % of those days lost were due to joint injuries (Dyson et al., 2008). A survey study conducted by the National Animal Health Monitoring System (NAHMS, 2000) reported that 50% of the surveyed equine operations reported having at least one horse with lameness during the previous year (1999), and that 60% of these lamenesses were attributable to osteoarthritis (Caron and Genovese, 2003). Therefore, due to the high prevalence, cost, and debilitating nature of joint disease, it is increasingly common for early detection and prophylactic measures to be sought out and employed by the equine community.

Synovial Joints: Basic Structure and Function

Todhunter (1996) describes the joint as an organ comprised of two long bones that come together at the diarthrodial joint interface. Hyaline cartilage covers the articulating ends of the bones in four layers, or zones and is mainly comprised of chondrocytes within a collagen, proteoglycan, and glycoprotein matrix. The joint space is encapsulated by modified mesenchyme consisting of a fibrous layer lined with a thin subsynovium. The synovium (synovial membrane) is vascularized (blood vessels and lymph), innervated, and in direct proximity to the synovial fluid, an ultrafiltrate of plasma containing approximately 0.5 mg/ml hyaluronan located in the intraarticular space. The whole joint structure is supported by collateral and intraarticular ligaments. This organ supports the musculoskeletal system and if it is healthy, provides efficient

and pain free movement. All of the components that make up the joint are subject to physical stress and tissue microdamage leading to inflammation of the synovium (synovitis), cartilage matrix erosion and changes in soft tissue and bone; all of which characterize the onset and progression of osteoarthritic disease (McIlwraith, 2005).

The Nutraceutical Industry

Walk into any feed room at a stable and you are more likely than not to find a variety of nutraceuticals claiming to prevent or solve a wide range of problems. Nutraceuticals are popular among horse owners, easily obtainable, easy to use, and encompass a huge repertoire of applications for horse health. The United States nutraceutical industry is a multi-billion dollar industry. Market surveys of the human segment indicate that sales reached $6.1 billion in 2007, a 7.5% increase over the previous year's sales, and it is projected that by 2012, annual sales of nutraceuticals will approach $8.5 billion (Packaged Facts, 2010). The pet segment is keeping pace with the human market, showing considerable growth in sales as well. In 2008, sales rose 7% to $1.4 billion (Nutrition Business Journal, 2009) and are projected to reach $1.7 billion by 2012 (Packaged Facts, 2010).

The terms "supplement" and "nutraceutical" are vocabulary often used interchangeably, and indicate products somewhere between a nutrient (essential dietary component of feed ingredients) and a pharmaceutical (drug), often containing very little if any nutritive value, and advertised as a solution for the prevention and/or treatment of clinical diseases and conditions (Duren, 2005). Nutraceuticals or dietary supplements are defined by the National Research Council as "substances for oral consumption by horses, dogs, or cats, whether in/on feed or offered separately, intended for specific benefit to the animal by means other than provision of nutrients regarded as essential, or provision of essential nutrients for intended effect on the animal beyond normal nutritional needs, but not including legally defined drugs". For consumers, the downside to the nutraceutical industry is that it lacks regulation. Unlike the United States animal feed industry, which must adhere to strict guidelines set and enforced by the Association of American Feed Control Officials, Inc., or the pharmaceutical industry regulated by the Federal Drug Administration, the nutraceutical industry is not monitored for safety, quality control, or proof of efficacy.

A step in the right direction in terms of achieving regulatory oversight of the animal nutraceutical industry was the formation of the National Animal Supplement Council (NASC). The NASC is a group of supplement companies that self regulates while working closely with state and federal regulatory agencies. This organization has created labeling guidelines, GMPs, an adverse reporting system as well as an independent auditing protocol for member companies, and science based oversight of product research and development (Brown, 2010). The NASC has a joint initiative with the FDA/CVM that requires all NASC members to be registered with the FDA and will assign affiliates facility and label identification codes by the end of 2010. This agency works to keep its members up to date on contemporary cGMP guidelines, which are comparable of those required for human nutraceutical companies (Brown, 2010). The NASC stamp of approval is one of the best tools consumers have when navigating the crowded nutraceutical market. When choosing a product with this stamp of approval, nutraceutical selection can be made with a certain level of confidence that the product is supported by a

science-based auditing system and a greater degree of product labeling consistency than non-NASC members.

Another appeal to consumers regarding the use of certain nutraceuticals, seems to be the "natural" designation. Natural is often perceived as "safer, better, healthier, and/or more effective" than alternatives; however, this may not always be the case. Some "natural" supplements, often designated as herbal in origin, can result in toxicities and drug-like interactions with other supplements, pharmaceuticals, and/or dietary components (Williams and Lamprecht, 2008). Many supplements are derived from plant sources that intrinsically contain prohibited substances, and when in sufficient concentrations can cause adverse reactions in horses and other species. The toxicology of phytomedicine in horses is reviewed in Miller (1998), Poppenga,(2001), Harman (2002), Izzo et al. (2005), and Williams and Lamprecht (2008). Additionally, the use of dietary supplements intended to improve performance may result in nutrient imbalances within the total diet, which may result in impaired or suboptimal performance. In any case, care should be taken when incorporating any nutraceutical or dietary supplement into a horse's diet, and consultation with an equine nutritionist and/or veterinarian is strongly recommended before implementing the supplementation regimen.

Rationale for Using Nutraceuticals

A majority of horses do not have a real need for nutraceutical supplementation. Good management practices including balanced nutrition, regular veterinary, farrier, and dental care as well as regular exercise go a long way to keep our horses happy and healthy, and are usually easier on the pocket book than nutraceutical provision. There are a handful of situations that warrant the use of nutraceuticals. Horses with clinical disorders/injuries, dietary deficiencies, and/or horses in heavy training and competition or simply equines that are advanced in years, may benefit from the addition of the appropriate nutraceuticals to their diet. As with the increasingly aged human population, largely attributable to the "baby boomers" (Helmick et al., 2008), a greater percentage of our equine companions and athletes are living longer and performing well into and past their "teens" due to improved nutrition and health care (McKeever and Malinowski, 1997; Horohov et al., 1999). Additionally, exercise-induced immune responses (Moldoveanu et al., 2001) and changes in cartilage metabolism following exercise (Frisbie et al., 2008) have been documented. Just a single bout of intense exercise has been shown to up-regulate both pro- and anti-inflammatory cytokine transcripts in peripheral circulation of horses following exercise (Lamprecht et al., 2009). Inflammatory processes result in the release of small hormone-like proteins, or cytokines, which mediate the wide range of symptoms associated with trauma and infection. Specifically, oxygen-derived free radicals and inflammatory processes in the joint result in compromised viscoelastic properties of synovial fluid and tissue degradation, causing pain and mechanical instability (Bertone et al., 2001). Therefore, prevention of extreme pro-inflammatory responses and/or a chronic inflammation is important for the maintenance of healthy joints (Han and Ulevitch, 2005).

Nutraceuticals are used within two primary roles; 1) as preventative measures against the development of degenerative joint disease, and 2) as treatment for an existing condition. The underlying principles of either therapeutic intervention are to maintain or regain homeostatic conditions in the joint as soon as possible and reduce or eliminate joint pain (McIlwraith, 2010).

If a condition is pre-existing (e.g. traumatic injury, advanced OA), therapeutic goals are still to minimize or eliminate pain in the joint, and to retard the progression of tissue (cartilage and subchondral bone) degradation thereby minimizing functional impairment.

Additionally, use of nutraceuticals may reduce dependence on non-steroidal anti-inflammatory drugs, which can have undesirable side effects. The ease of use and wide availability of nutraceuticals lends to their popularity, particularly as a prophylactic measure against the onset of joint disease in a variety of species. Despite aggressive research efforts, particularly in human medicine, and advances in detecting and treating arthropathies, a cure for osteoarthritis remains to be identified (Dinubile, 2010).

EVIDENCE OF NUTRACEUTICALS AS USEFUL JOINT THERAPIES

There is a growing body of scientific literature that provides insight into questions surrounding safety, bioavailability, and efficacy of oral joint supplements in horses. As with all scientific literature, information gleaned from these studies must be considered within the context of the soundness of experimental design, quality of analytical methods, and the extent to which data is interpreted and/or applied.

Glucosamine and Chondroitin Sulfate

Out of the plethora of available nutraceuticals for humans and horses, probably the most researched products are glucosamine and chondroitin sulfate; both being precursors or subunits of cartilage proteoglycans. Mechanisms by which glucosamine provides benefits to synovial joints are not fully characterized, however safety and bioavailability following oral consumption, as well as anti-inflammatory and matrix enhancing effects have been documented in horses and are reviewed in Goodrich and Nixon (2004), Neil and colleagues (2005), and Pearson and Lindinger (2009). Evidence of glucosamine sulfate and glucosamine hydrochloride bioavailability (2.5 – 12 %) has been documented in humans (Setnikar et al., 1993), dogs (Adebowale et al., 2002), and horses (Du et al., 2004; Laverty et al., 2005). Healthy mares provided a single dose of glucosamine via nasogastric intubation at a rate of 20 mg/kg bwt, had plasma glucosamine concentrations reach approximately 940±270 and 1080±170 ng/ml (Meulyzer et al., 2008; Meulyzer et al., 2009). Additionally, elevated synovial fluid concentrations of glucosamine (0.3 – 0.7 μm), following the same 20 mg/kg bwt oral dose were reported by Laverty et al. (2005) as well as Meulyzer et al. (2008, 2009) with glucosamine sulfate being approximately 9.4% and glucosamine hydrochloride 6.1% bioavailable. Interestingly, plasma and synovial fluid concentrations of glucosamine sulfate remained significantly elevated above baseline values 12 hours following administration when compared to glucosamine hydrochloride (Meulyzer et al., 2008).

Bioavailability of low molecular weight (8 kDa) chondroitin sulfate given orally has been shown in dogs (Adebowale et al., 2002) and horses (Du et al., 2004) after a 1600 mg and 3 g dose, respectively. Furthermore, cumulative concentrations of chondroitin sulfate, up to 208 ug/mL in plasma, has been reported in horses at the conclusion of a 14 day supplementation period (Du and Eddington, 2002).

Data from *in vitro* studies indicate that treatment with moderate to high concentrations (25–250 μg/ml) of glucosamine, chondroitin sulfate, and a combination of both on healthy cartilage explants have the potential to reduce glycosaminoglycan release from the cartilage matrix and improve total cartilage content via enhanced glycosaminoglycan synthesis (Dechant et al., 2005). In a similar study using cartilage explants, this time treated with interleukin-1 (pro-inflammatory cytokine), only the combination of glucosamine and chondroitin sulfate was successful in inhibiting cartilage degradation (Dechant et al., 2005), which supports claims that these two compounds work synergistically to produce desired effects in diseased joints (Das and Hammad, 2000; Orth et al., 2002). Studies by Fenton et al. (2000a,b) suggest glucosamine addition to LPS treated cartilage explant culture resulted in reduced nitric oxide and prostaglandin E2 production, whereas chondroitin sulfate had no effect on those inflammatory parameters. However, the combination of glucosamine and chondroitin sulfate in the same model demonstrated a reduction in matrix metalloproteinase activity and trends towards reduced matrix metalloproteinase-13 protein concentrations.

Just a handful of studies have examined effects of glucosamine and/or chondroitin sulfate supplementation on markers of inflammation or cartilage degradation *in vivo*. In one study, mares with an induced osteochondral defect were supplemented for 10 wks with 2.5 g per day of either a 9600kDa or 25,000kDa chondroitin sulfate nutraceutical, demonstrated reductions in synovial fluid prostaglandin E2, matrix metalloproteinase-3 synthesis and release, as well as decreased glycosaminoglycans (Verde et al., 2006). Improvements in maximum flexion angle as well as a decrease in joint circumference were also observed. Similar data was obtained from another study where geldings with were administered 15 g per day of an oral glucosamine and chondroitin sulfate-containing nutraceutical along with other functional ingredients prior to the induction of IL-1 mediated articular inflammation (Pearson et al., 2009). In horses that received the nutraceutical, IL-1beta intraarticular injection did not induce increases in synovial fluid concentrations of prostaglandin E2 or glycosaminoglycans compared to non-supplemented controls. This study also reported no adverse effects resulting from oral supplementation of the same nutraceutical up to 75 mg per day for 84 days.

Assuming clinical relevance of glucosamine/chondroitin supplementation at the recommended or tested doses cannot be made with absolute confidence despite evidence of bioavailability, chondroprotective and anti-inflammatory properties in several species including horses. Conclusions based on the *in vitro* data may be misleading when predicting outcomes *in vivo*, considering that blood and synovial fluid nutraceutical concentrations reported in horses are substantially lower than concentrations tested in *in vitro* osteoarthritis models (Laverty et al., 2005; Neil et al., 2005; McIlwraith, 2010). It is more likely that accumulation of these compounds in blood, synovial fluid and tissues over time (multiple oral doses), allows for clinically relevant concentrations (208 μg/mL chondroitin sulfate) to occur as reported by Du and Eddington (2002). More research is needed to determine synovial fluid and tissue (cartilage) concentrations of glucosamine and chondroitin following long term supplementation, which would provide more insight into efficacious concentrations both in plasma and targeted tissues. Relying solely on plasma concentrations may not reflect uptake of the nutraceuticals into tissues and could lead to inaccurate conclusions surrounding bioavailability and effective concentrations. Another consideration when investigating the mode of action of exogenous nutraceuticals is that indirect effects (e.g. modulation of signal transduction pathways),

potentially in other tissues and organs, may be responsible for the symptom modifying effects observed in joint tissues following supplementation (Laverty et al., 2005). It has been shown in cell culture and cartilage explants models that chondrocytes do have the ability to bring glucosamine into the cell (Windhaber et al., 2003), however other *in vitro* studies indicate that exogenous glucosamine is not efficiently utilized by these cells (Sweeney et al., 1993; Sandy et al., 1998; Mroz and Silbert, 2002).

Evidence of efficacy previously reported in animal trials involving supplementation of nutraceuticals containing multiple ingredients (Hanson et al., 1997; Clayton et al., 2002; Pearson et al., 2009), makes it difficult to tease out which component(s) of the product were responsible for the responses, and some conclusions were based on subjective observations and did not provide evidence of blood or synovial fluid levels of the active ingredient(s). It would be useful to have more *in vivo* data in horses to provide insight into efficacious doses, how long it takes to achieve biologically relevant concentrations in blood, synovial fluid and tissues following oral consumption paired with evidence of anti-inflammatory and/or chondroprotective effects in the target tissues. Additionally, more data is needed to identify appropriate concentrations of glucosamine and chondroitin sulfate for different applications; as a prophylactic measure before signs of osteoarthritis occur, and concentrations that designate these compounds as a treatment for an existing degenerative condition.

Going beyond Glucosamine

Hyaluronan. Other nutraceuticals commonly targeted towards joint health include hyaluronic acid or hyaluronan (HA), omega-3 fatty acids (n-3), and avocado soybean unsaponifiables (ASU). Aside from anecdotal reports of efficacy, very little controlled research in horses has been conducted to evaluate the efficacy of oral preparations of HA, and reports in the literature are conflicting. In one study, oral hyaluronan gel (100 mg/day) administered to 24 Thoroughbred yearlings, having undergone arthroscopic surgery to correct osteochondritis dessicans (OCD) lesions in the tarsus, was shown to significantly reduce joint effusion scores on day 30 when compared to placebo controls (Bergin et al, 2006). Results from a similar study by Carmona et al. (2009), during which young horses with osteochondrosis were provided 250 mg/day of an oral HA preparation for a 60 day duration, did not demonstrate significant differences in synovial effusion, or synovial fluid concentrations of prostaglandin E2, nitric oxide, or hyaluronan as compared to placebo control animals. More research is needed to identify the benefits of hyaluronan supplementation to horses. Understanding the impact molecular weight has on the bioavailability and efficacy of oral preparations of HA, as well as any synergistic benefits that might be realized with its combination with other oral joint health supplements would be useful information.

Avocado Soybean Unsaponifiables. One of the most recent additions to the equine joint health nutraceutical market is ASU. A critical review of studies on ASU, via meta-analysis was conducted by Christensen et al. (2008) and concluded that preparations of unsaponifiable fractions of avocado oil and soybean oil have been identified as safe and effective symptom and disease modifying agents for osteoarthritis in both human (Blotman et al., 1997; Maheu et al., 1998; Reginster et al., 2000) and animal (Cake et al., 2000; Kawcak et al., 2007; Heinecke et al., 2010) models. A recently published study by Heinecke et al. (2010) used an equine chondrocyte

culture inflammation model to evaluate the efficacy of ASU and epigallocatechin gallate (EGCG) as an alternative to non-steroidal anti-inflammatory drugs for the management of osteoarthritis. Pre-treatment of the chondrocytes with a combination of ASU (8.3 μg/ml) plus EGCG (4, 40, 400 ng/ml) resulted in a reduction in cyclooxygenase-2 expression and prostaglandin E2 production which was associated with an inhibition of nuclear factor kappa-B translocation after incubation with inflammatory cytokines when compared to controls. Furthermore, this anti-inflammatory effect was much more pronounced with the ASU/EGCG combination than with either component independently. Data from another study *in vivo* using an ovine meniscectomy model, suggested that oral supplementation of ASU (900 mg/d) for 6 months resulted in chondroprotective and anabolic effects on chondrocytes via stimulation of matrix production, compared to placebo controls (Cake et al., 2000). The beneficial effects in sheep were supported by results from a study in horses with experimentally induced osteochondral fragmentation in middle carpal joints. Horses received either a 1:2 avocado-to-soybean ratio mixed with 6 ml molasses or just a 6 ml molasses placebo treatment every day for 70 days. Supplementation with ASU extracts did not result in pain or lameness alleviation, however it did reduce articular cartilage erosion and synovial hemorrhage as well as a significant increase in articular cartilage glycosaminoglycan synthesis when compared to placebo controls (Kawcak et al., 2007). There is more evidence in the literature surrounding the effective use of ASU and mixtures containing ASU as a treatment for osteoarthritis. There is limited data that lends insight into its potential as a preventative measure against the development of degenerative joint disease, although this may be accomplished through its anti-inflammatory properties. More controlled research *in vivo* would be helpful to more fully characterizing the ability of ASU to prevent vs. treat joint disease in target species, as well as studies that tease out the specific mechanisms through which this may be achieved (e.g. mitogen-activated protein kinase pathways, nuclear factor kappa-B pathway, transforming growth factor-β, etc.).

Omega-3 Polyunsaturated Fatty Acids. Plant and fish oil sources of omega-3 polyunsaturated fatty acids (PUFAs) have become a popular joint health supplement for a variety of species including horses. Omega-3 PUFAs stem from alpha-linolenic acid, which is desaturated in the body to form eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Omega-6 PUFA (linoleic acid) is also desaturated and elongated in the body to form arachidonic acid (ARA). Both omega-3 and omega-6 PUFAs are crucial to normal, healthy immune responses, and their ratio to one another is very influential on the nature of the immune response. More specifically, oxidation of EPA and ARA result in the formation of eicosanoids, which are important mediators of inflammatory responses; however, eicosanoids formed from ARA are considered to be more pro-inflammatory than those from EPA. Achieving optimal balance of omega-3 to omega-6 PUFAs helps support a healthy immune response, whereas extreme imbalances may contribute to either an immunosuppressed state or a chronic inflammatory state, both of which are undesirable. Several studies in horses and pigs have looked at the effect(s) of supplementing with omega-3 PUFAs. Woodward et al. (2007) demonstrated increased plasma DHA concentrations and a tendency towards increased stride length at the trot in Arabians provided 5.95g/d of stabilized omega-3 fatty acids for 75 days compared to horses supplemented with corn oil. No changes were detected in lameness scores, plasma tumour necrosis factor-α, or prostaglandin E2 metabolite concentrations. Using a swine model, researchers demonstrated increased EPA and DHA in cartilage following dietary supplementation with protected long chain polyunsaturated fatty acids (1% of the total diet), compared to control animals (O'Connor-

Robison et al., 2010). Another study investigated the incorporation of long chain fatty acids into synovial fluid following a 90 day supplementation with either 69 mg/kg bwt per day (143.5 mg/kg bwt total ration composition) of fish oil (EPA/DHA), 68.6 mg/kg bwt (142.5 mg/kg bwt of flax seed supplement (omega-3 alpha linolenic acid), or a control diet in horses (Ross et al., 2010). Increases in synovial fluid EPA and DHA were only seen in the horses that received the fish oil supplement, when compared to the flax and control groups and it was suggested that inefficient conversion of alpha linolenic acid to EPA and DHA metabolites may explain the lack of response in the flax supplemented group. Further investigation into the enzymatic capacity of horses to desaturate and elongate alpha-linolenic and linoleic acids, as well as optimal dietary omega-3 to omega-6 ratios for different classes of horses would be a valuable addition to the scientific literature. More research is needed to identify if omega-3 PUFA supplementation is better designated as a preventative measure or a treatment option for degenerative joint disease in horses.

TAKE HOME MESSAGES

The animal nutraceutical industry is a booming billion dollar industry that continues to grow alongside the human nutraceutical market. Due to the negative economic impact and the debilitating nature of degenerative joint disease in horses, joint supplements are very popular among the equine community despite the lack of regulatory oversight in regards to product safety, quality, and efficacy. A growing body of scientific information regarding the usefulness of several common compounds found in joint supplements is contributing to our ability to provide optimal doses in the right context, whether intended for preventative or treatment purposes as well as the synergistic benefits or lack thereof, from combining several therapeutic agents. More information is needed regarding the ability to produce clinically relevant concentrations of a therapeutic compound *in vivo* following regular oral dosing intervals over time, and the specific mechanisms through which they exert their beneficial effects, whether anti-inflammatory, chondroprotective, or antioxidant. Well designed studies, conducted in the target specie, that include proper controls and sufficient statistical power are crucial for the generation of information that can be translated into practical application. Nothing replaces good horse management in the way of balanced nutrition, avoidance of over-supplementation, regular veterinary and farrier care, and a balanced exercise program appropriate for the horse's life-stage and fitness level; however, under specific circumstances nutraceuticals may provide a benefit beyond what the normal diet can provide.

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