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The Use of Nutraceuticals for Osteoarthritis in Horses

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In horses, lameness is often attributable to some degree of osteoarthritis (OA), a complex disease process that is highlighted by eventual degradation of articular cartilage. Conventional therapies for OA in horses are designed to relieve pain and discomfort and often include pharmacologic intervention with nonsteroidal anti-inflammatory drugs (NSAIDs) or intra-articular steroids. Treatments have generally been symptomatic, without well-documented influence on the duration of the disease or its progression. OA is often a chronic disease that requires long-term therapy, which can lead to side effects when conventional therapies are used. For example, NSAIDs can be ulcerogenic [1,2] and also have been shown to have adverse effects on chondrocytes and cartilage matrix formation [3,4]. In addition, long-term intra-articular steroid use has the potential to alter the metabolism of articular cartilage [5]. The ultimate goal for treating OA is to prevent further degradation while restoring function.

OA is the leading medical condition for which people use alternative therapies in an attempt to alleviate the side effects or incomplete relief of symptoms from conventional therapies [6]. These alternative therapies include exercise, physical therapy, acupuncture, chiropractics, electromagnets, and nutraceutical administration, just to name a few. Naturally, if owners perceive that their own symptoms are being relieved using these adjunctive therapies, they are likely to attempt to use them on their horses. Oral administration of nutraceutical products to the horse is common and easy and is perceived to be a benign treatment for OA in horses. The main goal for use of nutraceuticals is to use them in OA cases to attempt to lower the dose of other drugs that are more problematic while potentially preventing further degradation (disease or structure modifying). The remainder of this article attempts to define a nutraceutical, identifies areas that need to

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be considered when these products are used, and describes the known scientific effects of the most common compounds contained in currently available equine nutraceuticals.

Definition

The term *nutraceutical* was adopted in veterinary medicine from the medical profession and refers to compounds that are neither nutrients nor pharmaceutics. The nutraceutical category is broad, including nutrients, dietary supplements, functional foods, and phytochemicals (including herbs) that are not recognized by the US Food and Drug Administration (FDA) as foods or drugs. Legally, a supplement was initially defined by the Federal Food, Drug, and Cosmetic Act, but this was amended in 1994 by the Dietary Supplement Health Education Act (DSHEA). Technically, the DSHEA only covers human products and defines a supplement as a product intended for the diet that contains at least one of the following: vitamin, mineral, herb, amino acid, substance used to increase intake, concentrate, metabolite, constituent, extract, or any combination [7]. Jurisdiction of veterinary products is primarily the responsibility of the North American Veterinary Nutraceutical Council (NAVNC), which was formed in 1996 to promote and enhance the further quality, safety, and long-term effectiveness of nutraceuticals used in veterinary medicine [8]. Therefore, the NAVNC has defined a veterinary nutraceutical as a ''nondrug substance that is produced in a purified or extracted form and administered orally to provide agents required for normal body structure and function with the intent of improving the health and well-being of animals'' [8].

The DSHEA allows manufacturers to make claims with regard to health, structure or function, and nutrient content of a nutraceutical. The Center for Veterinary Medicine has allowed products to be marketed as nutraceuticals provided that they do not claim to treat, cure, or mitigate disease. The FDA perceives veterinary nutraceuticals as unapproved drugs, however, even though they are not labeled or marketed as drugs. The FDA does not regulate these products unless they become unsafe or are associated with labels that claim a drug use. Therefore, there is no requirement to prove safety or efficacy of a nutraceutical for its intended use. The DSHEA requires that a manufacturer ensures its product is safe before marketing but does not have a system set up to monitor this. It is important for veterinarians to realize that manufacturers do not have to register themselves or their supplements with the FDA. The only time a manufacturer technically must notify the FDA is if it intends to market a supplement that contains a ''new dietary ingredient.'' In general, a manufacturer needs to comply with the FDA ingredient recognition program, which basically entails applying for complete ingredient definitions as described by the not-for-profit organization of state and federal feed regulators, the Association of American Feed Control Officials (AAFCO) [7]. In addition, the manufacturer must

identify each dietary ingredient contained in the product on the ''supplemental facts'' panel and must adhere to the state licensing requirements [7]. It is important to realize that there is no requirement of good manufacturing practices (GMPs) for manufacturers to guarantee high quality and batch-to-batch consistency; product safety is completely up to individual manufacturers. Unsafe compounds should be reported to the manufacturer as well as to the United States Pharmacopeia Veterinary Practitioner's Reporting Program.

Critical evaluation

More than 60 equine nutraceuticals can be purchased, resulting in a $20 to $50 million per year veterinary market [8]. Many owners or trainers use nutraceuticals independent of their veterinarians; in fact, many equine practitioners examine a horse for lameness after their client has already tried some form of alternative therapy. Unfortunately, the lack of regulation, in effect, gives implied consent to the marketing and production of these substances even though there is little scientific information available regarding the quality, efficacy, tolerance, and safety of these products [7,8]. Many manufacturers come close to crossing ethical and legal advertising boundaries by implying medical claims or writing phrases that potentially misrepresent the product to the consumer [7]. Therefore, it is important for equine owners and veterinarians alike to examine and understand the quality, efficacy, tolerance, and safety of individual nutraceuticals. This should be taken seriously by veterinarians who are willing to recommend particular nutraceuticals to clients, because it has been suggested that veterinarians may be legally liable for adverse effects on horses administered these products [9]. Veterinarians should request documentation validating the contents, purity, and quality control procedures from the manufacturer before selection or recommendation of a product. It is also important for the veterinarian to realize that direct comparisons of efficacy of different formulations are lacking; thus, just because two products claim to contain the same ingredients, they cannot necessarily be expected to act in the same manner. As with most products, the consumer gets what he or she pays for, with most of the higher priced products generally having information regarding quality, efficacy, and safety readily available.

Quality

When a manufacturer makes a claim regarding the structure or function of a product, it must be determined that the product contains a realistic concentration of the substance of interest so that it can be safe and efficacious. Unfortunately, because these products are not strictly regulated, many have considerable variability in purity, formulation, and consistency between batches. Although these products are marketed with ''guaranteed analysis,''

only a small number consistently meet the label claims [10–14] In fact, truth-in-labeling problems have been documented, where ingredients listed on the container were not present at the claimed concentration or purity. In testing conducted by an independent laboratory, only 50% of the products tested met the label claims [14], with many human over-the-counter products varying widely in composition [15] and more than 84% not meeting label claims [11]. In a study of five glucosamine (GLN), five chondroitin sulfate (CS), and one combination equine products [13], the actual composition of GLN was 63.6% to 112.2% of the label claim, whereas the concentration for CS ranged from 22.5% to 155.7%. In addition, the GLN content was 86% of the label claim, and the CS content was 83.3% of the label claim [13]. This significant deviation between the content of active ingredients and what is stated on the manufacturer's label tends to be greater for CS, which may be attributable to variability in the permeability of CS raw materials as well as the increased expense of CS relative to GLN. It is important to note that because of these differences from the label claim, it is difficult to extrapolate data on one product to another. The products that have the most inconsistency in concentrations compared with the label claims tend to be those that state that they are a "complex," "formula," or "blend" of ingredients with no weight of each component listed [14]. In general, those products that do not have information available on the quality of the product as well as those that make exaggerated claims or those that rely on testimonials should be avoided. The presence of lot numbers and expiration dates generally offers some evidence of accuracy in labeling but does not guarantee it [8].

Efficacy

From a scientific standpoint, it is most important to be able to determine whether a product performs in such a way that it can affect the patient in the appropriate manner claimed on the label. Most consumers trust the claims placed on the label because they are not trained to discern fact from fiction or even wishful thinking [8]. Unfortunately, the mechanism of action of many of these products is unknown or unproven, especially in the horse. When examining efficacy, the veterinarian should determine what the active ingredient(s) is and whether peer-reviewed controlled studies have been performed using the doses recommended by the manufacturer [8]. The claims of efficacy for many veterinary nutraceuticals are based on subjective methods of assessment, including testimonials by owners or clinical trials not subject to peer review. As has been aptly stated previously, observational, descriptive, and anecdotal studies do not provide proof of efficacy [7]. Many manufacturers with similar products attempt to compete based on research conducted by the competing company even though there is no proof of comparable efficacy. There is little scientific evidence available for these products, and assessment becomes difficult, because the veterinarian must

extrapolate data from one species to another; this should be done with caution, because the differences in pharmacokinetics and pharmacodynamics among species may be too great to be able to make accurate extrapolations. Because these products are administered orally, it is also important to make sure that there is adequate evidence of absorption in the species of interest, or else the product is essentially useless. Nevertheless, just because a product attains therapeutic blood and tissue levels after oral administration, one should not consider this as indirect evidence of proof of effectiveness, which is typical [7]. To truly determine the efficacy of a product, a controlled study with the appropriate number of animals with a consistent level of disease (via a model) should be performed [7]. In addition, many of the proposed effects of these products on articular cartilage are based on in vitro work in which different concentrations of the ingredients are used to attempt to inhibit the catabolic responses to cartilage explants or cultured chondrocytes, but they do not clarify the mode of action [7]. In general, these doses are quite high compared with what would likely be delivered to articular cartilage in vivo. Therefore, these studies should be evaluated based on the concept of how the ingredient of interest can potentially affect articular cartilage metabolism when particular levels are obtained. *The Arthritis Foundation's Guide to Alternative Therapies* [16] essentially states that until more controlled research has been performed, it is probably best to find out which brand has been studied the most with the best beneficial effects and to buy that brand.

Tolerance

In general, for obvious reasons, the product needs to be acceptable to the animal as well as to the owner. In addition, as with many medications, a particular individual may respond in an adverse fashion to various ingredients contained in the product. This could be a reaction to the main ingredients (GLN or CS), to contaminants, or to what is considered to be an inert ingredient that is added to help disperse or dilute the product [8,17]. Possible interactions with traditional therapies (NSAIDs) should also be taken into account, because the nutraceutical could potentially render the traditional therapy useless [17]. Unfortunately, little to no information exists to date for these products, which is important to consider when discussing safety of the product with the owner or trainer. Most of the information is anecdotal and is based on the lack of any major adverse reactions after treatment.

Safety

When examining safety of nutraceuticals, the typical response from most doctors and veterinarians is that "it can't hurt." This type of thinking is risky, because without published information or review of the safety information of the product in the species of interest, one should not and cannot

assume that these products do not cause harm; any substance can be toxic for a given individual. Just because there is a lack of published adverse reactions to a nutraceutical does not mean that one should extrapolate that the product is safe. In fact, most manufacturers are not going to spend the money to prove safety; thus, the lack of side effects does not mean that a product is safe. Unfortunately, even when safety data are present for a particular product, the variability in concentrations of the ingredients within and between batches can make these data inaccurate.

With all that said, many of these products can provide rational, safe, and effective alternative methods for the management of equine OA. With the popularity of use among owners and trainers, the veterinarian in today's practice environment must have an adequate working knowledge of these products. Unfortunately, the lack of regulation and critical research often places the burden of scientific assessment on the veterinarian. The traditional rationale for nutraceutical use was that these products provided precursors of cartilage matrix in excess quantities so as to favor matrix synthesis and repair of articular cartilage, with most nutraceuticals marketed as substances that are necessary for supporting or improving normal structure and joint function. In addition, compounds may be added that help to decrease the amount of articular cartilage degradation that occurs by decreasing inflammation, degradative enzymes, or free radicals. The remainder of this article attempts to describe the known scientific effects of the most common compounds contained in currently available equine nutraceuticals.

Basic structure and pathophysiology of articular cartilage

To understand fully how some of the common nutraceutical ingredients affect the joint environment, one must first have a general knowledge about the structure and function of articular cartilage and how it responds when injured. Articular cartilage is composed of a sparse population of chondrocytes that are embedded within an extracellular matrix (ECM). The ECM is primarily composed of an intricate network of collagen (primarily type II) and proteoglycans (PGs; primarily aggrecan) that traps water within it to allow for its biomechanical distribution of the load evenly across a joint. The PGs consist of a primary protein core that is attached to a hyaluronic acid (HA) molecule, forming large chains of PGs interspersed between the collagen fibrils. The PG protein cores also bind glycosaminoglycans (GAGs), which are long unbranched polysaccharide chains that consist of repeating disaccharide amino sugars alternating between glucuronic acid and *N*-acetyl-galactosamine [18,19]. The most abundant GAG within articular cartilage and the body is CS. Most of the *N*-acetyl-galactosamine residues are sulfated, which forms a strongly charged polyanion. This negative charge to the GAGs allows the attraction of water, which, when combined with the collagen network, imparts the resistance to compression and tension to allow for load dissipation. GLN is an amino-monosaccharide

(2-amino-2-deoxy-α-D-glucose) that acts as the preferred substrate for biosynthesis of GAG chains as well as HA, with *N*-acetyl-glucosamine being essentially an acetylated GLN.

In a normal joint environment, there is homeostasis between synthesis and degradation. Once injury occurs, however, even though there is an initial increase in synthesis, a general catabolic process predominates. The main initiators of many of these catabolic events are the proinflammatory cytokines, interleukin-1 (IL-1α or IL-1β) and tumor necrosis factor-α (TNFα). These cytokines tend to cause release or activation of many other cellular activities or enzymes that all contribute to altering the metabolic state of articular cartilage. Included in this list is the activation of proteinases, such as matrix metalloproteinases (MMPs) or aggrecanase, as well as increasing prostaglandin E_2 (PGE_2), nitric oxide (NO), and superoxide radical levels, just to name a few. For an in-depth understanding of the structure and function of articular cartilage and its response to injury, the reader is directed to further reading [20,21].

Glucosamine

There are three commercially available forms of exogenous GLN: hydrochloride (HCl), sulfate, and *N*-acetyl-D-glucosamine. In vitro studies have demonstrated that GLN HCl and GLN sulfate seem to inhibit cartilage degeneration more consistently than *N*-acetyl-D-glucosamine [22–24]. GLN has been derived from marine exoskeletons or beef, or it is produced synthetically. The GLN sulfate form has been postulated to be potentially more efficacious, because serum or synovial fluid sulfate concentrations increase after GLN sulfate administration, indicating that there may be ample sulfur for incorporation into the articular cartilage matrix [25–27]. The GLN HCl is more stable than GLN sulfate preparations, however, and it is almost twice as bioavailable (different amount of actual GLN available for use in the body from an equal weight of each form). In other words, to provide an equal amount of active GLN as the HCl salt, approximately 50% more sulfate salt is required [28]. Interestingly, in controlled blind studies, those studies with positive outcomes used GLN HCl [29–32].

GLN is a small molecule (molecular weight of 179) and is quite soluble in water. Absorption of GLN is mediated via glucose transporters, however, whereas absorption of *N*-acetyl-D-glucosamine occurs via diffusion [33]. Early methods for determining GLN absorption in human beings and animals demonstrated approximately 90% absorption after oral administration, with subsequent incorporation into plasma proteins and biotransformation in the liver [34–36]. Because of its wide distribution and accumulation in various tissues, sensitive analytic methods had to be developed to detect its presence in plasma [10,37] and synovial fluid [38] after administration. The oral bioavailability of GLN HCl in the horse has recently been reported to range from 2.5% to 5.9% [37,38]. This is low when compared with that in

the dog (12%) [39] and was attributed to a large volume of distribution, poor absorption from the gastrointestinal tract, and extensive tissue uptake [37,38]. Most of the GLN fed is rapidly modified to glucose and fructose derivatives, incorporated into plasma proteins, or used for other biosynthetic processes during first-pass metabolism in the liver [34–37,40]. Because the parent compound is difficult to recover in the plasma as a result of this transformation, it has been difficult to study the pharmacokinetics [37,41]. Via radiolabeling studies, it has been shown that there is good distribution of GLN to articular cartilage, with levels in the cartilage exceeding those in the plasma and multiple dosing resulting in accumulation of GLN in the cartilage [35]; however, just because GLN was delivered to the articular cartilage does not necessarily mean that it was incorporated into the articular cartilage. In fact, after oral dosing in the horse, it has been shown that synovial fluid levels of GLN are less than 10% of those in the serum at the same time point [38]. This indicates that GLN does not diffuse readily into the synovial fluid from the plasma, and because GLN only gets to the articular cartilage via the synovial fluid, it has been suggested that the effects of GLN may lie in its effect on nonarticular tissues [38].

GLN is required for synthesis of glycoproteins, glycolipids, and GAGs as well as being a component of biologically active compounds, such as heparin. Because of this structural similarity to heparin, concurrent use with other platelet inhibitors, such as phenylbutazone or aspirin, has been cautioned [42,43]. GLN has anti-inflammatory effects that were initially suggested to be cyclo-oxygenase (COX) independent [44,45] and attributable to the enhanced production of HA by the synoviocytes [46]. In a more recent report, however, the reduction in PGE_2 levels in cartilage explants with GLN alone or in combination with CS was directly attributable to a decrease in COX-2 gene expression [47].

Many in vitro studies have been performed and generally have identified that exogenous GLN has chondroinductive and chondroprotective effects on normal as well as stimulated or osteoarthritic chondrocytes or cartilage explants in the presence of high concentrations of GLN. These concentrations are likely quite high in comparison to what articular cartilage would be exposed to in vivo after digestion. Therefore, extrapolation of these results to the in vivo setting must be done with caution; however, with continually improving technology for detection of these products, in vitro examination of clinically relevant doses can potentially be performed. Exogenous GLN has been shown in vitro to have no detrimental effects on normal articular cartilage explants [48] or on chondrocyte metabolism after long-term exposure [49]. With the high doses of GLN used in vitro, however, some have suggested that it may have detrimental effects on chondrocyte viability [50,51], whereas other studies using similar doses failed to see the same effect [22,23].

Initially, it was thought that the effects of exogenous GLN were from providing the raw materials required for the synthesis of articular cartilage.

Chondrocytes can normally manufacture the GLN that is incorporated into PGs or HA from glucose. Most of the physiologic benefits are likely to occur because there is increased availability of the monosaccharide building blocks (glucuronic acid and *N*-acetyl-galactosamine) created during digestion. When exogenous GLN is available, however, the rate-limiting steps in the hexosamine biosynthetic pathways are bypassed. In addition, there is some support for the use of exogenous GLN as the source of cartilage matrix components allowing the potential for preferential incorporation of GCM into galactosamine moieties of CS, making it a potentially less expensive way to increase the CS content [52,53]. Rather than providing the raw materials for prevention, GLN may regulate mRNA production of PGs and downregulate mRNA for MMPs and aggrecanase. Regardless of the species examined, most in vitro studies involving cartilage explants or cell culture have demonstrated that GLN stimulates PG or GAG synthesis [48,52,54–56] and inhibits PG degradation [22,23,56,57]. One study demonstrated that GLN inhibited PG synthesis and degradation but had a decreased PG turnover, potentially indicating that GLN may have a preserving effect on the cartilage [22]. When the type II collagen content or expression has been measured, however, GLN seems to have a minimal effect on type II collagen synthesis [52,58]. Another study has suggested that GCM could improve the repair process in OA cartilage by restoring the chondrocyte adhesion to fibronectin in fibrillated cartilage, a process likely activated by protein kinase C [59].

The anticatabolic effects of GLN have also been demonstrated in vitro by demonstration of the decreased expression, synthesis, or activity of MMPs [22,55–57,60]. Aggrecanase-mediated degradation of aggrecan has also been inhibited by exogenous GLN administration [61]. Part of this effect on the proteinases may be because GLN can reduce the transcription factors involved in the intracellular signaling of IL-1, decreasing the amount of IL-1 that can further upregulate or activate the proteinases [62,63], or via pretranslational or translational regulation of the proteinase itself [60]. GLN has also been demonstrated in vitro in equine cartilage explants and human chondrocytes cultures to suppress NO production, a process that may be driven by a decrease in the activity of inducible NO synthase (iNOS) [22,24,64]. PGE_2 levels were also decreased by GLN via repression of COX-2 gene transcripts in equine cartilage explants [47] as well as by human chondrocytes [62]. The effects of GLN on articular tissues could occur via cellular signaling from various other nonarticular tissues [38].

The most commonly used animal model to test GLN has been the anterior cruciate transection (ACL) model in the rabbit. GLN did not show any significant differences in the severity of the macroscopic grades after ACL transection but did tend to have lower grades in all joint compartments compared with placebos [65]. In a major instability model in rabbits via transection of the ACL and posterior cruciate ligament (PCL) as well as removal of the medial meniscus, GLN had no identifiable effects on histologic

parameters of OA severity even though it was administered at 20 times the standard dose [31], which was likely attributable to the severity of the model. In a chymopapain model in rabbits, however, GLN was shown to increase the GAG content in injured knees compared with controls [58]. There are two studies in young horses in training that evaluated oral GLN. Neither study found any significant treatment effect on serum marker concentrations [66,67].

When critically examining human clinical trials for the use of GLN in OA, most industry-sponsored trials had positive results, whereas non–industry-sponsored studies did not [68]. In addition, the trials are all heterogeneous with regard to the formulations of GCM used, the route of administration, dosage, severity of OA, and measurement of outcome, making it really difficult to assess the value of GLN [69–74]. Some of the most consistent information that could be identified from these trials is that GLN seems to take 4 to 8 weeks before it really begins to act and that GLN showed no toxic effects over placebo when administered to human beings for 4 weeks to 3 years. In addition, it has been suggested that based on these clinical trials, GLN would likely achieve its best effects if used preventatively when minor lesions are present before the advancement of the disease process [75].

Chondroitin sulfate

CS is used topically with sodium hyaluronate and is approved by the FDA as an ophthalmic product. It is often derived from shark and bovine cartilage. CS is rarely found in equine nutraceuticals on its own, partly because it is difficult to synthesize, difficult to extract, and tends to be more expensive. Because of this expense, there is even more concern than for GLN with regard to the quality and quantity of the ingredients in the supplement. The form and source of the CS can greatly affect its pharmacokinetic profile. In general, the molecular composition (processing and degree of fractionation, range of particle size, range of molecular mass, location and percentage of sulfation, and purity of CS) is dependent on the species or tissue of origin and can dramatically alter the metabolic fate and therapeutic results [40,76]. Because of different origins, concentrations, dosages, and molecular weights, the same clinical effects between products cannot be assumed, which is important, because many manufacturers attempt to do this.

When CS is administered orally, it is not known whether the product is efficacious in the intact, fragmented, or disaccharide form, with intestinal degradation and liver metabolism likely determining the ultimate form of available CS [77]. When low-molecular-weight CS was administered intact and was not exposed to GAG-degrading enzymes, it showed efficacy in stimulating chondrocytes and protecting them from degradation [31]. The gastrointestinal mucosa contains a variety of GAG-degrading enzymes

[77,78], however, which strongly suggests that the intact CS is not absorbed but is rather enzymatically degraded. Presumably, in addition, CS may be subjected to a large first-pass effect in the liver such as occurs with other GAGs [77–79], causing further degradation into unsaturated disaccharide units before reaching the systemic circulation [80,81]. Recent studies have demonstrated that CS is orally absorbed in the horse but that its molecular weight and source can have a direct influence on its permeability across the gastrointestinal tract. Higher intestinal permeability was reported for CS with a lower molecular weight, because horses with the 8.0-kd CS had 32% bioavailability compared with 22% for horses with the 16.9-kd CS [37,82]. The specific concentrations achieved in various tissues remain to be determined, but assays have been developed to detect the resulting disaccharides in blood [10,41,80,83]. Absorption of CS seems to be rapid [84] and has been shown via radiolabeling to have a tropism for articular cartilage and synovial fluid [85]. Cartilage concentrations have been shown to exceed plasma concentrations for prolonged periods after dosing [84,86], and synovial fluid concentrations actually exceed those in plasma by 66.5% [85,86]. After multiple dosing, CS disaccharides have been found to accumulate in the plasma, indicating a substantial carryover effect that may explain why CS has a delayed treatment effect of 2 to 4 months [87]. In addition, it may explain why human patients have noted continued improvement in clinical trials even after administration was discontinued, which may support administration of lower doses of CS in maintenance therapy [75].

Similar to GLN, many in vitro studies on the effect of CS on cartilage explants or cell culture have been performed, demonstrating a tendency for CS to be better for controlling the symptomatic effects of OA by decreasing inflammation. Exogenous CS has been shown in vitro to have no detrimental effects on normal articular cartilage explants [48]. Similar to GLN, CS has been shown in vitro to increase the synthesis or decrease the turnover of PGs and GAGs [48,64,88,89]. The effects of CS on the metabolism of type II collagen have also been reported to be minimal [88]. CS has been shown in vitro to inhibit MMP expression and activity [64,84] as well as that of aggrecanase in a dose-dependent manner [90]. It has also been shown to increase the concentration and improve the composition (viscosity) of HA [84,91], which may play a role in its anti-inflammatory effects. In addition, using equine cartilage explants as well as human chondrocyte cultures, CS has been shown to have the potential to affect the inflammatory cycle via regulation of PGE_2 [47,64,88] as well as NO production [47,64], such that both are decreased after CS administration.

In a complete Freund's adjuvant carpitis model in horses, CS was compared with polysulfated glycosaminoglycans (PSGAGs) administered intramuscularly and was shown to be markedly less effective than PSGAGs for relief of lameness, stride length, and carpal flexion [92]. In one complete Freund's adjuvant model in horses, there was evidence of therapeutic value of CS irrespective of the route of administration (oral or intramuscular) [93].

In rats, cartilage CS concentrations were greater with oral administration than with intramuscular administration [91]. CS prevented a reduction in cartilage PG content on chymopapain-induced articular cartilage injury in rabbits [89].

Similar to GLN, human meta-analyses of CS were heterogeneous with regard to CS formulation, route of administration, dosage, severity of OA, and measurement of outcome [28,69,70,74]. CS has generally been shown to have a longer duration of action, producing fewer side effects than NSAIDs, while improving the clinical signs associated with OA. In fact, CS administration may allow for the NSAID dose to be lowered or even discontinued after 6 to 8 weeks [28,69]. Treatment of CS may not have to be continuous either, because intermittent treatment has proven to reduce symptoms significantly in some patients. CS demonstrated no toxic effects over placebos when administered to people for 2 months to 6 years.

Glucosamine and chondroitin sulfate combination

In general, as stated previously, studies have suggested that GLN would be best for potential inhibition of OA progression, whereas CS may be best for controlling the symptomatic action of OA. Many equine nutraceuticals have some sort of combination of GLN and CS (GLN-CS). In fact, most of the reported equine nutraceutical research is based on combination products, with most of the research being performed on Cosequin. Cosequin is a combination product containing GLN HCl, CS, manganese, and vitamin C. Many products simulate Cosequin and attempt to compete on the basis of decreased cost or the addition of different ingredients without any proof of comparable efficacy [7].

In normal equine cartilage explants conditioned with IL-1, there were no detrimental effects of GLN-CS on cartilage metabolism [48], and a tropism for articular cartilage that is in a reactive state has been suggested [31]. In cell culture and equine cartilage explant studies, it has been suggested that the combination inhibits proteolytic activity potentially via retardation of the molecular and biochemical events associated with proinflammatory cytokines, including decreased PGE_2 and NO levels [47,64,94,95], while also preventing GAG degradation [48,64]. In addition, the doses used in the combination are lower in vitro than when each is used alone, which may be a result of complimentary or synergistic effects. Synergistic effects have also been suggested in a rabbit instability model in which the combination was more efficacious in retarding cartilage lesions than with either agent alone [31]. In addition to the effects of the GLN-CS combination on articular cartilage, it has been shown that the combination improves collagen synthesis in tenocytes and ligament cells as well, suggesting that GLN-CS may be important for use in accessory joint structures [96].

In a complete Freund's adjuvant model of chemically induced synovitis in horses, Cosequin showed no clinically detectable benefits in one study [97]

but demonstrated a significant treatment effect for the oral and intramuscular routes of administration in another study [93]. In studies of clinical cases, horses showed improved lameness, flexion, and stride length, whereas navicular horses showed significant improvement in soundness compared with placebo controls [98,99]. GLN-CS (plus methylsulfonylmethane [MSM]) was also evaluated in horses with tarsal OA using gait analysis [100]. In a small number of horses, this study found a significant improvement in the left-to-right symmetry of peak vertical ground reaction forces and impulses as well as tarsal range of motion and joint energy generation during stance. Based on the results of biomarker concentrations within the synovial fluid of dogs after transection of the cranial cruciate ligament, GLN-CS modulates the GAG structure in normal and OA joints, an effect that proved to be systemic and not localized to the OA joint [101]. Oral administration of GLN-CS at doses greater than those recommended in dogs and horses is associated with a good safety profile, with no alterations in hematologic or clotting profiles [102,103] and with adverse effects limited to gastrointestinal upset [104] and polyuria or polydipsia in dogs [105].

Methylsulfonylmethane

MSM is a normal oxidative metabolite product of industrial-grade dimethyl sulfoxide (DMSO). Other names for MSM include dimethylsulfone, crystalline $DMSO_2$, and $DMSO_2$. MSM is naturally found in fruit, alfalfa, and corn [106] and is quite soluble in water [107]. When MSM was administered orally to people, however, only 3% was recovered, suggesting further modification in the gastrointestinal tract or liver [106]. In a guinea pig study, MSM was radiolabeled with only 1% incorporation into serum methionine and cysteine, which are essential for the synthesis of proteins [106]. There is little known about the pharmacokinetics of MSM. Similar to DMSO, MSM has been suggested for the management of musculoskeletal pain as well as OA [108,109]. In genetically susceptible mice, MSM was effective in preventing inflammatory joint disease [110]. When human neutrophils were artificially stimulated to produce oxidative compounds, MSM demonstrated anti-inflammatory and antioxidant mechanisms [111] that are independent of the prostaglandin pathways [112]. In addition, similar to DMSO, MSM may relieve muscle discomfort by decreasing the nerve impulses via cholinesterase inhibition, and subsequently reducing muscle spasm [113]. Sulfur is an important component of matrix GAGs, and MSM is considered a sulfur supplement because it contains 34% elemental sulfur. What is not clear is whether the potential increase in serum sulfur after supplementation with MSM is more beneficial to the GAGs in the articular cartilage, the muscles, or either. Oral administration of MSM to rats for 90 days did not cause any adverse effects or increased mortality, but little is known about safety of the product [114]. No controlled or clinical studies have been published to support the use of MSM for the management of OA in horses. One

uncontrolled non–peer-reviewed study demonstrated improved performance in Standardbred racehorses in training [115].

Fatty acids

Polyunsaturated fatty acids (PUFAs) are natural products that are found in fish and plants. The ω-3 (n-3) PUFAs contain α-linolenic acid that is desaturated in the body to produce eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) analogues of arachidonic acid. In theory, dietary supplementation with n-3 PUFAs would potentially be useful in OA, because the metabolic byproducts of EPA tend to be less inflammatory and immunosuppressive than those of arachidonic acid while also being vasodilatory and antiaggregatory. In an in vitro cartilage explant model of OA, supplementation with n-3 PUFAs showed a decrease in the expression and activity of aggrecanases and MMPs as well as in the expression of COX-2, IL-1α, and TNFα [116]. The anti-inflammatory effects of fatty acids have been attributed to stabilization of cell membranes, inhibition of the formation of inflammatory mediators, and protection against oxidation [117–119].

Another fatty acid that is being used more in equine nutraceuticals is cetyl myristoleate (CM). CM is an ester of cis-9-tetradecenoic acid (myristoleic acid) and 1-hexadecanol (cetyl alcohol) and is a 14-carbon monounsaturated ω-5 fatty acid. CM may act by inhibition of the 5-lipo-oxygenase pathway [120], which is responsible for the metabolism of leukotrienes, potent inflammatory mediators, from the arachidonic acid cascade [121]. CM has been shown to confer protection against adjuvant-induced arthritis in rats [122]. In another rat study, orally administered CM significantly reduced the incidence and severity of adjuvant-induced arthritis [123]. Human beings with knee OA who were administered CM showed improvement in knee flexion and function [124]. There are no published reports on safety, absorption, metabolism, or clinical use of CM in horses, however.

Collagen hydrolysate

Hydrolyzed gelatin products have been used safely in human foods and pharmaceutics for years [125]. Collagen hydrolysate is derived from bovine or porcine skin and bones, with gelatin being the purified protein formed by hydrolysis of collagen. Oral administration does not seem to have direct analgesic or anti-inflammatory effects. There does seem to be some degree of immune response, however. For example, ordinarily, when rats are immunized with undenatured type II collagen, arthritis is induced [126]. If soluble type II collagen is orally administered before immunization, however, there is an immunologic hyporesponsiveness decreasing IgG levels and suppressing the incidence of the collagen-induced arthritis [127]. The main theory

behind using collagen hydrolysate in the management of OA is based on directly stimulating chondrocytes to synthesize collagenous matrix [28,125]. In a radiolabeled study in mice, approximately 95% of the orally administered gelatin hydrolysate was absorbed, with a pronounced and long-lasting accumulation in articular cartilage (twice as high as controls) [128]. In additional support of this theory, a chondrocyte cell culture model supplemented with collagen hydrolysate demonstrated a dose-dependent increase in type II collagen biosynthesis [129]. There are currently no published reports on the safety, absorption, metabolism, or clinical use of collagen hydrolysate in the horse.

Vitamins, minerals, and trace elements

Many vitamins, minerals, and trace elements are included to some degree within many equine nutraceutical products. The use of these ingredients is generally based on supplying those elements that are necessary for the maintenance of normal cartilage metabolism as well as providing protection against reactive oxygen species. The most commonly supplemented vitamin is ascorbic acid (vitamin C). Vitamin C is required for normal collagen and aggrecan synthesis [53,130,131]. In addition, vitamin C is a free radical scavenger that potentially provides antiarthritic effects by protecting chondrocytes from damage by pro-oxidants [132]. In a guinea pig model of OA, vitamin C has been shown to have a protective effect on cartilage degeneration [133,134]. α-Tocopherol (vitamin E) is the only lipid-soluble chain-breaking antioxidant in the plasma [135], and thus also has the potential to protect chondrocytes from damage by reactive oxygen species [132,136,137]. Vitamin E may also have anti-inflammatory effects in the joint [138] as well as the ability to enhance chondrocyte growth [139,140]. Vitamin D has been shown in tissue culture to have a direct effect on cartilage by stimulating mature chondrocytes to increase PG synthesis [141]. β-Carotene (provitamin A) is also a free radical scavenger that likely has a protective effect against reactive oxygen species [132]. Selenium, zinc, manganese, niacinamide, and bioflavonoids all have potential antioxidant effects as well, with manganese also being a cofactor in the biosynthesis of GAGs [142].

Herbs

There are many equine nutraceuticals that contain herbal ingredients. Although some of these may have some clinical merit, there are too many products available with not enough research performed on individual products. For herbal supplements, all the warnings stated previously should be amplified to the consumer; often, the thought is that because they are ''natural'' products, they do not cause any harm. There are many pharmaceutics

that are based on "natural" plants that can cause harm to a given individual, however. When examining a nutraceutical with herbal supplements, one should investigate each herbal ingredient carefully to determine whether there is any research available as to its effects on the joint as well as potential side effects.

Summary

In summary, the amount of scientific information on equine nutraceuticals is increasing all the time. In general, if a practitioner is going to recommend a nutraceutical to clients, he or she should lean toward products that have been scientifically examined. These products most likely have the potential to help equine lameness patients in the preventative or early stages of OA rather than in the advanced stages. Because of the lack of regulation, however, the equine practitioner should examine each nutraceutical individually with their clients and make them aware of the pitfalls of these products.

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