Critical review of research evaluating glucosamine-based nutraceuticals for treatment of joint pain and degenerative joint disease in horses

Wendy Pearson[1] and Michael Lindinger[2]
[1] *CANTOX Health Sciences International*
2233 Argentia Rd., Suite 308
Mississauga ON
L5N 2X7 Canada
(905)542-2900 ext. 288
(905) 542-1011 (fax)
wpearson@cantox.com
[2] *Department of Human Health and Nutritional Sciences*
University of Guelph, Guelph ON N1G 2W1

Abstract

It was more than 25 years ago that two German researchers quietly associated supplementation of glucosamine to horses with improvement of joint disease. In the 25 years since, glucosamine and its related chemical chondroitin have become the most extensively used non-allopathic treatment for articular inflammation and arthritis in horses. While the practice of supplementing arthritic horses with these products has, in earlier times, been considered a complete unknown in terms of safety and efficacy, the story is quite different today. An expanding body of knowledge on glucosamine continues to raise scientific curiosity about the general principles and cellular basis of treating equine osteoarthritis and inflammation with glucosamine-based nutraceuticals. This review critically interprets the published *in vivo* studies designed to investigate glucosamine-based nutraceuticals for horses with joint pain and/or inflammation. These studies have contributed valuable preliminary information as to the possible usefulness of glucosamine-based nutraceuticals in horses, and demonstrate an encouraging trend to manufacturers of these products investing in research. Importantly, however, these studies have significant limitations that until now have not previously been addressed. For example, measures of bioavailability in horses have not acknowledged fundamental binding of glucosamine to serum proteins and as such likely provide gross underestimates. Other more general limitations include underpowered studies, unbalanced research design, non-existent or inappropriate controls, unclear or non-representative experimental conditions and uncharacterized or complex, heterogeneous experimental materials. Shortcomings such as these have heavily influenced interpretation, as well as misinterpretation, of results. Because of these substantial limitations, the existing data on glucosamine-based nutraceuticals for horses cannot be considered conclusive evidence supporting the efficacy of glucosamine-based nutraceutical

Proceedings of the 4th European Equine Health & Nutrition Congress, April 18-19, 2008 – Wageningen University and Research Centre, Wageningen, the Netherlands. Page 81-91

products. Recommendations are made as to minimum scientific requirements for future research into glucosamine-based nutraceuticals for horses.

Introduction

Lameness is among the most important causes of poor performance in racing horses (Verheyen and Wood 2004). While non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids remain important therapeutic resources for treatment of overt clinical lameness, nutraceuticals are becoming widespread as therapeutic and prophylactic management strategies for horses with low-grade, sub-acute articular damage and for those at risk of developing articular problems (Trumble 2005; Neil et al., 2005). It was more than 25 years ago that two German authors quietly associated supplementation of glucosamine to horses with improvement in clinical signs of joint disease (Jaeschke and Steinbach 1982). In the 25 years since, glucosamine and its related chemical chondroitin have become the most extensively used non-allopathic treatment for articular inflammation and arthritis in horses (Trumble 2005). The contemporary scientific literature abounds with new papers almost daily on glucosamine sulphate (GS), glucosamine hydrochloride (GH) and chondroitin sulphate (CS) for treatment of cartilage inflammation using a wide variety of animals, including humans. With this expanding body of knowledge on glucosamine arises a new scientific curiosity about the general principle of, and cellular basis for, treating equine arthritis and inflammation with glucosamine-based nutraceuticals.

In an industry well-accustomed to extrapolating *in vitro* data and data generated from research in non-equine species, it is encouraging to see scientific research appearing that attempts to directly investigate anti-arthritic nutraceuticals in horses. Importantly, these publications illustrate an encouraging trend to equine supplement manufacturers investing in product research to demonstrate safety and/or efficacy. Often, however, there are methodological and/or analytical compromises made in these investigations which impact the interpretation of the data and limit the conclusions that can be drawn. The purposes of this paper are to provide a critical review of the *in vivo* equine literature published between 1994 and 2006 relevant to the use of glucosamine-based nutraceuticals, and to make recommendations for minimal essential criteria that should be adopted in future equine research in this area.

In vivo studies in horses

It is important that the experimental model chosen to test nutraceutical products provides a reasonable representation of the pathophysiological condition for which it is (to be) used as a prophylaxis or a treatment. For this reason, an early report of glucosamine-based nutraceuticals in horses (White et al., 1994) was not particularly useful for furthering our understanding of the effect of dietary glucosamine-based nutraceuticals for horses. However, White et al. (1994) made an important point with respect to the type of study designs that are not well

Proceedings of the 4th European Equine Health & Nutrition Congress, April 18-19, 2008 – Wageningen University and Research Centre, Wageningen, the Netherlands. Page 81-91

suited to the assessment of nutraceutical products. The authors evaluated the effect of a dietary nutraceutical "Cosequin" (Nutramax; 9g twice daily for 30 days) on inflammation induced by intra-articular injection of Freunds Complete Adjuvant (FCA). Cosequin is a dietary product formulated for performance horses, and is composed of glucosamine hydrochloride (GH; 60%), chondroitin sulphate (CS; 20%), manganese (0.5%), ascorbate (3.5%) and undisclosed filler (16%). The authors concluded that Cosequin had no inflammatory effect in an equine model of inflammation induced by intra-articular FCA. FCA is a concentrated formulation of mycobacteria (usually *Mycobacterium tuberculosis*) in mineral oil. Injection of FCA results in a large increase in tumour necrosis factor-alpha (TNF-α) (Geboes et al., 2007), pronounced effusion and neutrophilia (Levy et al., 2000) and marked and sustained lameness (grade 4, AAEP scale; Toutain and Cester 2004; White et al., 1996). It is highly likely that this very rapid onset and severe model of inflammation overwhelmed any inflammatory pathways that had the potential to be inhibited by dietary Cosequin. This model of inflammation did not emulate the condition for which the product was intended; powerful drugs are typically used to treat acute, severe inflammation. In contrast, dietary nutraceuticals are generally applied as a preventative and/or to treat low-grade, chronic lameness (Trumble 2005). If a horse turned up lame in a stable to a similar magnitude as that induced by intra-articular FCA, it is extremely unlikely that horse would be treated with a dietary nutraceutical. Thus a negative result in this model does not further our understanding of the effectiveness of the product within a context of intended use. A second important limitation of this study is that, while the composition of the experimental product was provided, there was no confirmatory analysis of product label composition conducted by the investigators. This is not to suggest that the label information for the product was inaccurate; rather it is to demonstrate independently that the information is *not* inaccurate. A well-known characteristic of the nutraceuticals industry is one of inconsistent quality control (Oke et al., 2006), and confirmatory analysis of experimental products should become standard due diligence on the part of investigators.

In contrast to the White study, authors of a subsequent *in vivo* study of Cosequin concluded that the product was beneficial in arthritic horses (Hanson et al. 1997). Twenty five horses with degenerative joint disease received 9 (for horses < 500 kg) or 12g (for horses > 500kg) Cosequin twice daily for 6 weeks, and the authors reported improvements in lameness grade and stride length. The most important limitation to this study is that there was no control group maintained under similar conditions in the absence of supplementation with Cosequin. Thus it is not possible to determine whether the improved outcomes were due to normal healing as a function of time, reduced activity, supplementation with Cosequin, or to other confounding factors. Indeed, many cases of lameness in performance horses improve over time without any treatment at all (Ross et al., 1999), which suggests that the null hypothesis tested in this study (i.e. "baseline measurement and the

following repeat examinations are the same") had a good chance of being rejected even in the absence of any treatment. Some important discussion points missing from this study include the duration and aetiology of lameness of the experimental horses, records of exercise and/or rest after inclusion of horses in the study, and potential influences of differing diets or management strategies for the horses over the course of the study. Without at least this information the experimental data provide little useful information on the efficacy of the supplement. Unilateral study designs (without appropriate control groups) such as this can provide some useful information. But importantly, balanced interpretation is critical in that researchers need to acknowledge potential confounding factors to the reader in order to support the interpretations and conclusions made. So, like the White study, the experimental design of this study prohibits a conclusion addressing whether Cosequin actually does work in horses or not.

Caron et al. (2002) reported the *in vivo* effect of GH on serum markers of bone [osteocalcin (OC) and pyridinoline crosslinks of type-I collagen (PYD)] and cartilage [keratan sulphate (KS)] metabolism. Sixteen juvenile standardbreds in early race training completed the 48-week study. Nine treatment horses received 4g GH twice per day and 7 control horses received 4 g glucose twice per day. GH had no effect on serum concentrations of OC, PYD or KS and, as such, the authors concluded that they were not able to obtain evidence that GH influences metabolism of bone or cartilage in growing and exercising horses. A number of factors influence the interpretation of these data. The choice of glucose as a placebo may be questioned because glucose can influence type I collagen expression *in vitro* (Zhang et al., 2007). Although the small amount of glucose fed in this study probably did not have a marked effect in the Caron study, there are no data in the literature to confirm this. The control group in this study should have demonstrated a significant decline in PYD over the time course of the protocol, an effect previously demonstrated in young exercising horses (Brama et al., 2000) and growing chickens (Pedrini-Mille et al., 1988). The fact that this decline was not observed in either the control or treatment group suggests that either the study was underpowered or that both the treatment (glucosamine) and the control (glucose) influenced this particular dependent variable. This raises a major concern regarding the absence of control groups for stage of growth or activity level of the experimental animals. The authors acknowledge that they did not use a sedentary and/or mature control group for this study, but they inappropriately dismiss this as unimportant to interpreting outcomes of the study. In fact, having no such controls leaves the authors and readers with no way in which to tease out effects of growth and/or exercise from effects of glucosamine (or placebo) on the serum levels of these biomarkers. The authors chose a model (exercise training) expected to significantly elevate OC (Smith et al. 2007) and keratan sulphate (Yoon and Halper 2005); such an elevation has the potential to mask any stimulatory effects of glucosamine on these biomarkers. An experiment

quantifying the effect of glucosamine on *steady-state* PYD, KS and OC, even *in vitro*, would be a useful prefix or adjunct to this study, and would improve the interpretations that can be made from it.

In an 8-year study of 10 show hunter-jumpers (2 years prior to treatment + 6 years of dietary treatment of 10g/day of an undisclosed glucosamine-based nutraceutical product), Rodgers (2006) reported a significant reduction in the frequency of intra-articular injection of hyaluronan and steroid from an average of 1.7 to 0.85 injections per year. Also, the mean duration between injections increased from 6.8 to 10.0 months after 2 or more years of supplementation, after which there was no additional improvement. Rodgers (2006) concludes that the dietary glucosamine-based nutraceutical decreases the need for distal tarsal joint injections to maintain soundness. This study did very well in considering a number of confounding factors, appeared to have good control over lameness evaluations, and was long term. But the study included only a small number of horses, and lacked any parallel control group. As such, it cannot be considered "proof" that the benefits noted over time accrued primarily as a result of supplementation with the glucosamine–based nutraceutical.

Forsyth et al. (2006) presents the most recent efficacy report of a glucosamine-based nutraceutical "Synoquin" for improving articular function in horses. In this study, 20 mixed breed, mixed age elderly geldings and mares were randomly allocated to treatment (n=15) or control (n=5) diet for 12 weeks. Treatment and control diets contained Synoquin (VetPlus) or an equal amount of 'filler' – the composition of which is not disclosed in the paper. Synoquin is composed of chondroitin sulphate (CS - 19% w/w), GH (50%), N-acetyl-D-glucosamine (5%) and filler (25%). The label composition was not confirmed by the investigators. Given the heterogeneous profile of the horses and the unbalanced allocation of horses to treatment and control groups, use of random assignment of horses to the groups is a limitation of this study. Allocation of horses to the control group should have been stratified to be representative of horses in the treatment group; this is particularly important because the control group (n=5) was substantially smaller than the treatment group (n=15). Each horse in the control group should have represented characteristics of 3 horses in the treatment group. Matching of control horses by sex, age, breed, and/or body mass with treatment horses would have improved the validity of the of the data obtained. Furthermore, the baseline characteristics of horses allocated to each group should have been provided, particularly as the data were only reported as mean change from baseline. Without any information on what the baseline data were for each group it is not possible to determine whether the results indeed reflect an improvement due to treatment, as concluded by the authors, or if the differences were simply artefacts of differing baselines. The authors do report that the data were of equal variance, but presumably it was the transformed data (i.e. '*change from baseline*') that were of equal variance, and not the actual baseline means from each group. This

is a major limitation which substantially limits the conclusions that can be drawn from the data.

Bioavailability and pharmacokinetics

Bioavailability may be defined as the total amount of ingested substance that is absorbed across the gastro-intestinal tract into the blood for distribution to tissues. Bioavailability of glucosamine is inherently difficult to measure accurately and, in general, these difficulties often result in underestimates of bioavailability. Important considerations when evaluating bioavailability of glucosamine in any species include: (1) there is substantial first pass removal of glucosamine by intestinal epithelial cells and the liver in all mammals which has been quantified (humans, dogs and rats; reviewed by Setnikar and Rovati 2001, Anderson et al. 2005); (2) the parent compound or its metabolites may be metabolized by cells of the blood, gastrointestinal tract and/or the liver; (3) glucosamine is very rapidly bound to plasma globulins, such that less than 1% of glucosamine remain free in plasma. Therefore when plasma proteins are precipitated prior to analysis of the supernatant for glucosamine, the glucosamine may effectively be precipitated along with the protein resulting in net analysis of only free glucosamine. Thus, it may be erroneously concluded that the bioavailability of glucosamine in horses is very low (2.5 or 5.9%; Du et al 2004 and Laverty et al. 2005, respectively). In this regard it is important to note that even though glucosamine is transported within the circulation bound to globulins, it does appear to remain in dynamic equilibrium with free plasma glucosamine and hence is capable of being rapidly extracted by virtually all cells receiving nutritive blood flow. In humans, dogs and rats the absolute oral bioavailability of glucosamine, based on the globulin-incorporated radioactivity of ^{14}C-labelled glucosamine, is 40-45%, with g.i. absorption as high at 88% of the administered dose (Setnikar and Rovati 2001).

There are other inherent difficulties in studying the bioavailability and kinetics of glucosamine and glucosamine-based nutraceuticals. Glucosamine is an endogenous substance that can be formed within cells using glucose as a precursor. Due to its structural similarity to glucose, it is a competitive inhibitor of glucose transport, probably via GLUT2 in hepatocytes (Uldry et al., 2002) or GLUT3 in chondrocytes (Windhaber et al., 2003). Glucosamine is taken up by virtually all cell-types in the body, leading to apparent volume of distribution that exceeds total body water (Setnikar and Rovati 2001, Anderson et al. 2005). Rapid tissue extraction of glucosamine could in and of itself contribute to the very low plasma concentrations measured by Du et al. (2004) and Laverty et al. (2005). But because ~99% of circulating glucosamine is bound to globulin, and lost from the plasma phase with current deproteinizing methods, highly sensitive analytical techniques are necessary to track free plasma glucosamine kinetics after oral dosing; techniques that have only recently become available.

The first report of GH and CS bioavailability in horses (Du et al., 2004) was composed of 2 parts. Initially 10 horses received i.v. or oral doses of GH + low molecular weight CS (LMWCS: 8kDa; 3 + 9 g) or GH + high molecular weight

CS (HMWCS: 16kDa; 3 + 9 g). Not surprisingly, given the rapidity and magnitude of glucosamine binding to plasma globulins, the authors were not able to detect GH in plasma with this protocol.

In their second experiment, 2 horses received i.v. or oral GH (125mg/kg; approx. 62.5 g per horse). An apparent bioavailability of GH based on free serum glucosamine (~2.5%) was significantly lower than both CS products. With respect to experiment 2, it is unfortunate that data from only 2 horses were used to estimate bioavailability of GH, because the two horses showed markedly different absorption profiles after oral administration of GH. The maximum plasma concentration (C_{max}) of GH in Horse 1 (~5 μg/mL) was achieved about 2.5 h after dosing, whereas Horse 2 reached a maximum of ~15 μg/mL in approximately 1.5 hours. Similarly, the Area Under the Curve (AUC) for GH was varied widely between the two horses (33.2 ± 23.8 μg/mL h).

Limitations of the Du et al. (2004) studies include the failure to account for the majority of glucosamine in plasma, the probable interaction between CS and GH because CS was administered in the presence of GH in the first experiment, the dose of GH administered to horses in the 2nd study (125 mg/kg) was far in excess of what would normally be given to horses prophylactically or therapeutically (usually ~20mg/kg), and the small number of horses used in the 2nd experiment.

The second equine glucosamine / chondroitin bioavailability and pharmacokinetics study (Laverty et al., 2005) addressed some of the limitations noted in the former study. In this study 8 horses received i.v. or oral doses of GH at 20mg/kg – a dose typical of what might be used in the field – after an overnight fast. The pharmacokinetic behaviour of a single oral dose was determined by collecting jugular venous blood over a 12 hour period. Synovial fluid was also obtained from each horse at 0, 1 and 12 hours after dosing in order to quantify hypothesized increases in synovial fluid GH. As with Du et al. (2004), only free glucosamine was determined, and the fraction bound to plasma globulins ignored. The mean apparent bioavailability of GH (5.9%) was similar to that reported by Du et al. (2004), and confirmed the high variability between horses with respect to magnitude (C_{max}) and time course of pharmacokinetic behaviour. Synovial fluid GH also increased, albeit to a negligible degree (>9-fold less than that seen in serum); a small increase in synovial fluid GH persisted beyond the time at which GH was no longer detectable in serum. The authors concluded that the appearance of ingested glucosamine in synovial fluid could not be expected to exert any substantial modification to chondrocyte metabolism, and thus *in vivo* bioactivity must result from a non-physiological, i.e. pharmacological, effect of GH on tissues other than cartilage.

The apparent disparity between serum and synovial fluid concentrations of post-dosing glucosamine in the Laverty et al (2006) study is challenged by others, who report human synovial fluid concentrations of glucosamine only about 25% lower that that of blood after dosing (Persiani et al., 2007). It is unlikely that this simply reflects a species difference. Perhaps most important is that synovial fluid glucosamine was determined after 2 weeks of oral dosing with GS (Persiani et al.,

2007) whereas Laverty et al. (2006) described accumulation 12 hours after a single oral dose. While there was no attempt at time-course assessment of glucosamine in human synovial fluid, Persiani et al. (2007) were likely detecting an accumulation of glucosamine over time, consistent with the reported accumulation of chondroitin sulphate in canine synovial fluid a period of repeated dosing (Adebowale et al., 2002).

Another important consideration is that the increase in synovial fluid GH represents only the net effect of free GH transport into synovial fluid. This includes uptake of glucosamine by cells within the joint, binding to other molecules, and incorporation into joint structures. Indeed, in a review of their detailed pharmacokinetic studies in mammals, Setnikar and Rovati (2001) noted that 2 h after administration of ^{14}C-labelled glucosamine to rats and dogs, the radioactivity accumulated in the knee cartilage of the treated animals to values 13-fold greater than in plasma. Therefore, it is unlikely that the discrepancy is just a species difference. It is not clear why the researchers performing the glucosamine bioavailability studies in horses did not quantify the total plasma glucosamine concentrations. They were seemingly aware of this literature, yet neglected to even mention its importance in determining bioavailability and discuss the impact on tissue distribution.

While a definitive conclusion as to the bioavailability and fate of oral glucosamine in horses has not been provided by these two equine studies, they do support at least 2 important hypotheses:

1.) glucosamine and chondroitin are absorbed from the intestinal tract of horses

2.) glucosamine does appear in the synovial fluid of horses

General recommendations for future research and Conclusions

Further studies, accounting for total plasma glucosamine, and preferably using larger numbers of horses to account for the wide variability in individual horse responses to dietary glucosamine, are needed to more clearly define the differential bioavailability and post-absorptive behaviour of GH, GS, and CS in horses. It might also be interesting to look at the post-dosing intracellular glucosamine or glucosamine metabolite content of equine erythrocytes as this may be a significant sequestration compartment for this species. Glucosamine is often administered to performance horses in order to pre-empt or treat joint disease (Trumble 2005). It is possible that glucosamine is actively transported into erythrocytes and because equine hematocrit can increase to more than 60% of total blood volume during high intensity exercise (Catalani et al., 2007), erythrocyte distribution and fate may be an important consideration in this species. Furthermore, glucosamine-based nutraceuticals are administered to horses almost exclusively as part of the diet. Thus, bioavailability and pharmacokinetics of glucosamine in the presence of a normal meal would provide

meaningful information on what can be expected of the supplement under normal conditions of use.

Species-specific research into glucosamine-based nutraceuticals for horses has, as yet, failed to produce substantial evidence for the functionality of these products in this species. Conclusions of both 'effective' and 'not effective' in the peer reviewed equine literature have been compromised to varying degrees by numerous experimental factors including underpowered studies, unbalanced research design, non-existent or inappropriate controls, unclear or non-representative experimental conditions (i.e. models) and uncharacterized or complex, heterogeneous experimental materials. Future studies on glucosamine-based nutraceuticals in horses need to embrace fundamental experimental paradigms in design and interpretation of data. Use of appropriate statistical methods for determining sample size will reduce the problem of underpowered studies. Studies should have balanced treatment and control groups, and the experimental material should be independently characterized by the investigators – i.e. investigators should perform confirmatory analysis on label composition and purity. Experimental research designs should reflect the expected conditions of use, and placebos should be chosen such that they have confirmed inactivity in the experimental model or condition. The improved application of sound scientific principles to the assessment of glucosamine and its related nutraceutical products will lead to a better understanding of the role that this class of products can play in supporting joint health in horses.

References

Adebowale, A., Du, J., Liang, Z., Leslie, J.L., Eddington, N.D., 2002. The bioavailability and pharmacokinetics of glucosamine hydrochloride and low molecular weight chondroitin sulfate after single and multiple doses to beagle dogs. *Biopharmaceutics and Drug Disposition*, 23, 217-225.

Anderson, J.W., Nocolai, R.J., Borzelleca, F.J., 2005. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. *Food and Chemical Toxicology*, 43, 187-201.

Brama, P.A., Tekoppele, J.M., Bank, R.A., Barneveld, A., Firth, E.C., van Weeren, P.R., 2000. The influence of strenuous exercise on collagen characteristics of articular cartilage in Thoroughbreds age 2 years. *Equine Veterinary Journal*, 32, 551-554.

Caron, J.P., Peters, T.L., Hauptman, J.G., Eberhart, S.W., Orth, M.W., 2002. Serum concentrations of keratan sulfate, osteocalcin, and pyridinoline crosslinks after oral administration of glucosamine to standardbred horses during race training. *American Journal of Veterinary Research*, 63, 1106-1110.

Catalani, G., Dottavio, M.E., Rasia, M., 2007. Acute training in racing horses at two different levels of effort: A haemorheological analysis. *Clinical and Hemorheological Microcirculation*, 37, 245-252.

Du, J., White, N., Eddington, N.D., 2004. The bioavailability and pharmacokinetics of glucosamine hydrochloride and chondroitin sulfate after oral and intravenous single dose administration in the horse. *Biopharmaceutics and Drug Disposition*, 25, 109-116.

Forsyth, R.K., Brigden, C.V., Northrop, A.J., 2006. Double blind investigation of the effects of oral supplementation of combined glucosamine hydrochloride (GHCL) and chondroitin sulphate (CS) on stride characteristics of veteran horses. *Equine Veterinary Journal Supplement*, 36, 622-625.

Geboes, L., De Klerck, B., Van Balen, M., Kelchtermans, H., Mitera, T., Boon, L., De Wolf-Peeters, C., Matthys, P., 2007. Freund's complete adjuvant induces arthritis in mice lacking a functional interferon-gamma receptor by triggering tumor necrosis factor alpha-driven osteoclastogenesis. *Arthritis and Rheumatism*, 56, 2595-2607.

Hanson, R.R., Smalley, L.R., Huff, G.K., White, S., Hammad, T.A., 1997. Oral treatment with a glucosamine-chondroitin sulfate compound for degenerative joint disease in horses: 25 Cases. *Equine Practice*, 19, 16-22.

Jackson, C.G., Plaas, A.H., Barnhill, J.G., Harris, C.L., Clegg, D.O., 2005. The pharmacokinetics of oral glucosamine and chondroitin sulfate in humans (Abstract). *Arthritis and Rheumatism*, 52. Late breaking abstract no L13.

Jaeschke, G., Steinbach, W., 1982. Causal treatment of arthrosis deformans in horses with glucosaminsulfate. *Deutsche tierärztliche Wochenschrift*, 89, 288-293.

Jia, L., Schweikart, K., Tomaszewski, J., Page, J.G., Noker, P.E., Buhrow, S.A., Reid, J.M., Ames, M.M., Munn, D.H., 2007. Toxicology and pharmacokinetics of 1-methyl-d-tryptophan: Absence of toxicity due to saturating absorption. *Food and Chemical Toxicology*, [Epub ahead of print] PMID: 17868966

Laverty, S., Sandy, J.D., Celeste, C., Vachon, P., Marier, J.F., Plaas, A.H., 2005. Synovial fluid levels and serum pharmacokinetics in a large animal model following treatment with oral glucosamine at clinically relevant doses. *Arthritis and Rheumatism*, 52, 181-191.

Levy, A.S., Simon, O., Shelly, J., Gardener, M., 2006. 6-Shogaol reduced chronic inflammatory response in the knees of rats treated with complete Freund's adjuvant. *BMC Pharmacology*, 6, 12.

Neil, K.M., Caron, J.P., Orth, M.W., 2005. The role of glucosamine and chondroitin sulfate in treatment for and prevention of osteoarthritis in animals. *Journal of the American Veterinary Medical Association*, 226, 1079-1088.

Oke, S., Aghazadeh-Habashi, A., Weese, J.S., Jamali, F., 2006. Evaluation of glucosamine levels in commercial equine oral supplements for joints. *Equine Veterinary Journal*, 38, 93-95.

Pedrini-Mille, A., Pedrini, V.A., Maynard, J.A., Vailas, A.C., 1988. Response of immature chicken meniscus to strenuous exercise: biochemical studies of proteoglycan and collagen. *Journal of Orthopedics Research*, 6, 196-204.

Persiani, S., Rotini, R., Trisolino, G., Rovati, L.C., Locatelli, M., Paganini, D., Antonioli, D., Roda, A., 2007. Synovial and plasma glucosamine concentrations in osteoarthritic patients following oral crystalline glucosamine sulphate at therapeutic dose. *Osteoarthritis and Cartilage*, 15, 764-772.

Rodgers, M.R., 2006. Effects of Oral Glucosamine and chondroitin sulfates supplementation on frequency of intra-articular therapy of the horse tarsus. I*nternational Journal of Applied Research in Veterinary Medicine*, 4, 155-162.

Ross, W.A., Kaneene, J.B., Caron, J.P., Gallagher, K.F., Gardiner, J.C., 1999. Factors influencing recovery from and duration of lameness in Michigan (USA) horses. *Preventive Veterinary Medicine*, 40, 127-138.

Rovira, S., Muñoz, A., Benito, M., 2007. Hematologic and biochemical changes during canine agility competitions. *Veterinary Clinical Pathology*, 36, 30-35.

Setnikar, I., Rovati, L.C., 2001. Absorption, distribution, metabolism and excretion of glucosamine sulfate. A review. *Arzneimittelforschung*, 51, 699-725.

Smith, M.Z., Goettsch, B.M., Van Ramshorst, R.D., O'brien, J.A., Jaque, S.V., Sumida, K.D., 2007. Resistance training and bone mineral density during growth. *International Journal of Sports Medicine* [Epub ahead of print] PMID: 17879877

Toutain, P.L., Cester, C.C., 2004. Pharmacokinetic-pharmacodynamic relationships and dose response to meloxicam in horses with induced arthritis in the right carpal joint. *American Journal of Veterinary Research*, 65, 1533-1541.

Trumble, T.N., 2005. The use of nutraceuticals for osteoarthritis in horses. *Veterinary Clinics of North America Equine Practice*, 21, 575-597, v-vi.

Uldry, M., Ibberson, M., Hosokawa, M., Thorens, B., 2002. GLUT2 is a high affinity glucosamine transporter. *FEBS Letters*, 524, 199-203.

Verheyen, K., Wood, J., 2004. Back problems and lameness in horses. *Veterinary Record*, 155, 751-752.

Vincent, H.K., Vincent, K.R., 2007. Influence of admission hematocrit on inpatient rehabilitation outcomes after total knee and hip arthroplasty. *American Journal of Physical and Medical Rehabilitation*, 86, 806-817.

White, G.W., Jones, E.W., Hamm, J., Sanders, T., 1994. The efficacy of orally administered sulfated glycosaminoglycans in chemically induced equine synovitis and degenerative joint disease. *Journal of Equine Veterinary Science*, 14, 350-353.

White, G.W., Jones, E.W., Stites, T., Hamm, J., Walls, R., Sanders, T., 1996. The efficacy of systemically administered anti-arthritic drugs in an induced equine carpitis model. *Journal of Equine Veterinary Science*, 16, 139-145.

Windhaber, R.A., Wilkins, R.J., Meredith, D., 2003. Functional characterisation of glucose transport in bovine articular chondrocytes. *Pflugers Archives*, 446, 572-577.

Yoon, J.H., Halper, J., 2005. Tendon proteoglycans: biochemistry and function. *Journal of Musculoskeletal and Neuronal Interactions*, 5, 22-34.

Zhang, X., Stewart, J.A. Jr, Kane, I.D., Massey, E.P., Cashatt, D.O., Carver, W.E., 2007. Effects of elevated glucose levels on interactions of cardiac fibroblasts with the extracellular matrix. *In Vitro Cell and Developmental Biology in Animals*, 43, 297-305.

Proceedings of the 4th European Equine Health & Nutrition Congress, April 18-19, 2008 – Wageningen University and Research Centre, Wageningen, the Netherlands. Page 81-91