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Low quality of evidence for glucosamine-based nutraceuticals in equine joint disease: Review of *in vivo* studies

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Summary

Nutraceuticals are increasingly applied to the management of equine arthritis and joint disease, particularly those based upon glucosamine and chondroitin sulphate. While the first report of using glucosamine in horses appeared more than 25 years ago, it was not until 1992 that isolated studies began to be reported. Since that time, 15 *in vivo* papers have been published in the equine literature, usually on products already commercially available and often seeking evidence for efficacy. These studies demonstrate an encouraging trend to manufacturers of these products investing in research, but most do not meet a quality standard that provides sufficient confidence in the results reported. This review discusses the entirety of published *in vivo* research on glucosamine-based nutraceuticals (GBN) for horses, including that on Cosequin, Cortaflex, Synequin, Sasha's EQ, Myristol, chondroitin sulphate, glucosamine sulphate and glucosamine hydrochloride; and considers experimental limitations of this research along with their impact on interpretation of results. A quality score was calculated for each paper according to preset quality criteria. A minimum quality standard of 60% was set as the threshold for confidence in interpretation of results. Of the 15 papers reviewed, only 3 met the minimum quality standard. Experimental limitations of each research paper are discussed. It is concluded that the quality of studies in this area is generally low, prohibiting meaningful interpretation of the reported results. New high quality research on GBN for horses is needed and recommendations for future research are discussed.

Abbreviations

GBN:	Glucosamine-based nutraceuticals
GS:	Glucosamine sulphate
GH:	Glucosamine hydrochloride
CS:	Chondroitin sulphate
QS:	Quality score
WF:	Weighting factor
WFP:	Weighting factor points

Introduction

Lameness is a perennial limitation to the utility and wellbeing of performance horses (Rossdale *et al*. 1985; Verheyen and Wood 2004; Steel *et al*. 2006). While nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids remain important therapeutic resources for treatment of overt clinical lameness, nutraceutical drugs are becoming commonplace as therapeutic and prophylactic management strategies for horses with low-grade, subacute 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 2 German authors associated supplementation of glucosamine to horses with improvement in clinical signs of joint disease (Jaeschke and Steinbach 1982). Since then, glucosamine and its related chemical chondroitin have become the most extensively used nonallopathic 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 in a wide variety of species, including man. With this expanding body of knowledge arises a new scientific curiosity about the general principle of, and cellular basis for, treating equine arthritis and inflammation with glucosamine-based nutraceuticals (GBN).

In an industry well-accustomed to extrapolating *in vitro* data and data generated from research in nonequine species, it is encouraging to see scientific research appearing that attempts directly to investigate GBN in horses. Importantly, these publications illustrate a heartening trend to equine supplement manufacturers investing in product research to evaluate target animal safety and/or efficacy. But in order to provide meaningful information about whether or not GBN are useful in horses with lameness, these studies must adhere to the fundamental principles of quality science. The purpose of this review is to assess objectively the quality of published *in vivo* literature on GBN for horses in order to determine the strength of evidence for their use in horses.

Defining GBN

For the purpose of this review, a GBN is defined as a dietary product intended for use in horses with lameness or that are

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maintained under conditions expected to increase their risk for developing lameness. A GBN is further defined as any product that does not contain any known allopathic anti-inflammatory drugs and which is promoted and/or marketed on the basis of containing: 1) GH, GS, CS or any combination of these; 2) molecular precursors to CS, GH or GS or any combination of these; or 3) raw materials from which glucosamine and chondroitin are derived, including bovine or shark cartilage and ocean molluscs.

Search strategy

A search of the literature was conducted in order to identify peer reviewed research publications describing *in vivo* effects of GBN in horses. This search was designed to be as broad as possible in order to encompass all published equine studies on GBN, regardless of the test product or experimental circumstances. The search strategy is described in Table 1.

A quality score (QS) (%) was calculated for each paper according to predetermined quality criteria (Table 2). This was done by allotting points for each quality criterion:

Paper meets criterion	= 2 points
Paper partially meets criterion	= 1 point
Paper does not meet criterion or criterion not reported	= 0 points

Each paper was scored individually. The number of points (P) obtained for each criterion was multiplied by a weighting factor (WF) in order to put emphasis on those criteria which are essential to the correct interpretation of data (Tier 1 criteria), and put less emphasis on those criteria which are less essential (Tiers 2 and 3 criteria). Tier 1 criteria were multiplied by a WF of 3; Tier 2 criteria were multiplied by a WF of 2, and Tier 3 criteria were multiplied by a WF of 1. The resulting weighting factor points (WFP) were added together, divided by the number of relevant criteria (RC), and then expressed as a ratio to the total possible number of WFP divided by 30 (maximum RC) (TWP). This ratio was then multiplied by 100 to derive the QS:

QS = {[sum(WFP)/RC]/TWP} x 100

TABLE 1: Literature search for studies pertaining to use of GBN in horses

Databases searched	MedLine, Agricola, Agris, ToxFile, Mantis, Allied and Complementary Medicine, Biosis Previews, Biosis Toxline, FoodLine, Food Science and Technology Abstracts, NTIS, EMBASE. Manual search of reference lists of identified papers also performed.	
Search terms	Glucosamine OR chondroitin()sulphate OR chondroitin()sulphate OR nutraceutical OR shark()cartilage OR bovine()cartilage OR perna()mussel OR GAG OR glycosaminoglycan OR shell()fish AND Horses OR ponies OR equine OR horse OR pony AND Joint()disease OR arthritis OR inflammation OR pain OR lameness OR lame OR articular OR knee OR carpal OR fetlock OR intercarpal OR tarsal OR hind OR front OR bioavailability OR pharmacokinetics	
Total number of titles identified	315	
Number of titles included in assessment	15	
	Inclusion criteria	**Exclusion criteria**
Source	Published or in press in a peer-reviewed source.	Published in a nonpeer-reviewed source (e.g. magazine, web-site).
Report type	Full length article/study report of original equine research, short communication, systematic reviews.	Nonsystematic review, opinion letter, abbreviated Unpublished study report, abstracts, human studies, nonequines or *in vitro*.
Language	English	Not in English
Research design	All (controlled/noncontrolled, randomised or nonrandomised, blinded or open).	N/A
Route of exposure	Oral	Nonoral
Treatment exposure	Product intended for use in horses; does not contain any known allopathic anti-inflammatory drugs, and meets any of the following additional inclusion criteria; 1) are promoted and/or marketed on the basis of containing GH, GS, CS or any combination of these; 2) are marketed on the basis of containing molecular precursors to CS, GH or GS or any combination of these; or 3) are composed of raw materials from which glucosamine and chondroitin are derived, including bovine or shark cartilage and ocean molluscs.	
Relevant control	Any or none	N/A
Relevant endpoint(s)	Endpoints or observations related to anti-inflammatory and/or chondroprotective effect of GBN in horses with relevance to potential effect in modulation of articular inflammation and/or arthritis. Also, endpoints related to safety or bioavailability of GBN on any organ and/or biological condition, system or function in horses.	Endpoints or observations not related to anti-inflammatory and/or chondroprotective efficacy, safety or bioavailability of GBN in horses
Study setting	Stables, veterinary clinics or research institutions. Any country.	N/A
Health status of study population	Generally healthy horses or horses with lameness	Subjects with underlying diseases or illnesses other than lameness.
Ages	All	N/A

GH = glucosamine hydrochloride; GS = glucosamine sulphate; CS = chondroitin sulphate; GBN = glucosamine-based nutraceuticals; N/A = Not available.

Study quality was arbitrarily categorised as Excellent (QS>80.0), Good (70.0<QS≤80.0), Fair (60.0<QS≤69.9) or Poor (QS<60.0). The minimum QS threshold was set at 60.0; studies with QS less than 60.0 were considered to lack sufficient strength of evidence to support their published result(s).

Results

A total of 15 papers were identified that satisfied the inclusion criteria. Quality scores ranged 25.9–68.7%, median quality score 41.5%.

Three studies (Meulyzer *et al*. 2008, 2009; Pearson *et al*. 2009) met the minimum QS of 60.0, corresponding to studies on Sasha's EQ (Pearson *et al*. 2009), GH and GS (Meulyzer *et al*. 2008, 2009). All other studies had a QS<60 and did not meet the minimum quality standard.

TABLE 2: Quality appraisal tool

Criterion	Weighting factor (WF)
Tier 1 criteria	
1. Outcome variables appropriate to test hypothesis and/or support purpose.	3
2. Study design appropriate to test hypothesis or purpose.	3
3. Randomisation.	3
4. Treatment compared with placebo control.	3
5. If cross-over study, appropriate wash-out included.	3
6. If clinical study, cohort appropriately characterised.	3
7. If bioavailability study, binding of CS and GH to plasma/ synovial fluid proteins accounted for.	3
8. Appropriate statistical model applied to data.	3
9. Subject inclusion/exclusion criteria appropriate for outcome variable.	3
10. Power calculation performed for primary outcome variable.	3
Tier 2 criteria	
11. Blinding of outcome assessors.	2
12. Dose of experimental product scientifically justified.	2
13. If clinical study, geographical distribution of subjects specified.	2
14. If clinical study, measures of compliance specified.	2
15. Treatment and control groups balanced or stratified for sex/age/weight of horses.	2
16. Subject inclusion/exclusion criteria specified.	2
17. Baseline levels of risk factors potentially influencing outcome variable specified.	2
18. Baseline levels of outcome variables specified.	2
19. Use of concurrent medications potentially affecting outcome variables reported.	2
20. Adverse events reported.	2
21. Composition of test product provided.	2
22. Composition of placebo provided.	2
Tier 3 criteria	
23. Purpose stated.	1
24. Hypothesis stated.	1
25. Diet of horses specified.	1
26. Health status of horses specified.	1
27. Bodyweight of horses specified.	1
28. Age of horses specified.	1
29. Gender of horses specified.	1
30. Assessments of product safety specified.	1
	QS = [sum(P x WF)/RC]/TWP] x 100

P = points; WF = weighting factor; RC = relevant criteria; TWP = maximum possible weighted points; QS = quality score. Points for criteria; 'Criterion met' = 2 P; 'Criterion partially met' = 1 P; 'Criterion not met' or 'Criterion not reported' = 0 P. Maximum possible number of relevant criteria = 30.

Detailed summaries of the 15 identified papers are provided in Table 3 (www.evj.co.uk/suppinfo). Distribution of quality criteria across the 15 papers is shown in Fig 1. Quality criteria met by <25% of those studies for which the criteria were relevant are listed in Table 4.

Discussion

In general, the strength of evidence for the utility of GBN in lame horses is low, primarily due to the poor quality of studies. Given the considerable commercial interest in these products, this finding is of great importance and underlines a critical need for new, high quality research in this area.

Philosophically, scientific research on GBN for horses appears to be evolutionarily immature, i.e. in a 'pre-paradigm state' (Kuhn 1962). An experimental paradigm is a set of rules that define "...what is to be observed and scrutinised, the kind of questions that should be asked in relation to the subject, how these questions are to be structured, how the results of a scientific investigation should be interpreted, how an experiment is to be conducted and what equipment is available to conduct the experiment" (Kuhn 1962). Without a well-developed scientific paradigm, it is virtually impossible to evaluate the body of evidence for equine GBN as a whole. Critical limitations of the equine GBN body of evidence are discussed below, with examples of studies that highlight the importance of these limitations.

'Power' in persuasion

Without statistical power, there can be little statistical persuasion. And across the 15 papers evaluated, there was a universal absence of statistical justification for the number of horses used in each study. A study that does not recruit an appropriate number of horses increases the risk of a *type II* (β) statistical error – i.e. a null hypothesis is not rejected when it is indeed false. The most common cause of the *type II* error is small sample size, leading to an 'underpowered' study. Thus, data suggesting no effect of GBN treatment on outcome variables (White *et al*. 1994; Fenton *et al*. 1999; Caron *et al*. 2002; Du *et al*. 2004 [*Experiment 2*]; Meulyzer *et al*. 2008; Pearson *et al*. 2009 [*Experiment 1*]) cannot be considered reliable in the absence of statistical justification for the number of animals included in the study. It is possible that power calculations were not reported for these studies but were in fact conducted (many institutional Animal Care Committees require statistical justification for the number of animals used in a study before the study is permitted to proceed). But it must become common practice in equine GBN studies to report this information, so the reader is able to determine whether conclusions drawn from the data are supported by a sufficiently powered study. Based upon the low numbers of horses in many of these studies and the well-known population variability of some of the outcome variables evaluated (Keegan *et al*. 2008) it is probable that many were statistically underpowered.

Blinding

Given that many studies on GBN for horses are probably underpowered, it is curious that the majority of studies report

TABLE 3: Overview of *in vivo* studies pertaining to the evaluation of glucosamine-based nutraceutical compounds (GBN) in horses

significant effect of GBN treatment on at least one outcome variable - an apparent violation of the statistical paradigm of 'power'. While it is possible that a null hypothesis may be legitimately rejected in an underpowered study, the almost universal lack of blinding in these studies probably also contributes to the apparent success of these products in research. The 'placebo effect' is very powerful in patients with osteoarthritis and is recognised as having *bona fide* biological activity (Zhang *et al*. 2008). This is not to say that researchers are reporting deliberately untruthful data; rather, in the absence of blinding there is a natural tendency for evaluators to want to see an effect. So when subjective outcome variables, such as lameness evaluations (Hanson *et al*. 1997, 2001; Rodgers 2006; Keegan *et al*. 2007), are used exclusively, there is a predisposition on the part of the evaluator to observe an improvement, even if no clinical improvement actually exists. Lameness grading is notoriously plastic and unrepeatable. Even experts are only 25% more likely to agree on the existence of lameness in the forelimbs than by random chance alone, and they frequently disagree on grade of lameness on a given day or even on which leg is lame (Keegan *et al*. 2008). Even consistently using the same evaluator for a study provides little more than a random chance that s/he will agree with his/her own evaluations on the same horse at any given time (Keegan *et al*. 1998). Lameness evaluations are fine, but for these data to provide meaningful insight into the biological effect of GBN on horses, they must be supported by objective outcome measures, within a context of experimental blinding. These may include synovial fluid and/or serum analyses for biomarkers of

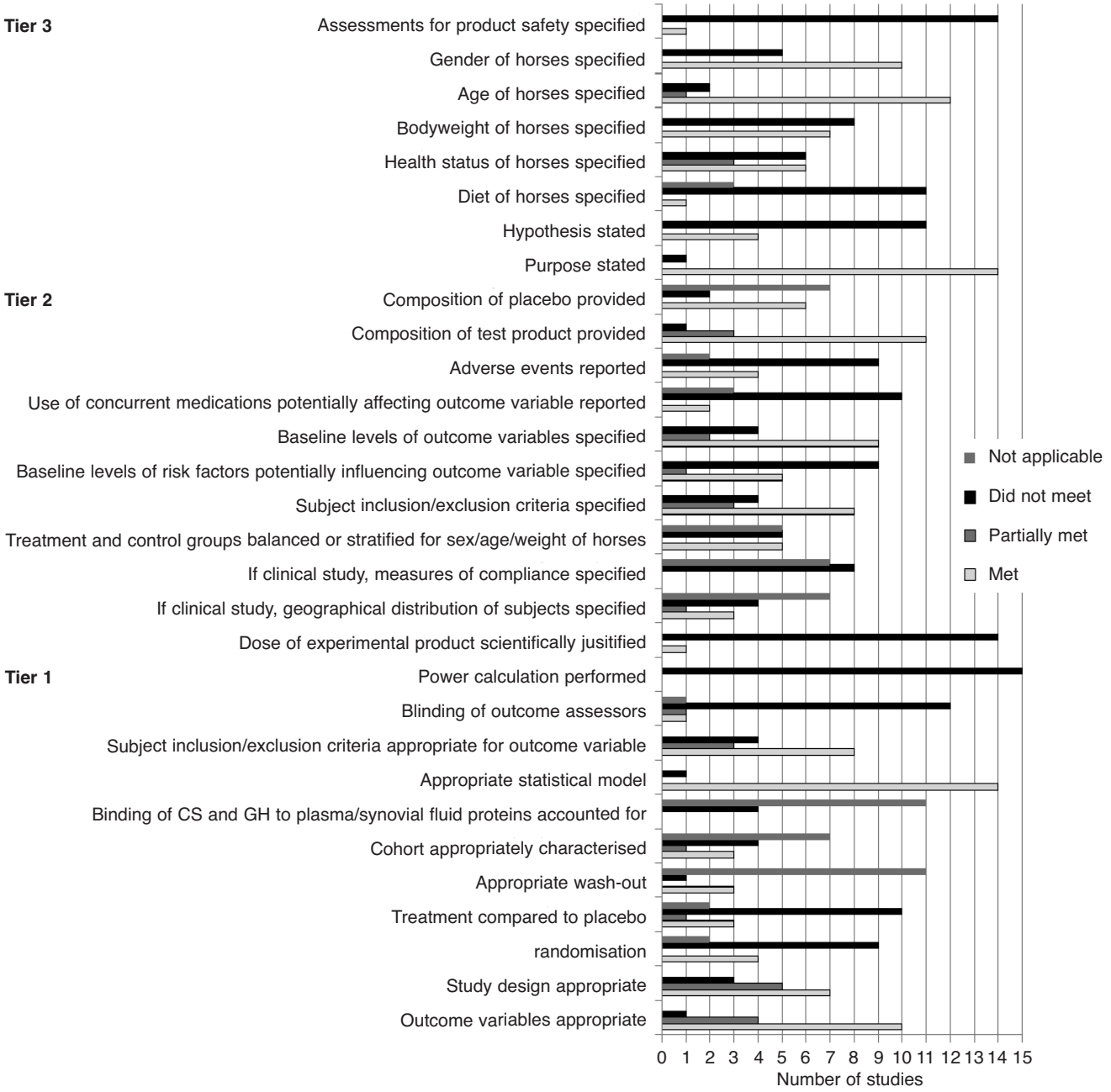

Fig 1: Distribution of quality criteria amongst research papers evaluating Glucosamine-based nutraceutical compounds (GBN) for horses. Bars represent % of papers that met quality criteria. Criteria are presented in increasing order of importance to quality scores.

inflammation and/or cartilage metabolism (White *et al*. 1994; Fenton *et al*. 1999; Caron *et al*. 2002; Verde *et al*. 2006; Meulyzer *et al*. 2008, 2009; Pearson *et al*. 2009), objective measures of gait analysis (Clayton *et al*. 2002; Forsyth *et al*. 2006), and/or imaging techniques, including radiographs (Hanson *et al*. 2001), ultrasound, arthroscopy or nuclear scintigraphy.

Experimental controls

Another common problem with studies evaluating GBN for horses was the lack of an appropriate placebo control. In some cases placebo controls were not used at all (Hanson *et al*. 1997; Rodgers *et al*. 2006) but, more frequently, the use of a placebo was reported but the composition not provided (Hanson *et al*. 2001; Forsyth *et al*. 2006; Verde *et al*. 2006; Keegan *et al*. 2007). Without this information, it is impossible for readers to determine if the dietary treatment was compared against an inert product as intended, or a product which might influence outcome measures in some way. Caron *et al*. (2002) provide an excellent example of how critical it is to select an appropriate placebo. In this study, the authors selected glucose as the 'placebo', a molecule since reported to influence *type I* collagen expression *in vitro* (Zhang *et al*. 2007) and likely to influence their outcome measures at some dose. The authors (perhaps justifiably) did not consider the small amount of glucose fed in this study important to their outcome measures, but there are no data in the literature to confirm this. To further complicate matters, the control data from this study are not consistent with an expected decline in pyridinoline crosslinks of *type I* collagen (PYD) over time, 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 question could have been addressed if the authors had maintained 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 glucose) on the serum levels of these biomarkers. The authors chose a model (exercise training) expected to elevate significantly osteocalcin (OC) (Smith *et al*. 2008) and keratan sulphate (KS) (Yoon and Halper 2005); such an elevation has the potential to mask any stimulatory effects of GH on these biomarkers. An experiment quantifying the effect of GH on steady-state PYD, KS and OC, even *in vitro*, would have been a useful prefix or adjunct to this study, and would improve the ability of the authors (and readers) to make balanced interpretations.

Defining the study group

'Lameness' is just a clinical sign; the human counterpart is 'limping'. Like limping, lameness can be due to a wide range of aetiologies and is influenced by many confounding factors. For a study on any putative remedy for lameness to be useful, the study group must be carefully characterised prior to study commencement and then reported in the paper. Loosely defined or loosely applied inclusion/exclusion criteria negate the value of a GBN study before it has even begun. This is a common weakness in studies on GBN for horses. In some cases inclusion criteria are minimalistic, generating a poorly defined and heterogeneous cohort (Hanson *et al*. 1997; Clayton *et al*. 2002; Forsyth *et al*. 2006; Keeton *et al*. 2007); in other cases inclusion criteria are not reported at all (White *et al*. 1994; Fenton *et al*. 2002; Rodgers 2006; Pearson *et al*. 2009). Particularly important for studies involving clinical field cases of 'lameness', which can be very difficult to standardise, basic information that should be made available in research papers, includes lameness aetiology, duration of lameness, exercise regimens before and after inclusion in the study, diet(s) and management strategies, sex, age and bodyweight(s).

Furthermore, these criteria should be sufficiently narrow to allow testing of a well-defined experimental hypothesis. Clayton *et al*. (2002) is an example of an equine GBN study that suffered heavily from problems with inclusion/exclusion criteria. The stated objective of the study was to "…assess changes in gait variables in horses with tarsal DJD…", yet 2 of the 8 horses recruited (25%) had only forelimb lameness, and a third had navicular 'changes' that were not localised to a front or hindlimb. Importantly, 6 of the 8 horses were described as bilaterally lame, which appears highly problematic as the primary outcome variable was the symmetry ratio between the lame leg and its contralateral leg. It is unclear how the authors could confidently interpret greater symmetry of gait in these bilaterally lame horses as improvement in lameness without at least addressing the possibility that observed differences may have resulted from a worsening in weightbearing or functionality of the one of the limbs, which could also have resulted in a net improvement in symmetry. Furthermore, gait

TABLE 4: Quality criteria fully met by <25% of studies for which the criteria were relevant

% of studies meeting criteria	Tier 1	Tier 2	Tier 3
0	• Power calculations performed for primary outcome variable • If bioavailability study, binding of CS and GH to plasma/synovial fluid proteins accounted for	• If clinical study, measures of compliance specified	
>25	• Treatment compared with placebo control (3 studies; Keegan *et al.* 2007; Meulyzer *et al.* 2008; Pearson *et al.* 2009)	• Blinding of outcome assessors (one study; Keegan *et al.* 2007) • Dose of experimental product scientifically determined (one study; Pearson *et al.* 2009) • Use of concurrent medications potentially affecting outcome measures reported (2 studies; Keegan *et al.* 2007; Meulyzer *et al.* 2009)	• Diet of horses specified. (one study; Pearson *et al.* 2009) • Assessments for product safety specified (one study; Pearson *et al.* 2009)

symmetry appears to be poorly correlated with athletic performance (Muñoz *et al*. 2006) and thus is not a particularly useful endpoint to evaluate clinical utility.

A matter of dose

With only one exception (Pearson *et al*. 2009), studies evaluating equine GBNs do not support the dose of product tested with objective experimental evidence. Rather, most rely on the advice of the product manufacturer (White *et al*. 1994; Hanson *et al*. 1997; Forsyth *et al*. 2001, 2006; Clayton *et al*. 2002) or on GS/GH doses utilised in other studies (Fenton *et al*. 1999; Caron *et al*. 2002), the majority of which also do not objectively defend their choice of dose. Therefore, data showing no effect of treatment (White *et al*. 1994; Fenton *et al*. 1999; Caron *et al*. 2002; Meulyzer *et al*. 2008) may have been vulnerable to sub- or supra-optimal dosing. Conventional veterinary health products which are subjected to careful regulatory control in the marketplace (a circumstance that does not yet fully define GBN for horses in the EU, USA or Canada), must, in most cases, show a biological response to dose in order to meet regulatory requirements. The dose response may be described by positive or negative linearity, bipolarity, sigmoidality etc., but no pattern at all disqualifies these products from the marketplace. The current body of knowledge on GBN for horses does not allow for an objective analysis of biological dose response, either for a single product or between products within the same category.

Related to the concern of dose is the aspect of safety. It is well-recognised that only dose differentiates a medicine from a poison; and it is essential to understand the threshold of safety for GBN. Only one study has attempted to titrate a GBN to horses in a manner seeking to observe adverse events at higher doses (Pearson *et al*. 2009). This needs to become standard practice for all research involving GBN for horses. While reporting such data may be scientifically 'dull', they are a fundamental piece of information that contributes to the overall understanding of the utility of this class of products.

Conclusions

While studies evaluating GBN for horses have appeared more frequently in the literature over the past decade, the quality of these studies is generally low. A poorly defined experimental paradigm makes balanced interpretation of individual studies difficult, and analysis of the body of literature as a whole virtually impossible. The pre-paradigm state of GBN for equine lameness can only progress to mature, systematic and standardised scientific inquisition when researchers are willing and able to define and adhere to a common strategy for empirical inquiry and, ultimately, to a global concurrence on the appropriate choice of methodology, terminology and the kind of experiments that are likely to contribute to deepened scientific understanding. Scientists must be ever mindful that clinicians and clients frequently rely on primary literature to devise treatment strategies for their cases. And, in most cases, reading of a published paper on equine GBN would not be accompanied by rigorous critical appraisal of study quality. Therefore, it is beholden upon the researchers to uphold an autocratic scientific standard when testing GBN for horses. Scientists and clinicians alike are invited to evaluate those scientific principles that should define future investigations into the subject and elevate the scientific standard to which GBN for horses should be held.

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Low quality of evidence for glucosamine-based nutraceutical compounds in equine joint disease: Review of *in vivo* studies

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TABLE 3: Overview of *in vivo* studies pertaining to the evaluation of glucosamine-based nutraceutical compounds (GBN) in horses

No. of horses	Age (years)	Description of animals	Study design	Intervention	Control	Duration of intervention	Reported treatment-related effects	Reference	Quality score (%)
Studies of 'Excellent' Quality (QS>80.0) No studies identified. *Studies of 'Good' Quality* (70.0<QS≤80.0) No studies identified. *Studies of 'Fair' Quality* (60.0<QS≤69.9)									
Experiment 1 4 treatment A 4 treatment B 4 treatment C 4 control	2.5–24	Healthy geldings and mares.	Randomised, controlled trial.	Sasha's EQ[i] A: 15 g/day B: 45 g/day C: 75 g/day	No treatment.	84 days	• No treatment effects on blood biochemistry, haematology or urinalysis.	Pearson *et al.* (2009)	68.7
Experiment 2 5 treatment; 5 control	5–12	Healthy geldings; articular inflammation induced by intra-articular IL-1 injection.	Randomised, controlled trial.	Sasha's EQ[i] plus flavouring and molasses 15 g b.i.d.	Flavouring and molasses	28 days	• Inhibition of IL-1-induced PGE_2 and GAG. • Reduction in synovial fluid PGE_2. • Significant increase in synovial fluid GAG. • Reduction in joint circumference.		
8 treatment A 8 treatment B 8 treatment C 8 treatment D	14.4 ± 2.1	Healthy mares	Randomised cross-over.	A: GS[r] 20 mg/kg bwt oral B: GS[r] 20 mg/kg bwt i.v. C: GH[q] 20 mg/kg bwt oral D: GH[q] 20 mg/kg bwt i.v.	none	Single dose	• Significantly higher synovial fluid levels of glucosamine are attained following oral administration of GS compared with GH. • No difference with i.v. dosing.	Meulyzer *et al.* (2008)	65.8
8 treatment (A–D)	14.4 ± 2.1	Healthy mares; articular inflammation induced by intra-articular LPS injection.	Randomised, cross-over trial.	GH[q] 20 mg/kg bwt in 500 ml sterile saline.	500 ml sterile saline.	Single dose 12 h after induction of lameness by LPS.	• No effect of GH administration on synovial fluid total protein or WBC. • LPS induces significant increase in synovial fluid glucosamine in treatment and control groups.	Meulyzer *et al.* (2009)	61.0

TABLE 3 (continued): Overview of *In vivo* studies pertaining to the evaluation of glucosamine-based nutraceuticals in horses

No. of horses	Age (years)	Description of animals	Study design	Intervention	Control	Duration of intervention	Reported treatment-related effects	Reference	Quality score (%)
Studies of 'Poor' Quality (QS≤60.0)									
8 treatment A 8 treatment B	6–15	Healthy mares	Randomised 2-way crossover.	A: GH[q] 20 mg/kg bwt i.v. B: GH[q] 20 mg/kg bwt oral.	none	Single dose	• GH 5.9% bioavailability in horses. • Other pharmacokinetic parameters described.	Laverty *et al.* (2005)	59.9
8 treatment; 6 control	10.8 ± 0.8	Geldings (n = 9), mares (n = 4) and stallions (n = 1) with lameness due to navicular syndrome.	Double blind, placebo controlled randomised clinical trial.	Cosequin[a] 16.5 g b.i.d.	Identical looking powder containing only excipients.	8 weeks	• Improvement in median lameness score. • Improvement in median algofunctional lameness index. • Improvement in overall clinical score. • No effect on radiographic signs of navicular syndrome.	Hanson *et al.* (2001)	59.1
39 (number of treatments and controls not reported).	N/A	Gender not reported Horses diagnosed with 'naturally occurring osteoarthritis'; lameness score 2–4, (AAEP scale).	Double-blind placebo controlled clinical trial.	Myristol[k] Days 1–14: 113 g/day Days 15–42: 75 g/day.	Undisclosed filler.	42 days	• Improvement in AAEP lameness score, lameness at walk, lameness after flexion, and pain upon manual joint flexion.	Keegan *et al.* (2007)	48.4
4 treatment A; 4 treatment B; 2 control	>2	Healthy nongestating Standardbred mares; articular inflammation induced by osteochondral defect.	Randomised, blinded controlled trial.	A: CS[h] (9600 kDa) 2.5 g B: CS[h] (25000 kDa) 2.5 g	Inactive placebo; composition not provided.	10 weeks	• Significant decrease in joint circumference. • Significant increase in maximum flexion angle. • Nonsignificant improvement in stride length. • Significant increase in synovial fluid GAG followed by significant decrease after 6 weeks. • Significant decrease in synovial fluid PGE_2. • Significant reduction in synthesis and/or release of MMP3.	Verde *et al.* (2006)	47.4
Experiment 1 10 treatment (A–D)	N/A	Mature horses	Randomised 4-way crossover study.	A: CS[n] (8 kDa) 3 g i.v. B: CS[n] (8 kDa) 3 g oral C: CS[n] (16.9 kDa) 3 g i.v. D: CS[n] (16.9 kDa) 3 g oral All treatments administered concurrently with 9g GH[o].	None	Single dose	• No measurable GH in peripheral blood. • CS (8 kDa) bioavailability 32.2%. • CS (16.9 kDa) bioavailability 22%[p]. • Other pharmacokinetic parameters described.	Du *et al.* (2004)	41.5
Experiment 2 2 treatment	N/A	Mature horses; health status not described	2-way crossover	A: GH[n] 9 g i.v. B: GH[n] 125 mg/kg bwt (~ 60 g) oral.	none	Single dose	• GH 2.5% bioavailability in horses. • Other pharmacokinetic parameters described.		

TABLE 3 (continued): Overview of *In vivo* studies pertaining to the evaluation of glucosamine-based nutraceuticals in horses

No. of horses	Age (years)	Description of animals	Study design	Intervention	Control	Duration of intervention	Reported treatment-related effects	Reference	Quality score (%)
9 treatment; 7 control	1.3–1.7	Healthy Standardbred horses in early race training.	Randomised, placebo-controlled trial.	GH[q] 4 g b.i.d.	Glucose 4 g twice daily.	48 weeks	• No effect on serum markers of bone (osteocalcin, pyridinoline crosslinks of *type I* collagen) or cartilage (keratin sulphate) metabolism.	Caron *et al.* (2002)	39.7
10 treatment	N/A	Healthy show jumpers	Open, uncontrolled field study.	Unidentified glucosamine-based product 10 g/day.	None	6 years (+ 2 years pretreatment)	• Significant reduction in frequency of intra-articular injection of hyaluronan and steroids. • Significant increase in mean duration between injections. • No further improvement beyond 2 years of supplementation.	Rodgers (2006)	36.8
25 treatment	6–20	Geldings (n = 17), mares (n = 7) and stallions (n = 1) with lameness due to degenerative joint disease.	Open, uncontrolled clinical trial.	Cosequin[a] 9[c] or 12[d] g b.i.d.	None	6 weeks	• Increase in stride length compared with baseline. • Reduction in lameness grade compared with baseline.	Hanson *et al.* (1997)	35.8
15 treatment; 5 control	15–35	Mixed breed geldings (n = 11) and mares (n = 9); health status not disclosed.	Double-blind, randomised controlled trial.	Synequin[e] Days 1–35: 20[f] or 30[g] g/day Days 35–60: 10[f] or 15[g] g/day Days 60–84: 10 g/day.	Filler; composition not provided.	12 weeks	• Significant improvement in % change in range of motion (weeks 8–12) in treated horses. • Significant increase in % change in stride length (weeks 8–12). • Significant increase in % change in swing duration.	Forsyth *et al.* (2006)	32.8
5 or 6/group (not specified)	1.2–1.7	Quarter horses; health status not provided.	2 x 2 factorial field trial [factors: longeing (+/-); GH treatment (+/-)].	GH[q] Weeks 1–4: 11.0 g/day Weeks 5–6: 7.0 g/day Weeks 7–8: 4.0 g/day.	No treatment	8 weeks	• No effect of treatment on serum osteocalcin. • Increase in serum keratan sulphate in walking horses.	Fenton *et al.* (1999)	31.1
6 treatment; 6 control	2–12	Healthy mares (n = 10) and geldings (n = 2); articular inflammation induced by FCA[b].	Parallel, unblended laboratory study.	Cosequin[a] 9 g b.i.d.	Untreated	36 days	• No effect on lameness grade, carpal circumference, synovial fluid protein or carpal flexion.	White *et al.* (1994)	28.2

TABLE 3 (continued): Overview of *In vivo* studies pertaining to the evaluation of glucosamine-based nutraceuticals in horses

No. of horses	Age (years)	Description of animals	Study design	Intervention	Control	Duration of intervention	Reported treatment-related effects	Reference	Quality score (%)
8 treatment; 8 control	N/A	Riding horses with *Grade 1* or *2* lameness	Double-blind placebo controlled crossover clinical trial.	Cortaflex[m]; Days 1–5: 60 ml/day Days 6–14: 30 ml/day.	Aqueous base vehicle containing dextrose, corn syrup, sorbitol, xanthan gum, sodium benzoate and yucca extract.	2 x 2 week treatments in cross-over fashion with 2-week washout between treatments.	• Significant improvement in gait symmetry of the tarsal joints.	Clayton *et al.* (2002)	25.9

Abbreviations: CS = chondroitin sulphate; GH = glucosamine hydrochloride; PGE_2 = prostaglandin E2; GAG = glycosaminoglycan; MMP3 = matrix metalloproteinase 3; MMP 9: matrix metalloproteinase 9; WBC: white blood cell count; N/A: not available; SD: standard deviation; LPS = lipopolysaccharide from *E. coli.* [a]Nutramax Laboratories, USA; glucosamine hydrochloride (GH; 60%), chondroitin sulphate (CS; 20%), manganese (0.5%), ascorbate (3.5%), undisclosed filler (16%). [b]Freunds Complete Adjuvant [concentrated formulation of mycobacteria (usually *Mycobacterium tuberculosis*) in mineral oil]. [c]for horses weighing <545 kg. [d]for horses weighing >545 kg. [e]VetPlus UK; chondroitin sulphate (CS - 19% w/w), GH (50%), N-acetyl-D-glucosamine (5%) and filler (25%). [f]for horses weighing <500 kg. [g]for horses weighing >500 kg. [h]Syntex SA, Buenos Aires, Argentina. [i]Interpath Pty Ltd, Australia; undisclosed percentages of NZGLM, SKC, Biota oil and AB. [j]arithmetic mean; no variability estimates provided. [k]Tryan Enterprises, USA; undisclosed filler (74.3%), cetyl myristoleate fatty acid complex (6%), GH (5.9%), methylsulfonylmethane (5.9%), hydrolysed collagen (3.9%), DL methionine (2%), ascorbate (1.3%), manganese (0.3%), zinc (0.3%), copper (0.07%). [m]Equine America, UK; vitamin B6 (1.1%), ascorbic acid (1.06%), glutamine (1.0%), glycine (1.0%), proline (0.31%), glutamic acid (0.30%), manganese (0.11%), glucuronic acid (0.05%), copper (0.04%), sulphur (0.03%), with the remaining 95% comprised of 'animal protein products', individual amino acids and yucca in undisclosed percentages. [n]TRH122, Nutramax Laboratories, USA. [o]FCHG49, Nutramax Laboratories, USA. [p]note the very high variability (SD = 22.5%). [q]G1514 Sigma Aldrich. [r]Dona, Rotta Pharmaceuticals Inc, New Jersey, USA. [s]Tryan Enterprises, Texas, USA.

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