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SAGE

Evaluation of a nutritional supplement for the alleviation of pain associated with feline degenerative joint disease: a prospective, randomized, stratified, double-blind, placebo-controlled clinical trial

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Abstract

Objectives The purpose of this study was to evaluate the pain-alleviating and activity-enhancing effects of glucosamine/chondroitin sulfate (Dasuquin) in cats that had degenerative joint disease (DJD) and owner-noted mobility/activity impairment. We hypothesized that the nutritional supplement would produce pain-relieving and activity-enhancing effects in cats with painful DJD.

Methods In this prospective, randomized, stratified, double-blind, placebo-controlled clinical trial, 59 cats with DJD pain were assigned to receive a placebo (n = 30) or supplement (n = 29) for 6 weeks after 2 weeks of placebo. Outcome measures (at-home accelerometry and client-specific outcome measures [feline (CSOMf); Feline Musculoskeletal Pain Index (FMPI); quality of life (QoL)]; and veterinarian examination) were collected at days 14, 28, 42 and 56.

Results Twenty-seven cats in the treatment group and 30 in the placebo group completed the trial. Within the first 2 weeks (placebo administration to all cats), 78% of all cats had an improvement in CSOMf scores. Both groups showed significant improvement at most time points in CSOMf, FMPI, QoL and pain scores, with the placebo group showing greater improvement than the supplement group (significant for CSOMf [$P = 0.01$]). Overall, no differences in activity were seen between the groups. Cumulative distribution function analysis indicated that for most levels of activity, the placebo-treated cats were more active; however, the least active cats were more active on the supplement ($P = 0.013$).

Conclusions and relevance This study showed a strong placebo effect. The glucosamine/chondroitin sulfate supplement did not show pain-relieving effects when compared with placebo.

Keywords: Glucosamine hydrochloride; chondroitin sulfate; nutritional supplement; mobility; accelerometer; activity

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Introduction

Degenerative joint disease (DJD) is associated with negative consequences, including pain, mobility impairment and decreased quality of life (QoL).[1–3] In cats, the prevalence of DJD is high: an estimated 90% of cats have radiographic signs of DJD, with at least 40% of these cats showing clinical signs related to pain.[1,4]

Currently, non-steroidal anti-inflammatory drugs (NSAIDs) are the first line and mainstay of effective treatment for the pain and inflammation that accompany DJD in humans and dogs. Meloxicam and robenacoxib are both approved for the treatment of chronic musculoskeletal pain in cats in various parts of the world, and a feline anti-nerve growth factor monoclonal antibody has recently received marketing authorization in the European Union.[5] These treatments were each evaluated using owner-assessed mobility impairment and objectively measured activity.[5–8] Currently, no other therapeutics are approved or licensed, and none are licensed in the USA.

Additionally, the effects of tramadol, gabapentin and amantadine on activity level and owner-assessed mobility impairment and quality of life (QoL) in cats with DJD have recently been evaluated in separate studies.[9–11] Compared with placebo, each of these treatments showed improvement in owner-assessed mobility impairment and QoL. However, gabapentin and amantadine caused decreased activity levels that were attributed to sedation, and adverse events (mydriasis, mild sedation, mild euphoria and vomiting) were reported with tramadol.[9]

A recent survey conducted primarily in the USA indicated that gabapentin and joint supplements were prescribed more frequently than NSAIDs for chronic musculoskeletal pain in cats.[12] Survey respondents also indicated that they prescribed 'joint supplements' for a longer median duration of treatment than NSAIDs, which was attributed to perceived lower risk of adverse effects from joint supplements. In that survey, details of which supplements were being used were not collected. Though joint supplements are commonly recommended in continuing education lectures for chronic musculoskeletal pain in cats, very little data on their efficacy (or safety) in treating musculoskeletal pain exist. This disparity between prescribing practices and evidence supporting the use of joint supplements creates a need for studies evaluating their safety and efficacy.

Dasuquin is a glucosamine hydrochloride/chondroitin sulfate supplement formulated for cats. Each capsule contains 125 mg glucosamine hydrochloride, 100 mg chondroitin sulfate and 25 mg avocado/soybean unsaponifiables (ASUs) (see https://www.dasuquin.com/dasuquin-sprinkle-capsules-for-cats/). In humans, there is no compelling evidence that these components alter disease progression, but they may reduce pain and improve function.[13] Little work has been performed in cats,[14,15] and no studies have evaluated the supplement Dasuquin in cats.

The aim of this study was to evaluate the effects of Dasuquin, compared with placebo, in cats with DJD and owner-noted mobility/activity impairment through the use of objective accelerometry and subjective owner assessments (client-specific outcome measures – feline [CSOMf], the Feline Musculoskeletal Pain Index (FMPI) and global QoL assessment) and veterinarian-assessed pain scores. We hypothesized that the nutritional supplement would produce pain-relieving and activity-enhancing effects in cats with painful DJD over a 6-week period of supplement administration.

Materials and methods

The study was conducted as a randomized, stratified, double-blinded, placebo-controlled clinical trial with each cat enrolled for 8 weeks. This trial was performed at the North Carolina State University College of Veterinary Medicine (NCSU-CVM), within the Translational Research in Pain (TRiP) Program, between June 2009 and August 2010. This study was approved by the Institutional Animal Care and Use Committee (IACUC number 08-125-O; approved 4 December 2008) and in all cases the owners signed a written consent form following a verbal explanation of the study protocol. The protocol included a provision that should an owner consider that any cat became significantly more painful during the study, the cat would be removed and treated as deemed appropriate by the cat's regular veterinarian or by the study veterinarian, whichever the owner preferred. Outcome measures included changes in owner ratings of how their cat was doing, as well as changes in objectively measured activity (as measured by collar-mounted accelerometers) after having been treated with the nutritional supplement or placebo. Additionally, adverse events and clinical pathology were evaluated. The study is reported following the CONSORT guidelines (http://www.consort-statement.org).

Study population

Potential study subjects were identified from clinic records by area primary care veterinarians, or were self-referred by owners who had seen advertisements for the clinical trial. Advertisements were placed on the NCSU-CVM website and flyers were sent to small animal veterinary practices in the area. Animals enrolled in the study were all client-owned cats with naturally occurring chronic DJD. All cats had lived with their owners for at least 2.5 months (range 2.5 months–16.5 years; mean 10.25). Details of sex, weight and age distribution are presented in the 'Results' section.

Inclusion and exclusion criteria

Cats were eligible to participate in the study if they had a qualifying degree of owner-noted mobility/activity impairment (see below), evidence of pain during

manipulation of at least one appendicular joint or spinal segment during the orthopedic evaluation (see below) and overlapping radiographic evidence of DJD in at least one of the painful joints or spinal segments (see below). Cats were required to be >1 year of age and weigh >1 kg (2.2 lbs), and not have received NSAIDs or other analgesics within the 2 weeks prior to enrollment. Predetermined exclusion criteria for all cats included the presence of suspected or diagnosed infectious diseases, symptomatic cardiac disease, immune-mediated disease, neoplasia, inflammatory bowel disease, urinary tract infection, hyperthyroidism and diabetes mellitus. These conditions were ruled out by careful review of the medical records, owner history, physical examination, complete blood count (CBC), serum biochemistry panel and urinalysis (UA). Cats with chronic kidney disease (CKD) up to and including International Renal Interest Society (IRIS) stage 2 were eligible to enroll,[16] provided the disease was stable as determined by repeated serum biochemistry panels and UA. For cats with evidence of CKD during screening, previous laboratory work was evaluated; if similar findings were present, cats were eligible to enroll immediately. If evidence of CKD was not present on prior laboratory work, or previous laboratory work was not available, serum biochemistry was repeated at least 2 weeks later; if findings were stable, cats were eligible to be enrolled. Cats with IRIS stage 3 or 4 CKD were excluded. Owners had to agree to stop the feeding of any glucosamine/chondroitin sulfate-containing preparations or other nutritional supplements at the start of the study (eg, glucosamine/chondroitin sulfate; Trixyn; green-lipped mussel; fish oil; ASUs).

Protocol design

The study design is shown in Figure 1. Following inclusion and randomization, the blinded portion of the study started. All cats received placebo for 2 weeks, and then placebo or nutritional supplement. The initial placebo phase was blinded (first 2 weeks), as well as the subsequent phase (supplement/placebo) that was 6 weeks in length. A stratified randomization scheme was used based upon a total target of 64 enrolled cats. The four strata were established upon initial client-specific outcome measures (CSOMf) of pain ('high' = CSOMf score 13–20; 'low' = CSOMf score of 7–12 [see below]) and prestudy supplementation status such that the strata were: (1) high pain/on supplements; (2) low pain/on supplements; (3) high pain/not on supplements; and (4) low pain/not on supplements. Within each of the four strata, 16 consecutive case numbers were randomized in blocks of two to maintain balanced treatments. Cats were assigned a unique case number upon enrollment into the study. Owners came into the clinic with their cats for screening on day 0 (D0) and at day 56 (D56). At other times (D14, D28, D42) owners came into the clinic alone to complete outcome assessments.

Treatments

The test material used in this study consisted of the commercial product, 'Dasuquin for Cats' or placebo. The placebo capsule weight and flavoring matched the supplement. Both materials were put into identical green-and-white capsules and packaged into blister cards. The blister cards were coded with either 'lot 001', 'lot 002' or 'lot 003'. The placebo test material was filled into blister

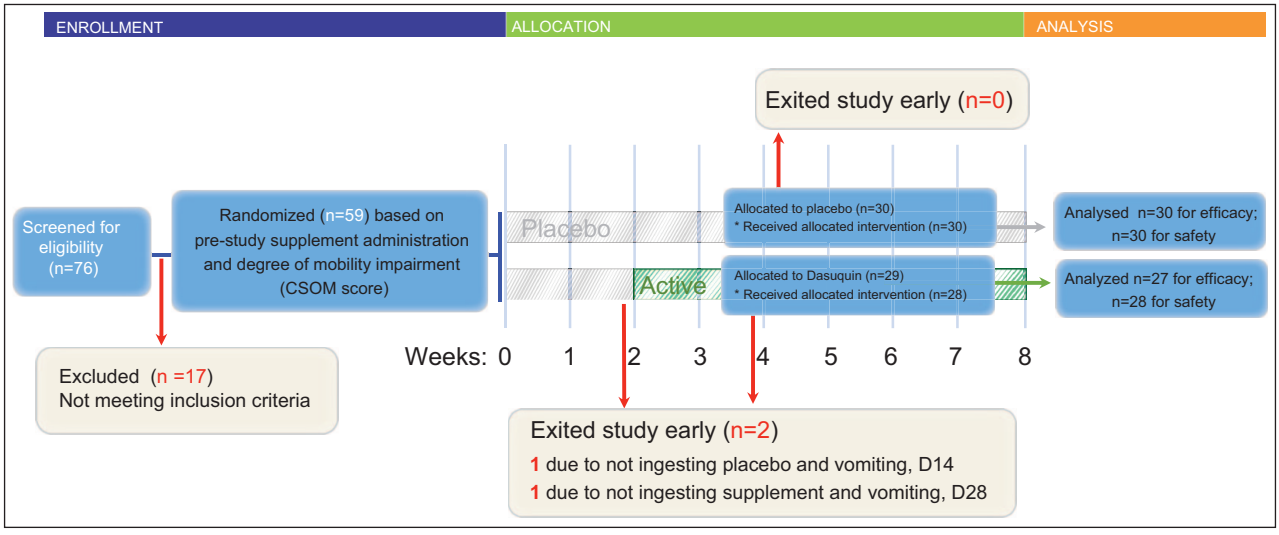

Figure 1 Diagram outlining the study protocol. During the 'allocation' period, administration of the test articles was performed under double-blind conditions (investigators and owners did not know what was being administered). CSOM = client-specific outcome measure

cards identified with lot numbers 001 or 002. The supplement was filled into blister cards identified with lot 003. The investigators were not aware of the identity of each lot until data analysis had been completed. As per manufacturer dosing recommendations, cats weighing <4.55 kg received one capsule daily and cats weighing >4.55 kg received two capsules. These were administered by owners once daily; as the product is considered palatable, owners were instructed to either administer the capsule by mouth, or open the capsule and sprinkle the contents on a small amount of food and monitor their cat to ensure the food was consumed.

Blinding

The blister cards were packaged in boxes containing an adequate quantity of capsules for any weight of cat to have sufficient capsules to last from one study visit to the next. Each box contained four blister cards of 10 capsules each. The outside of the box had a color-coded sticker with the case number and week number printed on it.

The randomization list was held by Nutramax Laboratories Veterinary Sciences for each subject recruited to the study. The study technician (AT) assigned a case number to use for each enrolled cat according to the block randomized design. Animals were randomized in sequential order based on randomization tables and the preassigned test materials were then dispensed in 2-week allotments. For the first 2 weeks all cats received placebo (lot 001). For the remainder of the study, cats received either placebo (lot 002) or supplement (lot 003). The identities of the individual lot numbers were not known to investigators until after data analysis. All lots were independently tested prior to initiation of the study and at completion of the study. Lot 003 (supplement) met the guaranteed amounts of glucosamine HCl (125 mg), chondroitin sulfate (100 mg) and ASUs (25 mg) per capsule at initiation and completion of the study (analysis independently performed at Silliker [Orem, UT, USA] at the request of BDXL). At the end of each study period, the owners returned their used boxes; any remaining capsules were counted and recorded for each patient.

Outcome measures

The primary outcome measures were the subjective CSOMf and objectively measured accelerometry. Several secondary outcome measures were used and included the FMPI, global QoL assessment and owner-assessed side effects.

Subjective outcome measures

CSOMf At the screening visit, owners were interviewed and a general questionnaire was used to gather demographic information and determine if the owner had noticed altered activity in their cat. Several activities commonly affected by DJD and associated pain were listed for owners to consider in their cat, and owners were asked to indicate whether these were affected for their cat. After this, the specific activities that were problematic for each cat were defined in more detail, as described previously.[17] Owners were directed to describe five time- and place-specific activities they considered to be altered, and to grade the degree of impairment vs when their cat was normal, or what a normal cat would be (see File 1 in the supplementary material). A single investigator (AT) directed the construction of each CSOMf. This resulted in a unique set of five activities for each cat (similar to more recent studies where only three activities were defined).[5,7,17,18] After completion of the CSOMf on D0, the same unique set of activities was assessed at each visit – D14, D28, D42 and D56. The owner was not permitted to see how they had graded severity of activity impairment at the previous assessment.

Dependent measures included total CSOMf scores, changes in CSOMf scores (change between D14 and subsequent time points) and treatment success/failure proportions. These proportions were determined on CSOMf scores during the placebo period (D0–D14) and then at each time point compared with D14 as a baseline. Treatment success was defined as no deterioration in any individual CSOMf activity score vs baseline and an improvement in total CSOMf activity score of ⩾3 (~20–30% change, on average, given the initial CSOMf scores).

FMPI The FMPI used was version 5, as described in Benito et al.[19] This version consisted of 21 questions. Eighteen questions asked about the cat's ability to perform different activities (activity factors); two related to pain intensity (severity factors); and one related to overall QoL. Activity questions were scored on a descriptive rating scale with seven descriptors (from left to right: 'above normal'; 'normal'; 'not quite normal'; 'somewhat worse than normal'; 'barely, or with great effort'; 'not at all'; 'does not apply or I don't know'). Scores allotted to these options were –1, 0, 1, 2, 3 and 4, respectively, with 'does not apply or I don't know' not being scored. Pain severity factor questions were scored on a descriptive rating scale with five descriptors (from left to right: 'no pain'; 'little pain'; 'mild pain'; 'moderate pain'; 'severe pain'). Scores allotted to these options were 0, 1, 2, 3 and 4, respectively. The overall QoL question was scored on a descriptive rating scale with four descriptors (from left to right: 'excellent'; 'good'; 'fair'; 'poor').

Global QoL assessment On D14, D28, D42 and D56 the owners were asked to complete a simple, unvalidated global assessment of change of QoL (QoL Change) (see File 1 in the supplementary material). They were asked to rate the change in overall QoL, compared with before the cat received the test supplement (D0). These ratings were scored from 0 to 6, where 0 = very deteriorated, 3 = no change and 6 = very improved. Owners were not given any specific instructions as to what constituted QoL.

Pain scores On D0 and D56, a musculoskeletal pain evaluation was performed by the study veterinarian (BDXL). Response to palpation of every joint (the manus and pes were considered single joints) and each part of the axial skeleton (cervical, thoracic, lumbar and lumbosacral) were graded on a 0–4 scale, as previously described.[20]

Objective measures

Actimetry using accelerometers An accelerometer (activity monitor [AM])[2] was placed on a neck collar for each cat. Epoch length was set at 1 min and monitors were worn for the duration of the study. At D14, D28, D42 and D56 the monitor was removed from the cat, and placed on a telemetric reader to download the data to a personal computer. The AM and collar were then replaced on the cat. Owners were asked to indicate any times when the collar and/or AM was removed and to report any adverse events that occurred during the study.

CBC, serum chemistry and UA

Standard CBC, serum chemistry and urinalysis were performed at the start and the end of the study.

Statistical analysis

The sample size was based on information available at the time of the study, and assumed a change in CSOMf scores similar to that seen in a trial of a joint support diet. Using these data (delta 2.5, SD 2.3), sample size estimation suggested group sizes of 18 at an alpha of 5% and beta of 0.9. Thus, a target of 30 cats per group was considered reasonable and allowed for dropouts.

The baseline data of the two groups were compared using χ^2 tests, *t*-tests and Wilcoxon rank sum tests, as appropriate, for sex distribution, age, weight, initial CSOMf score, and distribution of high and low impairment.

Subjective data CSOMf, FMPI, QoL and pain score data were initially evaluated using a split-plot repeated-measures ANOVA with a grouping factor of treatment and a repeat factor of time. If the errors were not normally distributed, data were log transformed and reanalyzed. Homogeneity of the variance–covariance matrix was assessed using Akaike's information criterion (AIC). Compound symmetry of the variance–covariance matrix was used unless there were serious differences of AIC and/or the ANOVA *P* values. Change within groups over time was evaluated, and the change between D14 and D56 was compared between groups using *t*-tests or Wilcoxon rank sums, depending on whether the data were normally distributed. The treatment success/failure proportions using the CSOMf for the supplement and placebo groups were compared at each time point using χ^2 tests.

For the pain score data, the average score per joint/part of the axial skeleton was used in the analysis owing to missing data from some cats who were unable to be fully examined due to their temperament. For all subjective data analyses, a Bonferroni correction was made to protect the type I error rate, where critical $P = 0.05/m$, where m = number of comparisons (eg, five comparisons between groups, D0, D14, D28, D42 and D56). Uncorrected *P* values and new critical thresholds are reported where necessary.

Accelerometry data Daily activity count data were evaluated using a repeated-measures ANOVA with a grouping factor of treatment, and a repeat factor of time (day). If the errors were not normally distributed, data were log transformed and reanalyzed. The variance–covariance matrix was modeled using compound symmetry, or AR1 for log-transformed data. Paired comparisons were made within groups, and unpaired between groups using *t*-tests and ANOVA. The mean daily activity was calculated for each week of the study. Changes from week 2, and the average of week 1 and 2 (baselines) were calculated for each week and compared between groups. Night-time activity has been reported as being improved in cats receiving an analgesic,[21] and so we compared groups for the change in night-time activity between baseline (average of weeks 1 and 2) and each week. The mean daily activity over weeks 1 and 2 was calculated, as was the mean daily activity over weeks 6–8, and for each cat the change was expressed as positive or negative, and the distribution of positive and negative changes was tested between groups using a χ^2 analysis.

Using the average daily activity of each cat, cumulative distribution functions (CDF) of the two groups were created, plotted and examined. The CDF plots the cumulative probability (*y*-axis) that each group will show a certain level of activity, against level of activity, allowing any differences between the groups to be assessed across the spectrum of levels of activity.

Blood and UA data Changes over time (D0 and D56 data) were evaluated using appropriate paired statistical tests (Wilcoxon, *t*-test), and the change over the study period was compared between groups using a Wilcoxon rank sums test for each variable. No adjustment was made for multiple comparisons on the blood and urine results.

Results

A total of 76 cats were screened. Seventeen cats were not eligible for enrollment due to a pain response and radiographic DJD not being detected in the same joint (n = 3); no radiographic evidence of DJD (n = 5); abnormal values on laboratory work (n = 1); evidence of neoplasia (n = 6);

Table 1 Demographics of all cats successfully recruited to the study, and comparison of the groups at day 0 (screening)

	Placebo group (n = 30)	Supplement group (n = 29)	*P* value
Sex (n)	M (4); MC (7); F (3); FS (16)	M (3); MC (11); F (5); FS (10)	0.40
Mean ± SD (range) age (years)	12.2 ± 4.2 (5.8–19.5)	11.7 ± 3.1 (5.0–16.6)	0.56
Mean ± SD (range) weight (kg)	5.5 ± 1.7 (3.0–11.0)	5.2 ± 1.9 (2.6–10.4)	0.52
Median (range) initial CSOMf score	14 (7–19)	14 (8–19)	0.51
Mean ± SD initial CSOMf score	13.2 ± 4.1	13.9 ± 3.4	
High or low impairment (n)	High: 18; low: 12	High: 18; low 11	1.00

M = male; MC = male castrated; F = female; FS = female spayed; CSOMf = client-specific outcome measure – feline

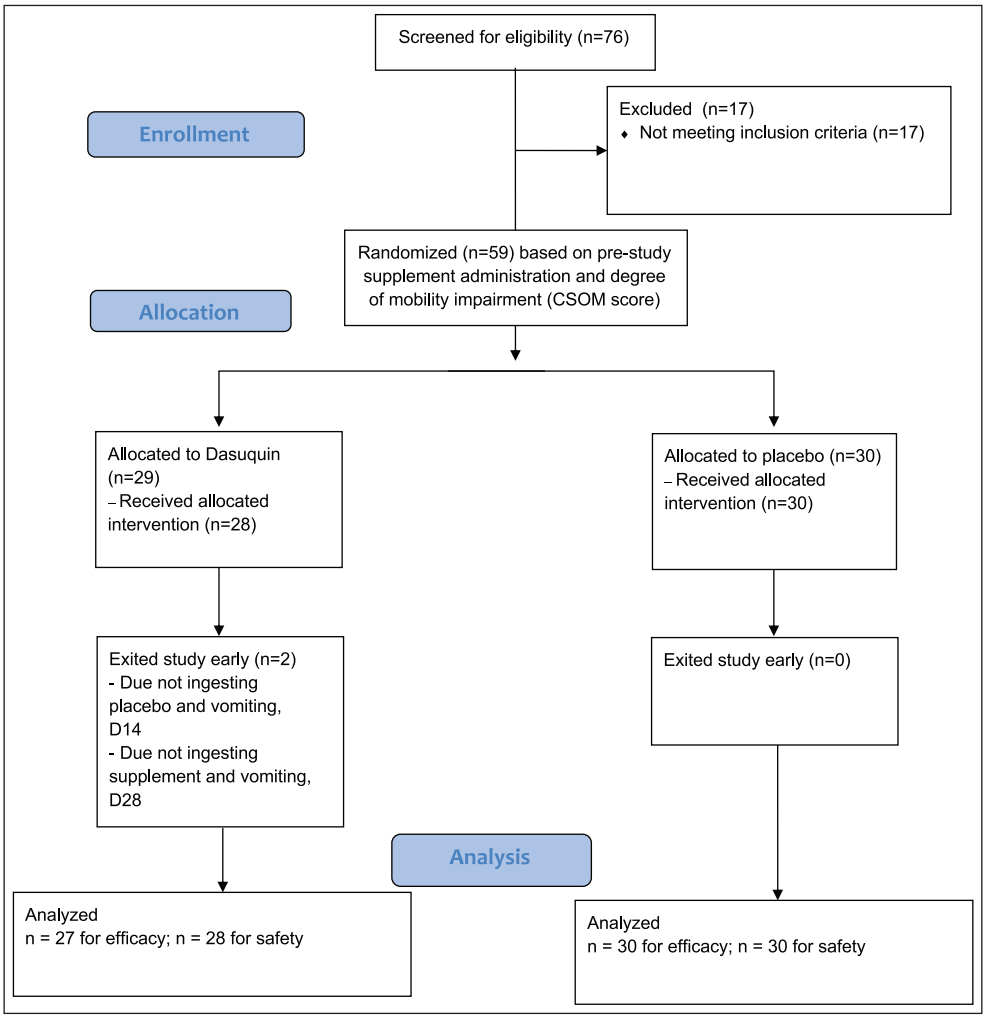

Figure 2 CONSORT flow diagram detailing the flow of subjects through the study protocol, reasons for early study exit and the numbers analyzed for safety and efficacy. CSOM = client-specific outcome measure

evidence of clinically significant cardiac disease (n = 1); ongoing steroid administration (n = 1 [asthma]).

A total of 59 cats were recruited to the study: 30 in the placebo group and 29 in the supplement group. Demographics are detailed in Table 1. The placebo and supplement groups showed no significant differences in regard to sex distribution, age, weight, initial CSOMf score, or distribution of high and low impairment at the start of the study. Figure 2 shows the flow of subjects through the study.

Two cats exited the study prior to D56; both were in the supplement group. One cat (cat #27, exited at D28) did not eat food with the supplement on it, started vomiting and was reported not to tolerate the collar. The other cat (cat #7, exited at D14) would not eat the placebo (prior to beginning supplement) and suffered a bout of vomiting,

Table 2 Summary of tests of fixed effects for client-specific outcome measure – feline (CSOMf), global quality of life (QoL) change and Feline Musculoskeletal Pain Index (FMPI)

	CSOMf		Global QoL		FMPI (average per question)	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value
Group	5.5	0.02	1.64	0.20	1.84	0.166
Day	64.26	<0.0001	13.86	<0.0001	23.74	<0.0001
Day × group	1.7	0.46	3.32	0.02	5.02	0.04

Table 3 Summary of owner-assessed outcome measure scores for each group at each time point and statistical comparisons

	CSOMf			Global QoL			FMPI		
	Placebo	Supplement	*P* value	Placebo	Supplement	*P* value	Placebo	Supplement	*P* value
D0	13.07 ± 4.18	13.90 ± 3.44	0.45	NA			1.63 ± 0.63	1.52 ± 0.71	0.51
D14	8.8 ± 4.55	10.79 ± 4.11	0.07	3.40 ± 1.04	3.52 ± 0.69	0.67	1.29 ± 0.72	1.48 ± 0.56	0.26
D28	6.9 ± 4.40	9.61 ± 4.33	0.02	4.00 ± 1.05	3.61 ± 1.03	0.17	1.07 ± 0.79	1.40 ± 0.64	0.08
D42	6.2 ± 4.41	9.00 ± 4.31	0.02	4.37 ± 1.35	3.70 ± 1.03	0.02	0.95 ± 0.82	1.34 ± 0.68	0.04
D56	5.7 ± 4.45	8.70 ± 4.04	0.01	4.37 ± 1.35	4.11 ± 0.80	0.35	0.84 ± 0.75	1.18 ± 0.68	0.07
Change between days 14 and 56	–3.1 ± 3.09	–2.26 ± 3.39	0.64	0.97 ± 0.96	0.59 ± 0.84	0.56	–0.45 ± 0.48	–0.31 ± 0.44	0.64
Within-group comparison P value	<0.0001	0.0009		<0.0001	0.0015		<0.0001	0.01	

Data are shown as mean ± SD. For client-specific outcome measure – feline (CSOMf) and Feline Musculoskeletal Pain Index (FMPI), higher numbers indicate greater impairment. For Global quality of life (QoL), higher numbers indicate more improvement in QoL. For comparisons between groups at each time point (days [D] 0, 14, 28, 42 and 56), the critical *P* value is 0.01 (ie, 0.05/5). No adjustment of *P* value is needed for between group comparisons of the change between D14 and D56. For within group comparisons of the change between D14 and D56, the critical *P* value is 0.025 (0.05/2)

which resolved after several days without any specific treatment, though other analgesics (buprenorphine) were administered during this period by the primary veterinarian.

Percent of capsules administered

There was no difference between the groups regarding the percent of the target number of capsules that were actually used ($P = 0.589$). The mean ± SD percent administered was 98.9 ± 6.4 in the placebo group and 98.0 ± 6.8 in the supplement group.

Analysis of capsule content

To check the capsule assignment, samples were analyzed by Silliker. Results indicated correct assignment of capsules.

CSOMf

Overall, there was a significant treatment effect (favoring placebo) and significant day effect (improvement over time) but no day × group interaction (Table 2). The groups were not significantly different at baseline (D0). Additionally, the groups were not significantly different on D14, D28 or D42, but were verging on being significantly different at D56 ($P = 0.01$), with CSOMf scores being lower (indicating less impairment) in the placebo group (the critical *P* value was 0.01) (Table 3). The evolution of total CSOMf scores over time are shown in Figure 3. Within-group comparisons indicated a

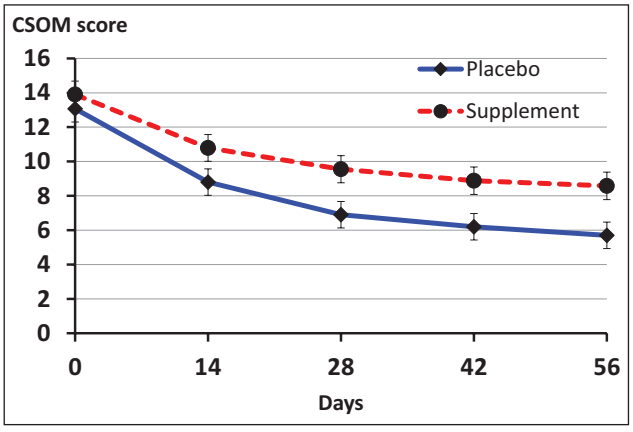

Figure 3 Graphical representation of client-specific outcome measures (CSOM) – feline scores over time for the two groups Values plotted are mean ± SEM. Absolute CSOMf scores were significantly lower in the placebo group compared with the supplement group at D56 ($P = 0.01$); however, the change in scores between day (D)14 and D56 did not differ between the groups ($P = 0.64$)

Table 4 Effect sizes (±95% confidence interval) based on total scores on days 28, 42 and 56

	CSOMf	QoL	FMPI
Day 28	–0.61 (–1.15 to –0.09)	0.36 (–0.15 to 0.90)	–0.39 (–0.92 to 0.13)
Day 42	–0.63 (–1.17 to –0.11)	0.55 (0.02–1.08)	–0.451 (–1.04 to 0.02)
Day 56	–0.69 (–1.24 to –0.16)	0.23 (–0.29 to 0.75)	–0.47 (–1.00 to 0.06)

For client-specific outcome measures – feline (CSOMf) and Feline Musculoskeletal Pain Index (FMPI), negative effect size (ES) values indicate a greater positive effect of placebo. For quality of life (QoL), positive ES values indicate a greater positive effect of placebo

Table 5 Effect sizes (±95% confidence interval) based on change in score from day 14 to days 28, 42 and 56

	CSOMf	QoL	FMPI
Day 28	–0.15 (–0.68 to 0.37)	0.48 (–0.05 to 1.01)	–0.39 (–0.92 to 0.14)
Day 42	–0.22 (–0.75 to 0.30)	0.66 (0.13–1.20)	–0.47 (–1.00 to 0.05)
Day 56	–0.26 (–0.78 to 0.26)	0.41 (–0.12 to 0.94)	–0.30 (–0.82 to 0.22)

For client-specific outcome measures – feline (CSOMf) and Feline Musculoskeletal Pain Index (FMPI), negative effect size (ES) values indicate a greater positive effect of placebo. For quality of life (QoL), positive ES values indicate a greater positive effect of placebo

significant improvement (decrease) in scores at all time points compared with D14, for each treatment group ($P < 0.0001$ for placebo and $P = 0.0009$ for supplement; critical P value was 0.017) (Table 3). There was no difference between the groups for change in CSOMf over this time period ($P = 0.64$).

The effect size (ES) (95% confidence interval [CI]) (treatment over placebo) based on CSOMf values at D56 was calculated to be –0.67 (95% CI –1.21 to 0.14), meaning there was a greater positive effect of placebo (Table 4; Table 5 details ES for the change in CSOMf scores over time).

A total of 46/59 cats (78%) responded positively (a CSOMf score decrease of ⩾1) between D0 and D14, and 35/59 cats (59%) were classified as treatment successes during this period. Over this time period, all cats were receiving placebo. The proportion of treatment success/failures was not significantly different between treatment groups at any time point.

FMPI

Overall, for the total FMPI score (mean score per question), there was no treatment effect, a significant day effect (improvement over time) and day × group interaction (favoring placebo) (Table 2). The groups were not significantly different on D0 (baseline) nor were they significantly different on D14, D28, D42 or D56 (the critical P value was 0.0125) (Table 3). At all time points, results numerically favored the placebo group. Within-group comparisons indicated a significant improvement (decrease) in scores between D14 and D56, for both treatment groups ($P < 0.0001$ for placebo and $P = 0.01$ for supplement; critical P value was 0.017) (Table 3). There was no significant difference between the groups for the change in total FMPI score between D14 and D56 ($P = 0.64$). Effect sizes for group comparisons based on total FMPI scores, and change in FMPI scores, are detailed in Tables 4 and 5, respectively.

Global QoL improvement

Overall, there was no treatment effect, a significant day effect, and day × group interaction (favoring placebo) (Table 2). The groups were not significantly different on any assessment day (Table 3). Overall, Global QoL change scores were higher (indicating more improvement) in the placebo group.

Effect sizes for group comparisons based on global QoL scores, and changes in scores, are detailed in Tables 4 and 5, respectively.

Pain scores

There was no treatment effect ($P = 0.814$) and a significant day effect ($P = 0.038$), but no day × treatment interaction ($P = 0.774$). Total pain scores were lower in both groups on D56 than D0, and this change over time was significant for the placebo group ($P = 0.022$) but not for the supplement group ($P = 0.507$). Total pain scores at D56 were not different between the groups ($P = 0.60$), and there was no significant difference between the groups for change over time between D0 and D56 ($P = 0.21$).

Accelerometry data

Overall, 7.4% of activity data were not included in the analysis owing to missing data within one of the time periods. When evaluating total daily activity counts, there was no overall effect of treatment ($P = 0.36$) or of day ($P = 0.087$) or day × treatment interaction ($P = 0.94$). Though analysis of the raw data suggested a possible day effect, analysis of the log-transformed data did

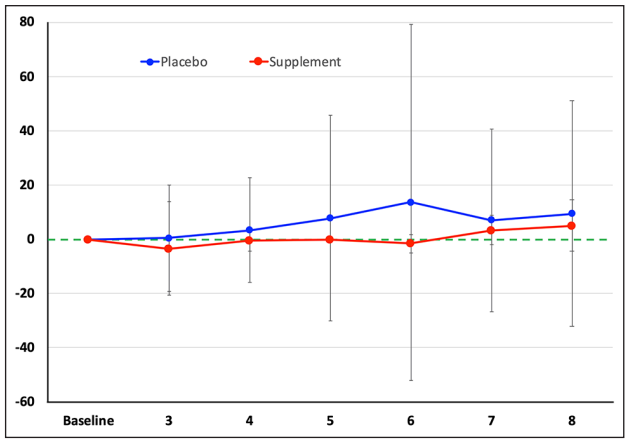

Figure 4 Plot of total mean daily activity expressed as a percentage change from baseline for each group, for each week. Values for each week represent the mean values for the whole of that week

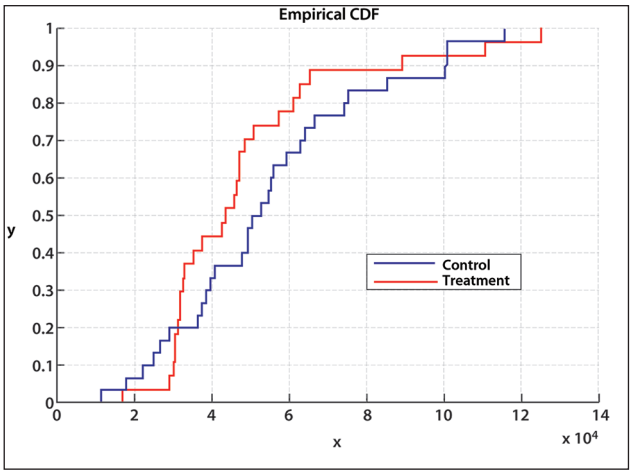

Figure 5 Cumulative distribution function (CDF) curves for the two groups. The CDF plots the cumulative probability (*y*-axis) that each group will show a certain level of activity against average daily levels of activity, allowing any differences between the groups to be assessed across the spectrum of levels of activity (*x*-axis). When the line is to the right, it indicates overall higher activity for that group at a certain overall level of activity. The plot shows a location-scale treatment effect, ie, there is a positive treatment effect for the supplement (red line) in the cats who were least active, but at activity levels of between 30,000 and 100,000 counts per day, cumulative probability shows the cats in the placebo group were more active; then, at higher levels of mean daily activity, there was no difference between the groups

not ($P = 0.35$). The lowest skewness and kurtosis were with box Cox transformation; again, there were no significant effects of treatment ($P = 0.47$), day ($P = 0.83$) or day × treatment interaction ($P = 1.0$). Figure 4 shows the plot of the mean daily activity as change from baseline (weeks 1 and 2), expressed as a percentage.

Using the mean daily activity over each week of the study, and calculating change from either week 2 activity, or the average of week 1 and 2, no differences between groups was found for any week in the study, or any comparison. The change (increase in activity) from the average of weeks 1 and 2 was numerically greater in the placebo group at weeks 4 and 5, and numerically greater in the supplement group at weeks 7 and 8. There was no significant difference between the groups for change in night-time activity at any week following baseline. At week 8, the change from baseline (mean ± SD) in night-time activity was –705 ± 5700 in the placebo group, and 702 ± 4682 in the supplement group ($P = 0.32$ for group comparison).

When expressing the change in mean daily activity between the mean of weeks 1 and 2 vs the mean of weeks 6, 7 and 8 as negative or positive, there was a positive change in 19/27 cats, and negative in 8/27 cats in the supplement group; and a positive change in 13/30 cats and negative in 17/30 cats in the placebo group. These distributions were not significantly different ($P = 0.06$).

The CDF of the two groups is shown in Figure 5. With CDF, a positive treatment effect (in terms of increased activity) is indicated for the group where the curve is shifted toward the right side (expected if treatment increases activity). The supplement group (red) distribution is to the right of the control group at the lower activity levels; at the medium levels of activity (as indicated on the *x*-axis), the distribution of the supplement group is to the left of the control; and at higher activity levels (higher 'x' on the *x*-axis), there is little difference between the groups. If just the tenth percentile of the two independent samples is considered (a total of 12 cats), the difference between the groups in terms of the probability of having a certain level of activity is significant ($P = 0.013$), with greater a probability of higher activity in the supplement group (six cats), compared to the placebo treated cats (six cats). These 12 cats with the lowest activity levels were compared to the rest of the study cats, and no differences were found in sex or initial CSOMf value; however, these 12 cats were significantly older than the rest of the group (14.2 ± 2.7 vs 11.37 ± 3.7 years; $P = 0.015$), and verged on being significantly less heavy (4.77 ± 1.2 vs 5.90 ± 1.9 kg; $P = 0.051$). There was no difference in CSOMf response between the six cats receiving placebo and six receiving supplement ($P = 0.50$).

Adverse events, CBC, chemistry and UA

There were no significant changes over time for any parameters or any significant differences between groups in the change over time for any parameter. The only change of clinical significance was one cat (cat #1; placebo) where creatinine and blood urea nitrogen increased dramatically (from 2.8 mg/dl at screening to 4.1 mg/dl at D56). There were no additional reported side effects in either group.

Discussion

In this randomized, stratified, double-blinded, placebo-controlled clinical trial assessing the effect of Dasuquin in cats with DJD, there was no difference between the groups for the change in any outcome measure over the 6-week period when the supplement or placebo were being administered. Therefore, we rejected our hypothesis that this supplement would produce pain-relieving and activity-enhancing effects in cats with painful DJD. The placebo group had significantly lower CSOMf scores at D56 and showed a statistically significant improvement in joint pain scores between the two examinations (D0 and D56). The placebo group also showed greater improvement in global QoL score, though this was not significant, and this scale has not been validated. There was no significant treatment effect on activity count, but CDF analysis showed that the placebo group had increased activity over the supplement group for all cats except those cats with the lowest activity levels (representing 12 cats), where the cats on the supplement (n = 6) were more active than cats on placebo (n = 6).

This study employed a design in which both the supplement group and placebo group underwent an initial placebo period that was intended to mitigate the impact of the placebo effect. The owners were blinded to this placebo period, with the idea being that this would allow the placebo effect to stabilize over the first 2 weeks of the trial and allow any subsequent improvement seen in the supplement group to be attributed to administration of the supplement. During this period of placebo administration, 78% of the cats showed an improvement in the CSOMf score by one or more points, indicating a strong placebo effect. At the time, no studies in cats had been performed using this design to mitigate the placebo effect. Since this study was performed, other study designs aimed at circumventing the large placebo effects seen in chronic pain studies in cats have shown promise, including evaluating deterioration in owner-assessed mobility and activity following active treatment;[14,18] however, a recent study exploring this approach of measuring deterioration did not detect deterioration on withdrawal of a medication that was shown to be effective on other measures.[6] Gruen et al described a profound placebo effect in clinical trials using subjective owner assessment of activity and mobility in cats receiving analgesic treatments for DJD-associated pain, which may confound the ability to show effectiveness of analgesics over placebo.[22] In other studies, using similar inclusion criteria, owners were not able to detect a treatment effect of meloxicam over placebo.[23] Since these studies were performed, our group has increased the required severity of impairment (more impaired CSOMf scores and a requirement for two joints to be affected with pain and radiographic DJD) based on the idea that a greater degree of initial impairment may make it easier to detect treatment effects if they exist.[5,7,18] In the present study, the inclusion of less-impaired cats may have made the detection of improvement more difficult. However, in the present study we analyzed the data by impairment level (analysis not presented) but found no differences in our conclusions.

CDF analysis of accelerometry data from this study suggests that the supplement had a significant positive effect on activity for the least active cats. For cats with the lowest activity levels, the CDF curve of cats in the supplement group was to the right of those on placebo, while the curve for cats on placebo was to the right at the intermediate activity level and there was no difference between the groups at the highest activity levels. This means that for cats with the lowest levels of activity, there was a greater probability that cats on supplement would have higher activity than cats on placebo; but for cats showing higher levels of activity, this was reversed. This phenomenon is termed a location-scale treatment effect. Overall, this suggests that supplement increases activity in the cats with DJD who show the least activity (approximately 20% of the cats), but for intermediate levels of activity, activity was increased with placebo. In the Glucosamine/chondroitin Arthritis Intervention Trial (GAIT), the subgroup of human patients with moderate-to-severe knee pain was the only group to show improvement on glucosamine/chondroitin sulfate, suggesting that the supplement may be effective for those with the most severe disease.[24] A follow-up study, which included only patients with moderate-to-severe radiographic knee osteoarthritis and moderate-to-severe pain, showed that glucosamine/chondroitin sulfate was as effective as celecoxib in reducing pain, stiffness, functional limitation and joint swelling over a 6-month period.[25] Activity data in our study showed a similar trend of improvement for the least active cats who received the supplement, though caution should be taken in comparing these groups as it is unknown if the least active cats represent those with the most severe disease or if other factors contributed to their low activity level.

The findings in this study are similar to studies in dogs and humans that show inconsistent and inconclusive evidence for the efficacy of glucosamine/chondroitin sulfate supplements. A similarly designed clinical trial assessing the efficacy of a glucosamine/chondroitin sulfate-based supplement (Dasuquin) in dogs with osteoarthritis using the Canine Brief Pain Inventory (CBPI) and actimetry (accelerometer measured activity) as outcome measures showed no treatment effect for the supplement and a strong caregiver placebo effect was seen in the CBPI scores.[26] Systematic reviews of studies in dogs and meta-analyses in humans on the efficacy of glucosamine/chondroitin supplements show inconsistent evidence for supplements producing significant improvement compared with placebo.[27,28] Differences in glucosamine/chondroitin combination, proportions and salt preparation, and variance

in dosage amount, frequency and form are often cited in these analyses as issues confounding the ability to assess the efficacy of glucosamine/chondroitin supplements in treating DJD-associated pain. Another issue is the duration of administration required to see any positive effects. By looking at our data in various ways, we did find that more cats in the supplement group had a positive change in activity when the mean activity over weeks 1 and 2 was compared to the mean of activity over weeks 6, 7 and 8. Although this was not reflected in the subjective assessment data, it is possible that a longer duration of administration of the supplement may allow for treatment effects to be seen. Previous studies in cats have also failed to show conclusive evidence that glucosamine/chondroitin supplements are effective in treating DJD-associated pain. In a study comparing glucosamine/chondroitin and meloxicam, cats on meloxicam showed significant improvement in mobility and pain scores compared with the cats on glucosamine/chondroitin.[14] Though the cats on the supplement did show improvement overall, this was not significant. Lascelles et al found that a diet containing glucosamine and chondroitin, in addition to omega-3 fatty acids and green-lipped mussel extract, significantly improved objectively measured activity in cats with DJD compared with a placebo diet.[29] However, the authors attributed these benefits to the omega-3 fatty acids, which have been deemed to have positive effects in dogs with OA pain.[15,30]

Dasuquin also contains ASUs (25 mg/capsule), and some studies of ASUs in humans have shown positive effects. However, the formulations and doses (even when adjusting for metabolic weight) are different between these studies and ours, and results of studies in humans have varied depending on the joints affected by OA.[31,32] Additionally, results have been inconsistent in relation to duration of the study, although the shorter-term studies in humans are generally 3 months in duration – longer than the period of administration in the study reported here. This, again, speaks to the need to perform additional studies with a longer duration of administration, and also to ensure doses are proportional across species.

Some of the study limitations have already been outlined: in retrospect, differentiation of the groups may have been clearer if only more highly impaired cats were included. Additionally, recently discussed approaches to analyzing patterns of activity might have been useful to employ; however, the validity of this approach needs to be confirmed.[33] It could certainly be argued that the length of administration of the supplement was not long enough, and future studies should assess administration over a 12-week period. It is always easy to argue for the need for more animals in any clinical study, but, in this case, there was a statistically significant difference at D56 between the groups on one parameter, and the difference was in line with the assumptions in the sample size estimation, except that the sample size estimation was based on the treatment being more effective. With the exception of the CSOMf, the tools used as outcome measures in this study were either not validated (QoL) or have since been refined (FMPI).[34] While the results are unlikely to have been different, it is not known what effect use of the refined FMPI (FMPI-sf) would have on study outcomes. The CSOMf used was constructed in an identical manner to the current recommendations for the CSOMf, except that five activities were selected rather than three. Finally, this study was conducted prior to the introduction of 'Dasuquin Advanced', which contains additional ingredients, such as *Boswellia*, that may have pain-alleviating effects.[35] Future work could evaluate this product in cats.

Conclusions

In this randomized, double-blinded, placebo-controlled clinical trial, we found that a glucosamine/chondroitin sulfate containing supplement (Dasuquin) did not have pain-relieving and activity-enhancing effects in cats with painful DJD when compared with placebo. There was a suggestion that cats with the lowest activity (20% of the population studied) may gain activity-enhancing effects from Dasuquin.

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Supplementary material The following file is available online:
File 1: Actual data capture formats for the Client Specific Outcome Measures – feline (CSOMf) and the Global Assessment of Change in Quality of Life.

Conflict of interest Nutramax Laboratories Veterinary Sciences contributed to the study design and supplied the test article and placebo. BDX Lascelles has received remuneration for continuing education lectures sponsored by Nutramax Laboratories Veterinary Sciences.

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Ethical approval The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognized high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed consent Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s)

described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

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References

1 Slingerland LI, Hazewinkel HA, Meij BP, et al. **Cross-sectional study of the prevalence and clinical features of osteoarthritis in 100 cats.** *Vet J* 2011; 187: 304–309.
2 Clarke S and Bennett D. **Feline osteoarthritis: a prospective study of 28 cases.** *J Small Anim Pract* 2006; 47: 439–445.
3 Bennett D and Morton C. **A study of owner observed behavioural and lifestyle changes in cats with musculoskeletal disease before and after analgesic therapy.** *J Feline Med Surg* 2009; 11: 997–1004.
4 Lascelles BD, Henry JB, 3rd, Brown J, et al. **Cross-sectional study of the prevalence of radiographic degenerative joint disease in domesticated cats.** *Vet Surg* 2010; 39: 535–544.
5 Gruen ME, Thomson AE, Griffith EH, et al. **A feline-specific anti-nerve growth factor antibody improves mobility in cats with degenerative joint disease-associated pain: a pilot proof of concept study.** *J Vet Intern Med* 2016; 30: 1138–1148.
6 Adrian D, King JN, Parrish RS, et al. **Robenacoxib shows efficacy for the treatment of chronic degenerative joint disease-associated pain in cats: a randomized and blinded pilot clinical trial.** *Sci Rep* 2021; 11: 7721.
7 Gruen ME, Griffith EH, Thomson AE, et al. **Criterion validation testing of clinical metrology instruments for measuring degenerative joint disease associated mobility impairment in cats.** *PLoS One* 2015; 10: e0131839. DOI: 10.1371/journal.pone.0131839.
8 Xu Z, Laber E, Staicu AM, et al. **Novel approach to modeling high-frequency activity data to assess therapeutic effects of analgesics in chronic pain conditions.** *Sci Rep* 2021; 11: 7737. DOI: 10.1038/s41598-021-87304-w.
9 Monteiro BP, Klinck MP, Moreau M, et al. **Analgesic efficacy of tramadol in cats with naturally occurring osteoarthritis.** *PLoS One* 2017; 12: e0175565. DOI: 10.1371/journal.pone.0175565.
10 Shipley H, Flynn K, Tucker L, et al. **Owner evaluation of quality of life and mobility in osteoarthritic cats treated with amantadine or placebo.** *J Feline Med Surg* 2021; 23: 568–574.
11 Guedes AGP, Meadows JM, Pypendop BH, et al. **Assessment of the effects of gabapentin on activity levels and owner-perceived mobility impairment and quality of life in osteoarthritic geriatric cats.** *J Am Vet Med Assoc* 2018; 253: 579–585.
12 Adrian DE, Rishniw M, Scherk M, et al. **Prescribing practices of veterinarians in the treatment of chronic musculoskeletal pain in cats.** *J Feline Med Surg* 2019; 21: 495–506.
13 Percope de Andrade MA, Campos TV, et al. **Supplementary methods in the nonsurgical treatment of osteoarthritis.** *Arthroscopy* 2015; 31: 785–792.
14 Sul RM, Chase D, Parkin T, et al. **Comparison of meloxicam and a glucosamine-chondroitin supplement in management of feline osteoarthritis. A double-blind randomised, placebo-controlled, prospective trial.** *Vet Comp Orthop Traumatol* 2014; 27: 20–26.
15 Vandeweerd JM, Coisnon C, Clegg P, et al. **Systematic review of efficacy of nutraceuticals to alleviate clinical signs of osteoarthritis.** *J Vet Intern Med* 2012; 26: 448–456.
16 IRIS. **Staging of CKD.** http://www.iris-kidney.com/pdf/IRIS2009_Staging_CKD.pdf (2009, accessed May, 2010).
17 Lascelles BD, Hansen BD, Roe S, et al. **Evaluation of client-specific outcome measures and activity monitoring to measure pain relief in cats with osteoarthritis.** *J Vet Intern Med* 2007; 21: 410–416.
18 Gruen ME, Griffith E, Thomson A, et al. **Detection of clinically relevant pain relief in cats with degenerative joint disease associated pain.** *J Vet Intern Med* 2014; 28: 346–350.
19 Benito J, Depuy V, Hardie E, et al. **Reliability and discriminatory testing of a client-based metrology instrument, feline musculoskeletal pain index (FMPI) for the evaluation of degenerative joint disease-associated pain in cats.** *Vet J* 2013; 196: 368–373.
20 Lascelles BDX, Dong YH, Marcellin-Little DJ, et al. **Relationship of orthopedic examination, goniometric measurements, and radiographic signs of degenerative joint disease in cats.** *BMC Vet Res* 2012; 8: 10. DOI: 10.1186/1746-6148-8-10.
21 Guillot M, Moreau M, Heit M, et al. **Characterization of osteoarthritis in cats and meloxicam efficacy using objective chronic pain evaluation tools.** *Vet J* 2013; 196: 360–367.
22 Gruen ME, Dorman DC and Lascelles BDX. **Caregiver placebo effect in analgesic clinical trials for cats with naturally occurring degenerative joint disease-associated pain.** *Vet Rec* 2017; 180: 473.
23 Benito J, Hansen B, Depuy V, et al. **Feline musculoskeletal pain index: responsiveness and testing of criterion validity.** *J Vet Intern Med* 2013; 27: 474–482.
24 Clegg DO, Reda DJ, Harris CL, et al. **Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis.** *N Engl J Med* 2006; 354: 795–808.
25 Sawitzke AD, Shi H, Finco MF, et al. **Clinical efficacy and safety of glucosamine, chondroitin sulphate, their combination, celecoxib or placebo taken to treat osteoarthritis of the knee: 2-year results from GAIT.** *Ann Rheum Dis* 2010; 69: 1459–1464.
26 Scott RM, Evans R and Conzemius MG. **Efficacy of an oral nutraceutical for the treatment of canine osteoarthritis. A double-blind, randomized, placebo-controlled prospective clinical trial.** *Vet Comp Orthop Traumatol* 2017; 30: 318–323.
27 Bhathal A, Spryszak M, Louizos C, et al. **Glucosamine and chondroitin use in canines for osteoarthritis: a review.** *Open Vet J* 2017; 7: 36–49.
28 DiNubile N. **Glucosamine and chondroitin sulfate: what has been learned since the Glucosamine/chondroitin Arthritis Intervention Trial.** *Orthopedics* 2018; 41: 200–207.
29 Lascelles BD, DePuy V, Thomson A, et al. **Evaluation of a therapeutic diet for feline degenerative joint disease.** *J Vet Intern Med* 2010; 24: 487–495.
30 Fritsch DA, Allen TA, Dodd CE, et al. **A multicenter study of the effect of dietary supplementation with fish oil**

omega-3 fatty acids on carprofen dosage in dogs with osteoarthritis. *J Am Vet Med Assoc* 2010; 236: 535–539.

31 Cameron M and Chrubasik S. **Oral herbal therapies for treating osteoarthritis.** *Cochrane Database Syst Rev* 2014: CD002947. DOI: 10.1002/14651858.CD002947.pub2.

32 Christensen R, Bartels EM, Astrup A, et al. **Symptomatic efficacy of avocado-soybean unsaponifiables (ASU) in osteoarthritis (OA) patients: a meta-analysis of randomized controlled trials.** *Osteoarthritis Cartilage* 2008; 16: 399–408.

33 Gruen ME, Alfaro-Cordoba M, Thomson AE, et al. **The use of functional data analysis to evaluate activity in a spontaneous model of degenerative joint disease associated pain in cats.** *PLoS One* 2017; 12: e0169576. DOI: 10.1371/journal.pone.0169576.

34 Enomoto M, Lascelles BDX, Robertson JB, et al. **Refinement of the Feline Musculoskeletal Pain Index (FMPI) and development of the short-form FMPI.** *J Feline Med Surg*. Epub ahead of print 18 May 2021. DOI: 10.1177/1098612X211011984.

35 Yu G, Xiang W, Zhang T, et al. **Effectiveness of *Boswellia* and *Boswellia* extract for osteoarthritis patients: a systematic review and meta-analysis.** *BMC Complement Med Ther* 2020; 20: 225. DOI: 10.1186/s12906-020-02985-6.