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Evaluation of a Therapeutic Diet for Feline Degenerative Joint Disease

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Background: Feline degenerative joint disease (DJD) is common and there are no approved therapies for the alleviation of the associated pain.

Objective: To test a diet high in eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) content and supplemented with green-lipped mussel extract and glucosamine/chondroitin sulfate (test-diet) for its pain-relieving and activity-enhancing effects in cats with painful, mobility-impairing DJD over a 9-week period.

Animals: Forty client-owned cats.

Methods: Randomized, controlled, blinded, parallel group, prospective clinical study. Cats with no detectable systemic disease, and with at least 1 appendicular joint with radiographic evidence of DJD where manipulation elicited an aversive response were included. Cats were randomly allocated to the test-diet or control diet (C-diet). Outcome measures were subjective owner and veterinarian assessments, and objective activity monitoring (accelerometry). Nonparametric statistics were used to evaluate changes within and between groups for both subjective and objective data, and locally weighted scatterplot smoothing regression analysis was used to predict activity changes.

Results: The primary objective outcome measures indicated that activity declined significantly ($P < .001$) in the C-diet group, significantly increased ($P < .001$) in the test-diet group and there was a significant difference between the groups ($P < .001$).

Conclusion and Clinical Importance: A diet high in EPA and DHA and supplemented with green-lipped mussel extract and glucosamine/chondroitin sulfate improved objective measures of mobility. Dietary modulation might be 1 method to use to improve mobility in cats with DJD-associated pain.

Key words: Arthritis; Cat; Mobility; Nutrition.

Radiographic evidence of degenerative joint disease (DJD) is common in cats,[1–4] and 93 of 100 randomly selected cats had radiographic evidence of DJD in some part of the axial or appendicular skeleton.[5,6] This DJD can be associated with pain,[7–10] and further, this pain can result in decreased mobility.[9] Although NSAIDs are the mainstay treatment for the alleviation of clinical signs in dogs and symptoms in humans with painful DJD, there are understandable concerns about using drugs such as NSAIDs in cats in which chronic kidney disease is common.[11] There has been increasing interest recently in the effect of dietary modulation on DJD-associated pain in dogs.[12–17] There has been particular interest in diets rich in long-chain fatty acids of the n3 series (eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], and eicosatetraenoic acid).[12–14,16,17] There have also been studies that have found a beneficial effect of green-lipped mussel extract in treating pain associated with osteoarthritis in dogs,[14,18–21] and recently a large human study suggested glucosamine/chondroitin sulfate might have a mild analgesic effect in some patients with arthritis pain.[22]

Abbreviations:

BCS	body condition score
CSOM	client-specific outcome measures
DHA	docosahexaenoic acid
DJD	degenerative joint disease
EPA	eicosapentaenoic acid
ETA	eicosatetraenoic acid
LOESS	locally weighted scatterplot smoothing
PL	phospholipid
QOL	quality of life
VAS	visual analogue scale

Although very little is known about the etiology of DJD in cats,[23] therapeutic diets could be a way of decreasing DJD-associated pain in cats. Some work has evaluated the difference in putative serum markers of arthritis in arthritic and nonarthritic geriatric cats, and evaluated the effect of several ''wellness'' foods on these markers.[24]

Our hypothesis was that a diet high in fish oil derived EPA and DHA and supplemented with green-lipped mussel extract and glucosamine/chondroitin sulfate (test-diet) would produce pain-relieving and activity-enhancing effects in cats with painful, mobility-impairing DJD over a 9-week period when compared with a control diet (C-diet) not supplemented with fish oil, green-lipped mussel extract, or glucosamine/chondroitin sulfate. The specific aims of the study were to measure the activity enhancing effects of the test-diet in cats with naturally occurring osteoarthritis using subjective owner assessment

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of activity-related behaviors and also objective activity monitoring using accelerometry. In addition, we aimed to evaluate the palatability of the test-diet and the effects of the diet on blood and urine analysis parameters.

Materials and Methods

This study was approved by the Animal Care and Use Committee at North Carolina State University.

Animals

The intent was to recruit 40 client-owned cats with DJD-associated pain and impaired mobility. Cats whose owners considered they had reduced activity or impaired mobility were recruited from faculty, students, and staff of the North Carolina State University College of Veterinary Medicine (NCSU-CVM), local practices, and the NCSU-CVM Integrated Pain Management Service. Recruitment was performed using e-mails, newspaper advertisements, and direct contact. All owners gave informed signed consent. Group size was based on power calculations using client-specific outcome measure (CSOM) data from previous studies,[9] and also based on pilot clinical data (8 cats) generated in cats with DJD that were administered nutritional supplements. We assumed that half the change in CSOM seen in the pilot data might be caused by placebo effect, and thus calculated that 16 cats would be required per group for a study power of 0.8 (at a 2-sided 5% significance level), and 21 cats in each group for study power of 0.9. We did not have enough preliminary data generated from appropriate subjects to calculate power based on the activity monitor (AM) data.

Evaluation of Potential Study Candidates ("Screening")

Cats whose owners considered them as having reduced activity or impaired mobility (see "Study Protocol" for details) were screened with a physical examination, orthopedic and neurological evaluation, CBC, blood chemistry, urine analysis, and orthogonal radiographs of suspected painful appendicular joints. Each cat was weighed, and body condition score (BCS) recorded (on a scale of 1–5: 1, emaciated; 2, thin; obvious abdominal waist; 3, ideal; 4, overweight; no observable abdominal waist; 5, obese).[25] The number of hours the owner spent with their cat each week was recorded. A single evaluator (B.D.X.L.) performed the physical, orthopedic, and neurological evaluations and was blinded to the treatment groups. Exclusion criteria included the presence of infectious diseases, symptomatic cardiac disease with exercise intolerance, suspected unidentified internal organ disorder, immune-mediated problem, neoplasia, moderate or severe renal disease (see later), inflammatory bowel disease, diagnosed urinary tract infection, hyperthyroidism, diabetes mellitus. These diagnoses were ruled out by careful review of the medical records, owner history, physical examination, blood work, and urine analysis. The orthopedic evaluation consisted of careful palpation of every joint for pain and instability. Additionally, assessment was made of the musculoskeletal system and neurological system for any conditions other than joint pain that might affect mobility. Cats were excluded if non-DJD orthopedic disease (eg, cruciate ligament rupture, joint luxation) or neurological disease (eg, lumbo-sacral nerve impingement) that might have affected mobility was detected. During the orthopedic evaluation, the 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 lumbo-sacral) was graded on the following scale: 0, no resentment; 1, mild withdrawal; mildly resists; 2, moderate withdrawal; body tenses; may orient to site; may vocalize/increase in vocalization; 3, orients to site; forcible withdrawal from manipulation; may vocalize or hiss or bite; 4, tries to escape/prevent manipulation; bite/hiss; marked guarding of area. Physical examinations were performed and recorded before radiographs were made.

Each cat was sedated for radiographic examination with a combination of ketamine (3–5 mg/kg), butorphanol (0.4–0.5 mg/kg), and medetomidine (10–15 μg/kg) administered IM. Doses were reduced or altered where it was considered clinically appropriate. Orthogonal radiographs of appendicular joints that were determined to be painful on examination were taken under sedation with indirect digital flat panel imaging system.[a] After radiography, a CBC, chemistry panel, and urinalysis obtained by cystocentesis were also performed.

Cats with no detectable systemic disease, and with at least 1 appendicular joint where manipulation elicited an aversive response and whose radiographs showed the presence of DJD, were included. Criteria used to determine the presence of radiographic signs of DJD were those previously reported.[26] Briefly, these radiographic signs were osteophytes, enthesophytes, joint associated mineralization, subchondral bone sclerosis, subchondral erosions, and cysts and intra-articular mineralizations (including meniscal calcifications).

Additionally, to be included in the study, it was decided a priori that eligible cats were required to be not currently receiving any anti-inflammatory medications; not have received any nutritional supplements (such as glucosamine/chondroitin sulfate) for at least 6-weeks before the study; free from clinically important abnormal hematological or blood chemistry values (BUN and creatinine increases up to 20% over the top of normal range were acceptable if they had been stable for at least 2 weeks before the study and this was documented via blood work); and an indoor only cat. Only cats whose owners were considered to have a stable routine of daily living that was unlikely to change over the 10 weeks of the study were included. Owners were required to agree to feed only the test-diet to the cat and in multicat households to agree to feed all the cats the study diets, or separate cats at feeding times.

Study Protocol

The study was a blinded, parallel group, placebo controlled, prospective clinical study over a 10-week period. Cats that were screened and deemed eligible for inclusion were randomized to 1 of 2 diets, in blocks of 4, stratified by high and low impairment groups. The primary subjective assessment outcome measure (CSOM) was used during the screening process to determine if owners considered their cat had mobility impairment, and also designate cats as either "high impairment" (score 11–20) or "low impairment" (score 1–10). The diets were only identified by the code names "Felix" (C-diet) and "Peewee" (test-diet). Blinding was maintained until after data had been analyzed.

On day 0 (D0), owners completed the subjective assessments (CSOM, Activity visual analogue scale [VAS]), cats were fitted with AM and owners instructed on transitioning cats onto the test diets over a 7-day period. On days 14 and 42 (D14 and D42) owners completed the CSOM and an assessment of palatability of the food. AM data were downloaded at these times. D14 and D42 assessments were used to give face-to-face contact with the study personnel, to allow AM data to be downloaded, and to help owners stay focused on the study. Subjective data from these time points were not analyzed.

On day 70 (D70), owners completed the subjective assessments (CSOM, VAS-assessed activities [Activity VAS]), palatability score, fecal score, and quality of life (QOL) score. Body weight was measured, BCS assessed and a physical and orthopedic examination performed. Blood was collected for CBC, chemistry, and blood EPA and DHA levels. The protocol is outlined in Figure 1. All

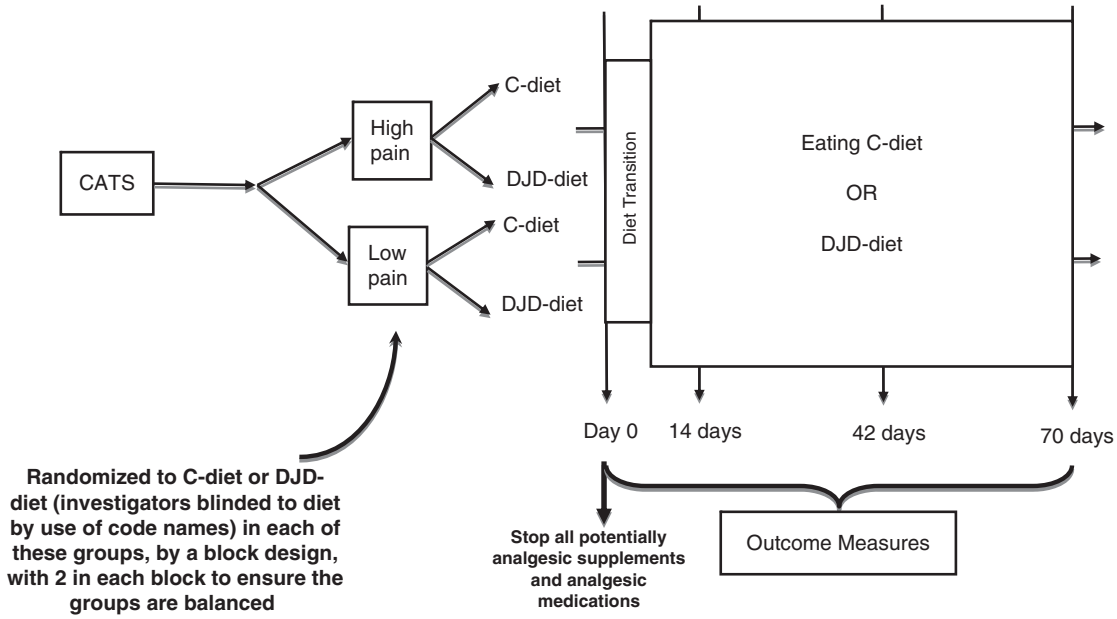

Fig 1. Schematic outline of the study protocol.

personnel involved in the study were blinded to the treatment groups at all times until data analysis had been completed.

Outcome Measures

Primary outcome measures were CSOM and AM counts. Secondary outcome measures were orthopedic evaluation pain scores, VAS activity scores, fecal score, palatability score, overall QOL score, temperament score, and blood and urine parameters.

CSOM

At the screening visit, owners were questioned on the activity of their cat. A general questionnaire (yes/no type) was used to determine if the owner had noticed altered activity. After this, the specific activities that were problematic for their cat were defined in more detail as described previously.[9] Owners were directed to describe 5 time- and place-specific activities that they considered were altered, and to grade the degree of impairment compared with a precise age when they considered their cat's activity was normal. A single investigator (A.T.S.) directed each CSOM construction. This resulted in a unique set of activities for each cat. After completion of the CSOM form on D0, the same unique set of activities was assessed at each visit—D14, D42, and D70. In addition to the CSOM described previously[9] owners also completed a CSOM that used a VAS to rate the degree of activity impairment (CSOM-VAS). The owner was not permitted to see how they had graded severity of activity impairment at the previous assessment.

AM

AM[b] were placed on a neck collar, with the directional arrow pointing upwards.[9] Epoch length (the length of time over which a datum value was created) was set at 1 minute and the time stamp was synchronized with local time (eastern standard time). After fitting of the cats with the AM on D0, they were worn for the duration of the study. At each visit (D14, D42, and D70) the monitor was removed from the collar, 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 in a diary any times when the collar and/or AM was removed. The diary was also used to record adverse events.

Orthopedic Evaluation Pain Scores

The orthopedic evaluation performed during the screening process was repeated at D70, with pain scores being recorded in the same manner. The most painful appendicular joint that also had radiographic sings of DJD was designated the "index joint." The "total pain score" (T-pain score) was the addition of all the scores for each appendicular joint and each segment of the axial skeleton. The "total appendicular pain" score (T-appendic pain score) was the addition of the scores for each appendicular joint. The "maximum pain score" (max pain score) was the highest appendicular or axial segment pain score at that evaluation.

Activity VAS

Because of the fact that there is no validated owner-based assessment system for feline musculoskeletal pain, several activities and behaviors were evaluated using a VAS system. On D0 and D70, the VAS system completed by owners. On D70, they were not permitted to see the D0 data. The activities and behaviors assessed are detailed in Appendix S1.

Fecal Score

At each visit, owners were asked to score the consistency of the cat's feces using a 5-point pictorial scoring chart (1, liquid diarrhea; 4, optimal well formed feces; 5, dry and hard feces).[c]

Palatability Score

On D14 and D70, palatability was assessed on a 5-point scale: (1) Cat refuses to eat food; (2) Cat does not like the food, but will eat it; (3) Cat eats the food normally; (4) Cat appears to enjoy the food; (5) Cat loves the food/is crazy about it.

Global QOL Evaluation

On D14, D42, and D70 the owners were asked to complete a simple global assessment form to evaluate the change in their cat's QOL as a result of the diet. The global assessment used is shown in Appendix S2. Owners were not permitted to see their previous evaluation.

Temperament Score

On D0 and D70, the temperament of the cat on physical examination was scored on a scale of 0–4: 0, neutral attitude, purring, kneading; 1, resistance to restraint; 2, resistance to restraint, growling, and hissing; 3, resistance with biting and scratching, hissing, spitting, and vocalizing; 4, resistance with biting, scratching, vocalizing, spitting, hissing, urinating, or defecating.

Blood and Urine Parameters

A standard CBC and chemistry profile was obtained, and a standard urinalysis performed at D0 and D70. Additionally, on D0 and D70 plasma (from a blood sample collected into EDTA) was collected and stored at −80°C. On completion of the study, total lipids were extracted from the samples and lipid classes subfractionated by thin-layer chromatography. Total phospholipid (PL) subfractions in plasma were derivatized to fatty acid methyl esters and gas chromatography used to generate fatty acid profiles.[27,28] EPA and DHA were expressed as a percentage of total plasma PL fatty acids.

Test Diets

The C-diet (Felix) and test-diet (Peewee) were identical dry expanded diets, except for the addition of anchovy oil, green-lipped mussel powder, glucosamine hydrochloride, and chondroitin to the test-diet. The composition of the diets used is detailed in Table 1. In the test-diet, the anchovy oil replaced part of the poultry fat of the C-diet and the green-lipped mussel powder, chondroitin, and glucosamine replaced part of the rice of the C-diet. Diets were given to owners in 3 and 6 kg unbranded foil bags labeled only with the code name, amount to feed (calculated as 55 kcal metabolizable energy/kg body weight), feeding instructions and the "best before" date.

Statistical Analysis

Data were analyzed by nonparametric approaches because of the relatively small number of animals in each group. In all the statistical analysis, critical P value was considered to be $P < .05$. Data were analyzed by statistical software.[d]

The age, weight, BCS, sex, CSOM score, index joint pain score, whether hind limbs or forelimbs were affected, temperament scores, hours the owner spends with the cat, and fecal score were compared between the groups at D0 by Wilcoxon's rank-sum and Chi-square tests as appropriate.

Values at D0 and D70 were compared within groups by Wilcoxon's rank-sum tests, and the change between D0 and D70 was compared between groups by Wilcoxon's signed-rank tests for each of the following subjective parameters: CSOM, Temperament score, T-pain score, T-appendic pain score, Max Pain score, Overall QOL, each of the individual activity VAS, fecal score, palatability, BCS, blood and urine parameters and fatty acid profiles.

Activity counts over days 8–21 of trial diet feeding (the 1st 2 weeks following the 7-day transition to the test-diet) were compared with the activity counts in the last 14 days of trial diet feeding within each group and between groups for the following time periods (using daily averages over the appropriate 14-day time period): 12 midnight to 6:00 AM; 6:00 AM to 12 midday; 12 midday to 6:00 PM; 6:00 PM to 12 midnight; over the whole day. Comparisons were made by Wilcoxon's rank-sum tests and Wilcoxon's signed-rank tests. Additionally, a locally weighted scatterplot smoothing (LOESS) regression analysis was performed using model variables (time periods) as a function of diet, weight at the start and change in weight between D0 and D70. The predicted values from that model were summarized, and compared with each other (Wilcoxon's rank-sum test) and change by diet compared with zero (Wilcoxon's signed-rank test).

As a sensitivity analysis, we conducted a repeated measured analysis on the mean weekly activity, using baseline weight and weight at endpoint as covariates, in addition to the diet.

Table 1. Composition of the diets used in the study.

	Units	C-Diet	Test-Diet
Protein	g/1,000 Kcal	74.96	71.57
Fat	g/1,000 Kcal	37.67	42.23
Crude fiber	g/1,000 Kcal	10.33	7.93
NFE	g/1,000 Kcal	98.02	91.15
Ash	g/1,000 Kcal	14.36	13.37
EPA+DHA	g/1,000 Kcal	0.03	1.88
Total n3 fatty acids	g/1,000 Kcal	0.68	2.97
Total n6 fatty acids	g/1,000 Kcal	7.66	8.03
CS+glucosamine	mg/1,000 Kcal	0.00	250.00
GLM	mg/1,000 Kcal	0.00	74.00
Energy kcal ME/kg as fed	Kcal/kg as fed	3970	4070

Ingredient list (ingredients that were present in test-diet only are presented in italics): Pearled barley, corn, corn gluten meal, rice, wheat gluten, chicken, chicken fat, chicken meal, oat meal, *anchovy oil*, dried egg powder, powdered cellulose, dried beet pulp, ground psyllium seeds, soya bean oil, potassium chloride, calcium, calcium carbonate, fructo-oligo-saccharides, *green-lipped mussel powder*, DL-methionine, brewers yeast extract (source of mannan-oligo-saccharides), potassium citrate, choline chloride, taurine, *glucosamine* hydrochloride, vitamins], Trace Minerals oxide, manganese proteinate, copper [zinc oxide, ferrous, zinc proteinate, copper, manganous proteinate, calcium iodate, sodium selenite], green tea polyphenols, *chondroitin sulfate*, marigold extract, L-carnitine.

CS, chondroïtin; GLM, green lipped powder.

Results

Fifty-three cats were screened for inclusion in the study, of which 43 started the study. Three cats dropped out of the study: 1 because of vomiting and the collar constantly being removed (D14; test-diet); 1 because of a central neurological event (cause undiagnosed) (D19; test-diet); 1 because of not transitioning to the test-diet (D14; test-diet). Forty cats completed the study, 20 in each diet group.

On D0, before the start of the study, there were no differences between the groups with respect to age, sex distribution, CSOM score, index joint pain score, whether hind limbs or forelimbs were affected, temperament scores, hours the owner spent with the cat, fecal score, and BCS. Cats in the C-diet group were significantly heavier than the test-diet group (5.97 ± 2.23 kg versus 4.71 ± 1.04 kg, $P = .040$). There were 15 high-impairment and 5 low-impairment cats in the C-diet group; and 14 high-impairment and 6 low-impairment in the test-diet group.

Missing data consisted of an incomplete orthopedic evaluation in 2 cats on D0 (temperament of the cat did not allow a full evaluation to be performed—we were able to examine the joints with DJD); CSOM and Activity VAS data for 1 cat on D70 (data sheets lost); 480 hours of AM data that were not captured (1.5% of the total 67,200 hours planned) because of 1 AM malfunctioning, collars being removed by the cats and 23 days of data were not captured at the end of the study due to owner schedules and revisit times.

Table 2. EPA and DHA concentrations (mean ± SD), expressed as percentage of total fatty acids bound to phospholipids.

	D0	D70	D70−0
Mean EPA			
C-diet	1.33 (0.78)	0.54 (0.32)	−0.79 (0.73)
Test-diet	1.06 (1.15)	6.10 (2.16)	5.02 (2.91)
Mean DHA			
C-diet	3.86 (2.02)	2.09 (1.15)	−1.77 (1.39)
Test-diet	3.12 (1.64)	3.80 (1.1)	0.71 (2.07)

D0, day 0; D70, day 70; D70−0, difference between levels at D0 and D70; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

On D70, there was no difference in fecal scores between the C-diet and test-diet groups (median 4 [range 3–5] and median 4 [range 2–5], respectively). There was no difference between the groups in change in fecal score between D0 and D70. Within groups, there was a significant decrease in fecal score in the test-diet group ($P = .027$) but not in the C-diet group ($P = .359$). There was no difference between the C-diet and test-diet groups in palatability scores at D70 (median 4 and range 2–5 for each group).

Both groups lost weight over the study period: C-diet group averaged a 30 g loss (SD 201 g) ($P = .401$), test-diet group averaged a 159 g loss (SD 159 g) ($P = .001$). The difference in weight loss between the groups was not significant ($P = .094$). The C-diet group was significantly heavier than the test-diet group at D70 ($P = .024$). There was no significant change in BCS in either group, and no significant difference between the groups at D70 in BCS.

On D0 there was no significant difference between groups in 20:5n3 (EPA) ($P = .088$) or 22:6n3 (DHA) ($P = .152$) fatty acid concentrations in the plasma. On D70 there were significantly greater amounts of 20:5n3 ($P < .001$) and 22:6n3 ($P < .001$) in the plasma of the test-diet group cats (Table 2). In the C-diet group, 1 cat had slightly higher EPA and DHA on D70 than D0 (approximately 0.5% higher). In the test-diet group, 1 cat had lower EPA and DHA on D70 than D0 and 5 cats had lower DHA on D70 than D0.

Primary Outcome Measures

CSOM and CSOM-VAS scores for both groups significantly improved over time ($P < .001$ for both groups). There were no differences between the groups in the CSOM (Table 3) or CSOM-VAS scores at D70 or for the change between D0 and D70. When only the high-impairment cats were evaluated, again CSOM and CSOM-VAS scores for both groups significantly improved over time (CSOM: $P = .002$ and $P < .001$, CSOM-VAS: $P = .002$ and $P < .001$, for C-diet and test-diet, respectively).

When comparing the change in activity counts over time (D8-21 period versus the last 14 days) between the groups, there was a nonsignificant difference between the groups for the 24-hour ($P = .082$) and the 6:00 AM–12:00 PM ($P = .078$) time periods. This was due in part to a significant decrease in activity in the C-diet group for the 24-hour ($P = .027$) and 6:00 AM–12:00 PM ($P = .009$) time periods. Additionally, over the period of the trial, there was a significant decrease in activity counts in the 12:00–6:00 PM time period ($P = .029$) in the C-diet group (Table 4).

Table 3. CSOM scores (mean ± SD) at D0 and D70 of the study, and the change in score from D0 to D70 (D70–0).

	D0	D70	D70−0
C-diet	12.2 (3.42)	5.5 (3.4)	−6.6 (4.14)
Test-diet	11.8 (4.31)	6.6 (3.51)	−5.3 (3.78)

There were no differences between the groups.

CSOM scores for both diet groups improved significantly over time ($P < .001$ for both groups).

CSOM, client-specific outcome measures.

When LOESS regression analysis was used to model variables as a function of diet, weight at start and change in weight between D0 and D70, the model showed that the test-diet group significantly increased their activity in the 6:00 PM–12:00 AM time period ($P < .001$). Similarly, the model showed that the C-diet group significantly decreased activity over all time periods after 6:00 AM, and over the entire 24-hour period ($P < .001$, .001, .001, and .001 for 6:00 AM–12:00 PM, 12:00–6:00 PM, 6:00 PM–12:00 AM, and the entire 24-hour period, respectively). A significant difference between diet groups was predicted for all evaluated time periods except the 12:00–6:00 AM period ($P < .001$, .001, .001, and .001 for 6:00 AM–12:00 PM, 12:00–6:00 PM, 6:00 PM–12:00 AM, and the entire 24-hour period, respectively) (Table 5).

The sensitivity analysis confirmed the above results, and showed that diet had a significant effect ($P = .0298$), with the test-diet group having counts 10,736 higher than the C-diet.

Secondary Outcome Measures

For the global assessment of change in QOL, both diet groups significantly improved ($P < .001$) according to owners, and there was no difference between the groups. Both diet groups significantly improved when only looking at "High Impaired" cats; again, there was no difference between the groups. Over time, there was no significant change in the temperament score in either group nor was there a difference between the groups.

There were several changes in owner-assessed activity VAS scores between D0 and D70 in both groups. There was a significant decrease in aggression ($P = .045$), and eating ($P = .016$) in the C-diet group. There was a significant increase the ability to jump up ($P = .035$), increase in eating ($P = .030$), and decrease in time spent sleeping ($P = .005$) in the test-diet group. Evaluating the change in activities and behaviors between the 2 groups over D0–D70, there was a significant difference between the diet groups for playing and interacting with other pets (greater increase in the test-diet group, $P = .007$) seeking seclusion (greater decrease in the test-diet group, $P = .035$), and sleeping (greater decrease in time spent sleeping in the C-diet group, $P = .022$), and a slight difference between the groups for restlessness (greater decrease in restlessness in the test-diet group, $P = .056$).

Table 4. Changes in activity counts between the early (D8–21) and late (last 14 days) time periods, expressed as mean and median values for each diet group for the whole day and 6-hour segments of the day.

Time Period	C-Diet: Mean (SD)	Test-Diet: Mean (SD)	Wilcoxon Rank-Sum Test *P* Values for Difference between Diets	Wilcoxon Signed Rank *P* Values for Difference from Zero: C-Diet	Wilcoxon Signed Rank *P* Values for Difference from Zero: Test-Diet
12:00–6:00 AM	−1,142 (5,002)	−293 (4,700)	.809	.349	.674
6:00 AM–12:00 PM	−4,002 (6,986)	−384 (4,857)	.078	.009	.498
12:00–6:00 PM	−3,512 (6,246)	−994 (5,097)	.503	.030	.246
6:00 PM–12:00 AM	−3,140 (6,796)	521 (4,758)	.167	.133	.475
Total change over day	−11,948 (22,183)	−1,110 (14,984)	.082	.027	.596

Counts are arbitrary units.

Both the C-diet and test-diet groups had decreased T-pain scores ($P = .011$ and .037, respectively) and decreased T-appendic pain scores ($P = .019$ and .017, respectively) between D0 and D70. The maximum pain score decreased in the C-diet group (mean decrease of 0.5 $\pm$ 0.69) ($P = .008$) between D0 and D70, but not in the test-diet group (mean decrease of 0.4 $\pm$ 0.81) ($P = .11$). There were no differences between the diet groups for any change in orthopedic pain scores over D0–D70.

There were several significant changes in blood values both within and between groups over the D0–D70 time period (Table 6). The changes considered clinically significant were: the decrease in ALT in the test-diet group ($P = .030$); increase in lipase in the test-diet group ($P = .005$) and difference between the groups in lipase change ($P = .038$); increase in monocytes ($P = .003$) and eosinophils ($P = .027$) in the C-diet group.

Discussion

This prospective, randomized, clinical study demonstrated that cats fed a diet high in fish oil derived EPA and DHA and supplemented with green-lipped mussel extract and glucosamine/chondroitin sulfate had greater objectively measured activity than cats eating the C-diet. The primary (CSOM) and overall QOL subjective measures by owners and the veterinarian examination scores revealed that each of the diets significantly improved mobility and reduced pain on manipulation. However, there were several significant changes in specific activities assessed subjectively that resulted in significant differences between the groups (favoring the test-diet group) for playing and interacting with other pets, seeking seclusion, and sleeping. The fatty acid analysis of the plasma confirmed that the intended diets were eaten by cats in each group.

There are no validated subjective assessment instruments for the evaluation of DJD-associated pain in cats. Indeed, such owner-completed instruments are only just beginning to be developed in canine medicine.[29–33] A major criticism of this study is the use of a nonvalidated subjective owner outcome assessment. However, the primary subjective assessment system used in this study (CSOM) appeared promising in early pilot work, which suggested owners could tell when their cat's chronic musculoskeletal pain was alleviated.[9] However, in that study, there was a large placebo effect with the difference between the placebo effect and NSAID barely achieving significance. That was probably partly because of small numbers of cats, but it demonstrates the strong placebo effect. It is likely that any therapeutic difference between the C-diet and the test-diet, if it exists, might be less that between an NSAID and placebo drug, making it very difficult to detect differences between treatment groups using this form of assessment. Additionally, in the present study, we found the degree of improvement in CSOM scores to be greater when an NSAID was used in a previous small study.[9] This might reflect a greater effect of both diets, or, more likely, might reflect a strong desire on the part of study participants for intervention to work.

Table 5. Locally weighted scatterplot smoothing regression results for changes in activity in each diet group over the study duration, with model variables (ie, time period activity counts) as a function of diet, weight at start, and change in weight between D0 and D70.

Time Period	C-Diet: Mean (SD)	Test-Diet: Mean (SD)	Wilcoxon Rank-Sum Test *P* Values for Difference between Diets	Wilcoxon Signed Rank *P* Values for Difference from Zero: C-Diet	Wilcoxon Signed Rank *P* Values for Difference from Zero: Test-Diet
12:00–6:00 AM	−1,211 (2,531)	113 (1,636)	.110	.058	.812
6:00 AM–12:00 PM	−4,279 (3,075)	−117 (843)	< .001	< .001	.756
12:00–6:00 PM	−3,572 (2,510)	−330 (1,186)	< .001	< .001	.277
6:00 PM–12:00 AM	−2,898 (2,564)	1,517 (1,015)	< .001	< .001	< .001
Total change over day	−12,178 (9,225)	1,205 (3,951)	< .001	< .001	.105

Counts are arbitrary units.

Table 6. Changes in measured blood and urine parameters over D0–D70.

Laboratory Test (Units) Normal Range	C-Diet D0: Mean (SD)	Test-Diet D0: Mean (SD)	C-Diet D70: Mean (SD)	Test-Diet D70: Mean (SD)	Wilcoxon Rank-Sum Test *P* Values for Difference between Diets	Wilcoxon Signed Rank *P* Values for Difference from Zero C-Diet	Wilcoxon Signed Rank *P* Values for Difference from Zero Test-Diet
BUN (mg/dL) 15–41	−5.2 (4.8)	−1 (10.4)	23.6 (5.5)	29.9 (14.7)	.187	< .001	.153
Phosphorus (mg/dL) 2.5–5	−0.4 (0.7)	−0.5 (0.8)	4.0 (0.8)	3.8 (0.9)	.494	.018	.006
Cholesterol (mg/dL) 93–304	16.2 (39.9)	29 (36.6)	175.5 (48.4)	185.1 (37.5)	.437	.105	.002
Alkaline phosphatase (IU/L) 14–50	5.2 (6.7)	−0.1 (9.2)	33.3 (12.2)	28.4 (11.6)	.141	.003	.686
ALT (IU/L) 28–88	0.4 (87)	−15 (40.3)	74.5 (62.0)	46.1 (12.2)	.216	.993	.030
Bicarbonate (mmol/L) 14–23	−1.1 (2.6)	−2 (2.0)	19.0 (2.6)	17.8 (1.7)	.221	.105	< .001
Anion gap 15–32	1.6 (3.9)	2.2 (2.6)	19.5 (2.1)	20.2 (1.5)	.629	.048	.002
Amylase (IU/L) 580–1,520	101.6 (173.7)	95.6 (132.1)	1,131.8 (320.4)	1,171 (342.2)	.861	.007	.006
Lipase (IU/L) 10–64	0.2 (3.2)	7.1 (15.4)	22.4 (6.9)	30.9 (31.6)	.038	.954	.005
Magnesium (mg/dL) 1.8–2.6	−0.2 (0.2)	−0.1 (0.2)	2.3 (0.3)	2.3 (0.3)	.344	.001	.106
RBC ($\times 10^6$/μL) 6.91–10.49	0.9 (1.8)	0.9 (1.7)	8.24 (0.7)	8.6 (1.3)	.883	.040	.041
Hematocrit (%) 32.8–49.8	3.8 (8.3)	4.1 (7.4)	37.5 (3.8)	38.2 (5.2)	.946	.040	.040
MCH (pg) 13–17.7	−0.5 (0.7)	−0.6 (0.9)	15.2 (1.2)	14.5 (1.4)	.778	.004	.010
MCHC (g/dL) 31.1–34	−0.7 (1.8)	−1.4 (1.9)	33.5 (2.2)	32.5 (2.0)	.246	.130	.007
Plasma protein (g/dL) 6.5–8.4	0.3 (0.7)	0.4 (0.6)	7.9 (0.72)	7.8 (0.4)	.850	.040	.028
Monocytes ($\times 10^3$/μL) 0.068–0.78	0.1 (0.22)	0.1 (0.22)	0.34 (0.27)	0.26 (0.19)	.503	.003	.120
Eosinophils ($\times 10^3$/μL) 0.118–0.879	0.5 (1.18)	0 (0.54)	0.83 (1.37)	0.46 (0.30)	.503	.027	.330

During the study, we found that owners were very keen for the test-diet to work, citing that it would be a safe and easy way to provide their cat some relief, and would circumvent the need for medications and then negate the worry associated with medications. The study veterinarian performing all the orthopedic evaluations was blinded to diet identity, and yet their data indicated that both groups had significant reductions in pain scores. Again, this could be real, or more likely, is the result of the cats in both groups becoming more used to the clinic visits and the veterinarian, and reacting less. Our group has observed this in other studies of cats (unpublished data). Overall, this points to the need for validated and sensitive subjective assessment methods, and the knowledge of how to apply them in such clinical studies.

There were differences between the groups in the accelerometry data. Increasing accelerometer counts correspond to increasing activity and distance moved in cats[34] and cats with mobility impairment because of DJD have increased activity counts when administered an NSAID.[9] These latter data suggest that pain relief results in increased activity in cats with painful DJD. Controlling for weight and change in weight, the present study found a significant increase in activity in the cats fed the DJD diet, and a significant decrease in activity in the cats fed the C-diet, and thus a clear difference between the groups. There are several plausible explanations for this. The natural history of progression of DJD and decrease in mobility has not been investigated for cats, and it is possible that the test-diet prevented the gradual loss of mobility that occurred in the C-diet fed group. The C-diet fed group had significant reductions in plasma EPA and DHA between D0 and D70, and it might be that feeding reduced amounts of EPA and DHA resulted in a reduction in mobility over the time period of the study, but that feeding significantly increased amounts of EPA and DHA, as occurred in the test-diet group, does not result in correspondingly as large an increase in activity. The majority of cats in both groups were on what would be considered ''premium'' foods before the start of the study. We were not able to power the study appropriately for the activity data because of a relative lack of relevant AM data. It might be that the study was underpowered for the AM data, and with appropriate numbers of subjects, clearer changes would have been seen both within and between groups.

Additionally, there is little known about how activity changes with pain relief in feline DJD. A previous study suggested that activity will increase,[9] but the relative increase that can be expected, and the time at which this occurs are not known. It might be that activity does not increase dramatically, but the cats are able to move more easily. This would not be recorded by the accelerometers. Learned behaviors might also play a confounding role.

Because of the large variability in activity data both between and within cats, we were careful to compare each cat only to itself, and compare the same weekdays to each other when comparing the early and late time periods and therefore the differences seen between the groups are likely a result of the diets.

For the analysis, we chose to compare a 14-day period from the start of the study with a 14-day period at the end. The 14-day period at the start of the study commenced immediately after the transition to the test-diet was complete (7 days).

The weight loss in relation to the changes in activity needs some discussion. Although not significantly different between the groups, the test-diet group lost more weight than the C-diet group. One explanation for this might lie in the increased activity in the test-diet group, possibly as a result of the cats feeling more comfortable. Conversely, the weight loss might have resulted in more activity in the test-diet group, and it might be the weight loss that was more important than any pain relief. In dogs, weight loss has been shown to result in decreased lameness in dogs with OA pain,[35] but in that study the weight loss was between 11 and 18%. In the present study, the test-diet group lost only 3.4% of their body weight. In a recent human study, weight loss of 3.1% did not result in decreased pain or improved function.[36] It is possible that the test-diet promoted weight loss in this group of cats. There is accumulating evidence that diets high in DHA and EPA might be beneficial in preventing obesity and promoting weight loss by creating a physiological environment that promotes lipid oxidation and inhibits lipogenesis and formation of adipocytes,[37–39] and might increase energy expenditure through Na^+/K^+/ATPase activity.[40] The relationship between weight, weight loss, activity and diet needs more investigation in the cat.

We were surprised to find changes both within groups and between groups when using the "activity" VAS assessment. This is another subjective assessment composed of many individual questions. No multiplicity adjustments of the type I error rate were used; therefore it is possible that the significant changes seen were because of chance. However, the majority of the questions used in the activity VAS have been found to allow cats with and without painful DJD to be distinguished in ongoing work in our program.[41] Additionally, reviewing the direction of changes shows that most of the changes and differences between the groups suggest the test-diet group were more active and more interactive. Full interpretation of the data is hampered by a lack of objective knowledge about what behaviors are altered, and in what direction, in painful DJD. This point only underlines the need for a validated subjective owner assessment system to evaluate DJD-associated pain.

Much of the above discussion relies on the assumption that the n3 fatty acids have a beneficial effect in painful DJD. DJD involves an inflammatory component in the periphery, and also inflammatory processes are likely involved in the central nervous system.[42] It might be possible to modify the inflammation by nutritional components, specifically n3 long chain fatty acids; however, the extent to which DJD in cats is inflammatory is not known. Several studies have been published that demonstrate a beneficial response to n3 fatty acid incorporation into diets of human beings with rheumatoid arthritis,[43,44] but this is a more inflammatory disease than osteoarthritis. One study in dogs found that a diet high in n3 fatty acids decreased matrix metalloproteinases in the synovial fluid of dogs that had a ruptured cranial cruciate ligament and underwent surgery.[16] There are no published randomized controlled clinical trials evaluating the effects of n3 fatty acids in dogs with DJD; however, there is 1 randomized, blinded study evaluating green-lipped muscle (GLM) which showed an improvement in the treated dogs over the control dogs.[14] GLM was included in the test-diet in the present study. Although GLM does contain n3 fatty acids, the levels are very low. The mechanism by which GLM might be acting is not known. Glucosamine and chondroitin sulfate were added to the diet, and arguably the best clinical study so far in humans has recently suggested a mild analgesic effect of the combination.[22]

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Footnotes

[a] Canon Medical CXDI-50G Sensor, Eklin Medical Systems, Santa Clara, CA

[b] Actical, Mini Mitter, Bend, OR

[c] Royal Canin Fecal Scoring Chart for Cats, Royal Canin, Aimargues, France

[d] SAS 9.1, SAS, Cary, NC

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Evaluation of Changes in Mobility and Activity Using the VAS Assessment as an "Absolute Measure" of the Activity

Appendix S2. Global Assessment of Quality of Life

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