Title

Nutraceutical supplementation increases mobility in aged captive non-domesticated felids.

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Abstract

Background –

Musculoskeletal diseases (MSDs) are an increasing issue as the lifespan of captive animals increases. Extracts of green-lipped mussels have been linked to alleviation of MSDs in domestic carnivores. Understanding their efficacy in non-domestic felids could provide another tool in improved welfare of aged individuals in collections.

Methods –

A within subject study design quantified steps per minute in each of 18 cats of 13 species before and after addition of Antinol^{TM} to their diets. The age structure of four commonly kept subspecies of non-domestic cats was quantified to provide a demographic context to the need for managing aged individuals.

Results – Each of the 18 cats exhibited a higher rate of steps per minute after addition of Antinol in their diet. A paired t-test showed that at the group level the rate of steps after uptake was significantly higher than before addition of Antinol to the diet.

Limitations –While our results showed a strong significant increase, further studies that incorporate a placebo, more individuals, and more detailed metrics of mobility would provide a more detailed evidence-base for practitioners.

Conclusion – Nutraceuticals may yield benefits to aged individual felids including species kept widely in European collections. Their use warrants further, detailed research in collections.

Keywords

Antinol^{TM}, Felidae, green-lipped mussel extract, musculoskeletal disease, osteoarthritis

Introduction

Captive felids suffer a range of health issues and novel diseases that are not typically observed in their wild counterparts[1]. Specifically, the longer lifespan, and the reduced activity and behavioural repertoire of captive felids can lead to a range of musculoskeletal diseases (MSDs). Although the boundary between standard physiological ageing and MSDs is somewhat subjective, if there is pain, loss of mobility or functional impairment MSDs can be considered present[2,3,4]. In older domesticated cats, MSDs can be highly prevalent: degraded cartilage commonly leads to osteoarthritis, while more widespread degeneration of the joints is categorised as degenerative joint disease, both of which fall under the umbrella term of MSDs. MSDs are also one the most prominent issues within captive felids, being associated with a large proportion of deaths within some age-classes of some species (e.g., geriatric jaguars[5,6]), in addition to having considerable welfare implications in chronic conditions[2]. Long-term studies suggest that mortality because of MSDs is common across a range of species in captivity, including jaguars[5,6], Amur leopards[7] and Asiatic lions[8]. The presence and welfare impact of MSDs, specifically degenerative joint diseases, are not restricted to larger species, with the similar trends also having been observed in smaller species, including domestic cats[9,10,11].

Despite MSDs being a well-known issue among aged felids, there is limited research into the efficacy of different treatments for these diseases. However, empirical evidence for some successful methods of treatment does exist, including surgery, medication, environmental manipulation, and nutraceutical supplementation. Surgery has been conducted on Bengal tigers[12], and snow leopards[13], bilateral denervation of the coxofemoral joints in the former case, osteochondral autograft transfer in the latter. In each case surgery led to medium to long-term benefits, with few side effects. Environmental manipulation, including switching to carcass feeding in Asiatic lions[14] and social housing conditions in tigers[15], has been associated with improved locomotor activity and a wider range of behaviours, both of which can increase range of motion, mobility and maintain muscle strength, with a view to reducing the functional impairment associated with MSD. Medication is another option for treatment of long-term conditions. Different medications have been proven to be an effective treatment for osteoarthritis in domestic cats[16, 17, 18, 19], but there are concerns related to pharmaceutical intervention. For example, there are concerns about side-effects of long-term use of pharmaceuticals[20, 21] although these may be less widespread than some fear[22,23]. Further, some of the pharmaceuticals that are very commonly used to treat MSDs have been developed for use in humans, for example tramadol and gabapentin,[24,25] can have prohibitive costs or may not be widely licensed[3]. Another option for treatment of MSDs are nutraceuticals (supplements added to the food), which unlike surgery are non-invasive, and which compared to pharmaceuticals are relatively inexpensive, although evidence for their efficacy, while promising, is sparse even in domestic cats[26,27] while to our knowledge there is only one published study on their use in non-domesticate felids. In this study, Arabian leopards and cheetahs with pre-existing conditions both showed increased activity and orthopaedic status after addition of Steadfast™, an eggshell membrane supplement, containing components such as collagen, hyaluronic acid, glucosamine, chondroitin sulphate, durmatan sulphate, desmosine, amino acids, and peptides[21], to their food[28]. However, this study was based on a very small sample size in only two species. There is a significant gap in our knowledge of the treatment efficacy of nutraceutical interventions for MSDs across the felid family.

Domesticated species represent a useful analogue for understanding health issues in wild and non-domesticated captive animals. They offer a more amenable, flexible system to study the effects of a given treatment, and therefore an opportunity to transfer knowledge of treatment efficacy to larger, less abundant, and less tractable species. The evidence-base for nutraceutical use in treating MSDs in domestic animals could therefore inform treatment in other closely related non-domesticated species housed zoo collections or sanctuaries. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are a widely used nutraceutical for human and animal health conditions, yet evidence for their efficacy is limited and results have been mixed[29,30,31]. One group of nutraceuticals which have shown promise are extracts of green-lipped mussel (GLM) (*Perna canaliculus*), which are thought to act via anti-inflammatory activity stimulated by a suite of lipids present in the supplement[32]. GLM extracts have been shown to alleviate symptoms of osteoarthritis and degenerative joint diseases in both domestic dogs[33,34,35,36] and cats[29,31]. In the context of non-domesticated captive felids, the latter result is of particular interest. For example, the *Panthera* genus is very similar anatomically to the domestic cat, with studies into the thoracic limb[37] and the pelvic limb[38] of lions showing the main anatomical differences being subtle, mostly relating to shape changes to allow for increased load during hunting. Given the conserved anatomy among felids, supplementation with GLM extracts may have similar effects in non-domesticated cats, including big cats, to those observed in domestic cats. One specific product based on GLM is Antinol™, which has shown beneficial effects in domesticated dogs[39,40]. This nutraceutical performs well in treating MSDs, specifically osteoarthritis, relative to non-steroidal anti-inflammatory drugs[39], is safe and effective in combination with medication[40,41] and is known to be safe at high levels[42]. As yet, however, its potential for treating MSDs in felids generally, but specifically in captive, non-domesticated felids is unknown.

Here, we use a within subject study design to investigate whether the nutraceutical Antinol™ is effective in reducing joint pain and the effects of MSDs in aged non-domesticated cats. To do so, we measure steps per minute before and after treatment with Antinol in a collection of aged captive non-domestic cats. We predict that we will see an increase in steps per minute after receiving Antinol, as green-lipped mussel extracts have previously been associated with increases in subjective measures of activity in domestic cats[29,31]. To provide further context for the scope of the problem of MSDs in older non-domesticated captive felids, we examine and report on the age distribution of felids in European collections as reported in the Zoological Information Management System[43] (ZIMS; Species 360 2023) focusing on four of the most widely kept species/sub-species: African (*Panthera leo*) and Asian lions (*Panthera leo persica*), Amur tigers (*Panthera tigris altaica*), and Sumatran tigers (*Panthera tigris sumatrae*).

Methods

Study Site

The study was completed at the Big Cat Sanctuary (BCS), based in Kent, United Kingdom. The collection under observation contained 18 individuals representing 13 species of a range of sizes. Individuals varied in their sex and age (Table 1). All cats kept at the sanctuary were either actively or previously involved in international breeding programmes.

Ethical Statement

The changes to the already existing dietary supplement regime of the animals (described below) were part of ongoing husbandry developments and were conducted under supervision and with the authority of two veterinary surgeons who oversee the collections at BCS. The data collection was non-invasive and presented no changes to the daily routine of the animals involved.

Experimental design

A within-subject design was used to examine the mobility of each individual cat before and after treatment with a nutraceutical supplement, Antinol, which is based on an extract of green lipped mussels containing a range of 3-omega fatty acids. Data on each cat was collected in two phases, each of which lasted a total of six days (before supplementation: 23rd-28th August 2022; and after supplementation: 18th-23rd October 2022). Data were collected 8 weeks apart to allow Antinol to take affect (4-6 weeks being the estimated time for the supplement to take effect, based on manufacturer's documentation[44] Antinol The Executive Summary 2023). The Antinol used in this trial was bought by the research project team.

Following data collection during the pre-treatment phase, the focal cats received Antinol non-invasively, as a dietary supplement. Capsules were placed inside small food pieces that were then introduced into the animal's enclosure to provide enrichment. The number of capsules given to each individual was dependent on their body weight with one capsule being administered per 20kg of body mass[44]. Capsules were given daily for six weeks. To record whether all animals received the medication keeping staff completed a compliance form to record each day. Prior to and during the study, all individuals were on Carnivit and Flexadin supplements.

Data Collection

Animals were observed from outside the enclosures between 09:00 and 16:00 each day. The objective was to collect one hour of activity for each cat in each of the before- and after-Antinol supplementation blocks. Each day, all enclosures were systematically visited, and if the cat was already active, recording took place for as long as that activity continued.

However, if the cat began activity upon observer arrival no data was collected for several minutes to allow the cat to habituate to the presence of human beings. Food motivation may also play a role in increased activity or speed, so feeding times and days were kept at a fixed schedule throughout, with data being collected for each individual on both feeding and non-feeding days to get a representative average.

The number of steps per minute of observation time was our metric for comparison and all data were collected through video analysis. All recordings were collected using a camcorder (Sony Handycam HDR CX240E, 1280x720/25p) or a GoPro Hero 6 at 30fps with 1080p resolution. The baseline level of inactivity in cats made general activity patterns operationally impractical as a response variable. To collect data on steps per unit time the number of steps was totalled for every period of activity recorded. A step was defined as each time the front left foot was raised and then returned to the ground, and a period of activity defined as the time-window in which the cat was actively in motion. For each time window the number of steps taken was divided by the number of seconds within the time window to obtain a consistent measure of steps per unit time. For the purposes of analysis, we converted this number into steps per minute and took the mean steps per minute across all quantified time windows for each cat for each of the two data collection periods. This gave us, for each cat, an average steps per minute in each of the before and after supplementation data collection periods.

In studies of chronic pain, the caregiver placebo effect can have a strong effect[45,46] and so we quantified the level of inter-observer reliability between the primary data collector (MN), who knew which treatment the data related to, and one independent observer (JB) who was blind to treatment. For each cat, the independent observer (JB) scored the steps per minute on two randomly selected video clips of a maximum of 30 seconds in duration, one clip per cat from each of the pre- and post-treatment phases. The two clips were scored blind to treatment. The scores obtained were compared to those scored by the primary data collector (MN) by means of a Pearson's correlation.

Data Analysis

All data met the assumptions of a paired t-test, which was therefore used to analyse the data in the software R[47].

ZIMS was interrogated for demographic data on two widely kept species, *Panthera leo* and *Panthera tigris*, including data on two subspecies of each (*P. leo* and *P. leo persica; P. tigris altaica, P. tigris sumatrae*) within captive collections held within Europe. Age pyramids for each were constructed and for each we calculated the median age and proportion of the population of 15 years or older (often used as a definition of geriatric in big cats).

Results

Group-level analysis revealed an overall significant increase ($t=-7.80$, $df = 17$, $p<0.001$; Figure 1) in steps per minute (SPM) after treatment (113.28 ± 4.3) versus before treatment (98.2 ± 3.24). Every individual cat in the study experienced an increase in steps per minute regardless of species, age, sex, or body mass (Table 1 and Figure 1).

Table 1: Taxonomic and demographic data on cats included in the study along with mean pre- and post-supplementation steps per minute (SPM). 'Relative change' was calculated by (post-supplementation SPM/pre-supplementation SPM)*100. [TABLE FOLLOWS ON NEXT PAGE]

Inter-observer reliability between the main dataset and the subset of observations scored by a second observer blind to treatment was extremely high ($r = 0.99$, $N = 36$, $p < 0.001$).

Analysis of ZIMS demographic data highlighted non-pyramid shaped population structures, with many aged cats of each of the four sub-species we investigated (figure 2). The 'top heavy' nature of these plots suggests that for each of these commonly kept subspecies there was a high proportion of aged cats. For each of the four subspecies, the median age category and proportion recorded in ZIMs as being geriatric (15+ years of age) was as follows: African lion, median = 9-10 years, 0.20 of total population geriatric; Asian lion, median = 10-11 years, 0.12 of total population geriatric; Amur tiger, median = 8-9 years, 0.12 of total population geriatric; Sumatran tiger, median = 9-10 years, 0.21 of total population geriatric. These data suggest a high median age for each, and high proportion of geriatric cats, and therefore higher risk of MSDs, in European collections.

Species	Name	Body mass(kg)	Sex	Age	Total footage before (min)	Total footage after (min)	Mean steps min^{1} pre-supp (S.E.)	Mean steps min^{1} post-supp (S.E.)	Relative Change
Lion	Kasanga	220	Male	12	63.96	62.28	72.37 (1.66)	85.37 (1.66)	118.0
White Lion	Ngozi	190	Male	8	60.23	61.28	84.26 (1.79)	94.04 (1)	111.6
White Tiger	Baikal	140	Male	11	61.12	66.02	88.33 (1.7)	99.96 (0.73)	113.2
White Lion	Imara	130	Female	8	60.23	61.28	80.37 (1.5)	92.68 (3.76)	115.3
Amur tiger	Amasia	113	Female	9	57.05	67.28	91.1 (1.1)	97.34 (1.13)	106.9
Sumatran Tiger	Puna	75	Female	17	61.35	69.32	96.34 (2.6)	109.46 (1.51)	113.5
Sumatran Tiger	Kirana	75	Female	15	63.68	52.14	92.08 (1.46)	111.84 (0.79)	121.5
Jaguar	Neron	70	Male	4	60.72	58.28	102.72 (2.94)	124.06 (1.33)	120.8
Jaguar	Athena	60	Female	18	63.05	38.2	88 (0.9)	96.98 (1.29)	110.2
Amur Leopard	Xizi	40	Female	16	59.85	41.32	111.82 (1.57)	135.15 (1.28)	120.9
Snow Leopard	Yarko	40	Male	10	43.62	54.06	92.45 (1.25)	115.62 (1.27)	125.1
Puma	Valentina	40	Female	14	61.45	62.01	111.57 (1.41)	113.53 (0.78)	101.8
Puma	Yahzi	30	Female	11	64	34.54	94.22 (1.42)	111.39 (1.14)	118.2
Eurasian Lynx	Petra	18	Female	19	55.02	37.41	101.83 (1.98)	111.18 (1.21)	109.2
Serval	Samia	10	Female	12	61.04	58.38	115.02 (1.84)	123.06 (1.93)	107.0
Serval	Jua	10	Female	9	61.37	60.51	104.61 (2.28)	120.02 (1.09)	114.7
Fishing Cat	Aquarius	10	Male	10	15.58	24.45	118.57 (6.53)	155.9 (5.15)	131.5
Jungle Cat	Jack	10	Male	10	57.17	23.39	121.85 (1.43)	141.47 (2.75)	116.1

Discussion

The treatment of aged and geriatric animals in living collections is controversial, often requiring a trade-off between the welfare of the individual and the long-term sustainability of the reproductive population[48]. However, it is an issue that curatorial teams, population managers, and veterinary professionals will need to tackle with increasing frequency[49]. As husbandry and veterinary practice improves, the lifespans of captive animals will increase, and there will be an associated need to maintain their quality of life. Specifically, the risk of reduced welfare resulting from age-related musculoskeletal diseases (MSDs) such as osteoarthritis will become higher[1,49] and will require management of the affected populations and/or individuals. Our investigation of the ZIMS dataset shows that the median ages of four widely kept subspecies of big cats is at, or approaching, ten years of age, and that between 10 and 21% of individuals in the European population of each are 15 years of age or older. These data highlight that the European populations of big cats are aged, and combined with our observational study suggest that nutraceutical supplements could be an important addition to the toolkit that veterinary professionals have available with aim of maintaining quality of life in these animals. Specifically, in 18 aging felids of different age and sex, across 13 species of different size, the addition of dietary nutraceutical supplements was associated with a consistent and significant increase in a key metric of individual mobility (the steps per unit time of those cats). We discuss the implications of these findings, and their potential role in the field below.

These results can start to fill a major gap in research surrounding MSDs in big cats, with very few studies investigating pain, how to measure it, or how to alleviate it within the taxon. Most of the research into the efficacy of nutraceutical treatment has so far focussed on domesticated dogs[33,34,35,36] and cats[29,30,31]. Our results are consistent with this growing body of research and illustrate that green-lipped mussel extracts yield beneficial outcomes in captive non-domesticated felids. The 13 species included in this study represent a broad range of species of different size, geographic distribution, and included individuals of different sex, and age. The fact that the rate of steps per minute increased across all individuals in this range of species highlights the potential transferability of this supplement, and the possibility of it being a more general treatment for musculoskeletal problems in a broader range of taxa outside of domesticated carnivores and non-domesticated big cats.

The caregiver placebo effect can be a strong factor in studies of chronic pain, particularly in felids[45,46]. Ideally all data collection would have been performed blind such that the observers would not know which clips corresponded to pre- vs. post-supplementation, and future research should consider the inclusion of a control group if sample size permits. Although we cannot entirely rule out the possibility of it influencing our results, our analysis of inter-observer reliability suggests that the data collected are robust to the placebo effect. Green-lipped mussel extracts have been shown to lead to objective improvements in other taxa either alone[50], or in conjunction with other supplements[29]. In the latter case, a 2010 study[29] highlighted the possible positive effects of GLM extracts in cats, but their supplements also included higher than usual levels of eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), both of which may also be beneficial in the treatment of MSDs. Similarly, in our study we cannot rule out the possibility that the increased mobility of cats was not due to interactions between GLM extracts and Flexadin, which was also administered throughout the data collection period. Flexadin has shown beneficial impacts in domestic cats and dogs[51,52], but the diet of non-domestic felids in captivity contains a higher proportion of its active ingredient (undenatured type II collagen) than their domestic counterparts, suggesting that while an interactive effect is possible, it is unlikely to entirely explain our results.

Important extensions of our research would be to include blinded data collection to reduce the likelihood of the caregiver placebo effect, using other objective measures of MSDs severity, and to remove other supplements from the diet entirely to isolate the effects of the focal supplement.

Steps per minute was used as a metric of mobility for operational reasons, but through further detailed observations, alternative measures could provide further insight into how the effects of Antinol are manifest in the study species. Stride frequency has a significantly lower metabolic cost compared to other movement centred metrics such as stride length[53] and it is therefore likely that stride frequency would be one of the first parameters to improve as the symptoms of MSDs ease. Other metrics that could be used to may be more objectively measured, relating to the gait of the treated animals[3] (e.g., peak vertical force), or subjective measures such as orthopaedic assessment scores or more behaviour-oriented data[3]. Further research investigating the effects of nutraceutical supplementation on a suite of these metrics could provide a more holistic view the benefits and potential drawbacks of Antinol treatment.

During the first data collection block, the temperature ranged between 25-30 degrees Celsius, whereas in the second stint it was lower at 10-15 degrees Celsius. Although understudied in non-human animals, there is evidence that lower temperatures and changes in barometric pressure can increase the severity of MSDs, specifically osteoarthritis in humans[54,55] although this evidence is not unequivocal[56]. In this case we might expect this temporal variation in temperature to make it harder to detect, rather than amplify, any beneficial impacts of Antinol. In domestic cats, activity levels may be negatively associated with temperature such that higher temperatures result in reduced physical activity[57]. Whether this trend applies to captive non-domestic cats from such a range of climates is unclear, as is the question of whether the range of temperatures under which the study was conducted would have an effect. If such a trend were consistent across species, we might expect the higher temperatures to reduce overall movement in the pre-supplementation treatment, but how this would affect our output metric of steps per unit time is difficult to ascertain. So, while the temperature difference between data collection periods remains a possible contributing factor to our results, we think it unlikely that steps per unit time would increase uniformly across such a range of species in response to this difference.

The longer lifespans and associated increased frequency of novel diseases means that stakeholders within collection management have divergent views of how to deal with the issues presented by housing aged animals[42]. Broadly speaking there exist three options for managing the increased longevity of captive animals. First, we revise our views and review our policies on which animals should be kept in captivity if we judge that the increased lifespan of large-bodied, long-lived animals causes a significant compromise to the welfare of those animals[58]. Second, if the focus of captive population management is purely to keep sustainable, reproductive populations, aged animals could be euthanized at the point in time at which they no longer contribute to that population and a clear decision-making process and audit trail exists[59]. Finally, we could develop a set of treatments and tools that allow us to maintain an acceptable level of quality of life in aged individuals beyond their reproductive contribution to the population. Our results suggest that if some collections and population managers wish to pursue the third option, then nutraceutical supplementation could provide another tool to help alleviate age-related conditions in these animals.

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Footnotes

There are no conflicts of interest related to this study.

Data Availability Statement

A flat sheet of all data used in the analyses within this study will be available from the Liverpool John Moores University data repository upon acceptance of the paper.

Author Contributions

Conceptualization: Jon Bielby, Matthew Norfolk, Luiza Figueiredo Passos, Adam Reddon, Matthew Rendle.

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Figure legends

Figure 1: Group median (± IQR) of steps per minute before and after addition of supplement to the diet. Each separate line joining the before and after treatment represents an individual cat.

Figure 2: Age pyramids from ZIMS data from European collections on the following sub-species of big cats: **A**. African lions (*Panthera leo*), **B**. Asian lions (*Panthera leo persica*), **C**. Amur tiger (*Panthera tigris altaica*), **D**. Sumatran tiger (*Panthera tigris sumatrae*). The lengths of the bars represent the number of individuals within each one-year age category for each of male (yellow bars on the left) and female (green bars on the right) cats separately. Deviation from a pyramid shape suggests a high prevalence of aged cats.

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