Pilot Study

Preliminary evaluation of the efficacy of Steadfast\textsuperscript{TM} Joint Supplement in captive exotic felids (Arabian leopard and cheetah)

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Summary

Feline degenerative joint disease causes pain and disability and therefore represents a welfare concern for captive animals. Alternatives to traditional pharmaceutical therapies are gaining popularity and this study aimed to evaluate the efficacy of an eggshell membrane therapy in captive cheetahs and leopards. Seven animals were treated with Steadfast\textsuperscript{TM} and monitored for radiographic changes (n = 6) and lameness score (n = 7) over a 42 day period. A reduction in lameness was determined over the course of the study (p = 0.01), and improved orthopaedic status detected in 50% (3/6) of animals via radiographic examination. Surprisingly, a negative dose response was detected, whereby animals receiving lower doses exhibited the most marked changes in lameness score. Despite the small sample size and subjective scoring of lameness, this preliminary study demonstrates the need for further investigation into the use of an eggshell membrane-based supplement to improve mobility in captive felids with degenerative joint disease.

Keywords: Cheetah; egg-shell membrane; joint health; lameness; leopard; mobility

(Received 27 November 2014 – Revised 14 January 2015 – Accepted 16 February 2015)

Introduction

Feline degenerative joint disease (osteoarthritis) is reported to occur frequently in domestic cats (Lascelles et al., 2010) as well as non-domestic felids (Rothschild et al., 1998). The condition causes pain and reduced mobility (Rothschild et al., 1998; Lascelles et al., 2010; Gowan et al., 2011), and therefore represents a welfare concern. Typical veterinary treatment involves the use of analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs). However, there is no NSAID that is approved for long term usage in felids for the management of osteoarthritis, and non-pharmaceutical therapies are therefore becoming increasingly popular. Steadfast\textsuperscript{TM} is a nutraceutical supplement aimed at improving joint health, providing relief from discomfort and promoting mobility (Novus Nutrition Brands, LLC, St. Charles, MO). The dietary supplement is composed of eggshell membrane (NEM\textsuperscript{®}, ESM Technologies LLC, Carthage, MO), organic chelated trace minerals, antioxidant vitamins, and other nutrients that support joint, bone, and connective tissue health (Dierenfeld et al. 2014). The NEM\textsuperscript{®} includes components such as collagen, hyaluronic acid, glucosamine, chondroitin sulphate, dermatan sulphate, desmosine, amino acids and peptides. Although not marketed for non-domestic species, Steadfast\textsuperscript{TM} has been successfully used in captive cranes and camels (Bauer et al., 2014; Dierenfeld et al., 2014). A preliminary investigation into the use of Steadfast\textsuperscript{TM} Joint Supplement in captive Arabian leopards and cheetahs was conducted for a 56 day period to determine the supplement’s efficacy in captive carnivores.

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Methods

The study was opportunistically conducted at the Breeding Centre for Endangered Arabian Wildlife (BCEAW; Sharjah, United Arab Emirates) following veterinary recommendation to administer a commercially-available nutraceutical to animals with known compromised joint health in an attempt to improve mobility and welfare. A total of seven animals were used (two male Arabian leopard, Panthera pardus nimr, three male cheetah, Acinonyx jubatus soemmerringii, and two female cheetah). The mean age of the males was 12.8 years (± SD 1.5), and females averaged 3.3 years (± 0.1). Animals were selected for treatment on the basis of a pre-existing mobility issue; one was age-related, one animal had experienced seasonal lameness associated with increased activity, two mobility issues were subsequent to fractured limb bones, and three were subsequent to abnormalities during the growth phase related to poor nutrition prior to admission to the BCEAW. Animals were dosed orally with one Steadfast™ Canine Large Breed (230 mg Natural Eggshell Membrane, NEM®) tablet per day (equivalent to 5.90 - 10.95 mg/kg BW), mixed into their daily food ration. Two observations per day were made twice weekly once the supplement was started. Lameness scores were assigned according to a 1 – 10 scale (1 = mild, 10 = severe) and assessed by the principal investigator. Animals were maintained on the supplement for 42 days, after which time observations continued for a further 14 days. Radiographs (taken prior to supplementation, and at the cessation of the supplementation period) were performed as part of the routine veterinary care plan of six of the affected animals, and were subsequently made available for the purposes of this study. All veterinary and animal care procedures conformed with industry standards for accommodation and care of zoo animals (EAZA, 2008). Data analysis was performed using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Data were tested for normality using the Kolmogorov-Smirnov test and data tested for difference between lameness scores on day 0 and day 42 using a two-tailed paired samples t-test. Pearson correlation analysis was used to determine change in lameness score over time since treatment.

Results and Discussion

A moderate but significant negative correlation was detected for lameness scores following treatment (see Figure 1), decreasing by 1 scoring unit between day 0 (mean 7.13 (SEM ± 0.55) and day 42 (6.13 ± 0.63; R² -0.248, p = 0.01; Figure 1).

Mean lameness score had somewhat reduced (p = 0.06) after the initial 14 days of treatment from a mean

![Figure 1](image-url). Mean lameness score of captive exotic felids (n = 7) treated with Steadfast™ Joint Supplement from day 1 until day 42, where increasing score represents increasing lameness. Error bars represent standard error of the mean, and Pearson correlation analysis revealed a significant relationship between day and lameness score (p = 0.01).
starting score of 7.29 (± 0.52) to 6.29 (± 0.64) on day 14. No significant difference was detectable between scores on day 14 and day 42 (score 6.14 (± 0.63); \( p = 0.61 \)), and a trend towards significance was detected for the reduction in scores on day 0 and those on day 42 (\( p = 0.08 \)). Two weeks following cessation of treatment, scores increased slightly, and approached baseline (mean score 6.57 (± 0.61) on day 56; \( p = 0.08 \)).

The antioxidants present in Steadfast™, as well as its anti-inflammatory properties, and other nutrients that support joint, bone, and connective tissue health (Dierenfeld et al. 2014) may have contributed to the reduction in lameness score demonstrated in the current study. In vitro studies have shown NEM® to reduce a number of pro-inflammatory cytokines in human immune cells following inflammatory challenge (Benson et al., 2012). More recently, Ruff and DeVore (2014) demonstrated a reduction in circulating pro-inflammatory cytokines following oral supplementation with NEM® through in vivo studies in both healthy and inflammatory-challenged rats. These authors also found significant effects on nearly all of the chemokines currently known to be associated with osteoarthritis and rheumatoid arthritis inflammation and pathogenesis. Likewise, positive changes in a cartilage synthesis biomarker (N-propeptide type IIA collagen) were reported in camels following treatment with Steadfast™ equine (Dierenfeld et al., 2014). The antioxidants present in Steadfast™, as well as chondrocyte-supportive nutrients, may also have contributed to the reduction in lameness score demonstrated in the current study.

A significant relationship between dosage and response (change in lameness score) was detected, whereby reduction in lameness decreased as dose increased (\( p = 0.04; \ R^2 = 0.785 \); see Figure 2). This unexpected finding may be explained by the fact that the animal exhibiting the most marked reduction in lameness score was also the largest animal in the study (and therefore received the lowest dose), but was concurrently the only animal to suffer from activity-related joint mobility issues. Radiographs of this animal on the final day of treatment demonstrated reduced soft tissue swelling. Two other animals exhibiting improved lameness scores also showed improved joint definition and reduced osteocyte formation. As such, radiographic changes were detectable in 50% (3/6) of animals. Radiographic examination of animals exhibiting negligible changes in lameness scores failed to reveal changes in any measured parameter. These animals were known to experience compromised mobility due to previous skeletal injuries or growth abnormalities, and the reduced effect of Steadfast™ in these animals may relate to its mechanism of action.

Although the use of radiography was included as an objective measure and provided some support for the

![Figure 2](image_url)
subjective (and non-blinded) lameness score results, additional testing is required to confirm our findings given the small sample size and variable nature of joint health issues.

Conclusions

This preliminary study has provided encouraging support for the use of an eggshell membrane-based supplement to improve mobility in captive leopards and cheetahs suffering from degenerative joint disease of varying causes. Subjective measures of lameness were determined to decrease over the study period during which animals received the Steadfast™ supplement. However, somewhat surprisingly, a negative dose response was detected, whereby reduction in lameness was most apparent in animals receiving lower doses (due to their greater body weight compared to other animals).

Results from the current study should be interpreted with caution as it was limited in duration and sample size, and lameness scores were subjectively assigned by assessors who were aware of the animal treatments. Future studies are planned and will include the use of additional objective parameters (e.g. blood biomarkers, orthopaedic evaluations and a defined activity status evaluation protocol), as well as the use of a case-control study design, more accurate dosing of individuals on a body weight basis, and assessors blinded to the treatments.

Acknowledgements

The Steadfast™ product was kindly supplied by Novus Nutrition Brands, LLC, USA. The authors gratefully acknowledge the sponsorship of His Highness Dr. Sheikh Sultan bin Mohammed al Qassimi, Ruler of Sharjah and Member of the UAE Supreme Council. Thanks are extended to the BCEAW veterinary nurses, Ms. Theyse and Ms. Strick and animal keepers Mr. Seyed Ali and Mr. Madda Wattage. A version of this article has previously been published in the Proceedings of the Tenth Comparative Nutrition Society Symposium, Flat Rock, NC (2014).

Declaration of interests

One author was employed by Novus International Inc. (of which Novus Nutrition Brands (NNB) is a division) during the initial phases of the study (design and data collection). However, this author did not report directly to NNB and data analysis was conducted only after the author had left the company; therefore no current conflict of interest exists. Moreover, the author was not directly involved in data collection or analyses.

References


European Association of Zoos and Aquaria. (2008) EAZA Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria. Amsterdam, the Netherlands.


