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Abstract: Osteoarthritis is a problem that threatens the reproductive capabilities of captive populations of endangered cranes. In our pilot study, we used 5 cranes with a history of unilateral, chronic tarsal pathology in a cross-over design to gauge the effects of the primary ingredient (NEM®, ESM Technologies LLC, Carthage, MO) of the nutraceutical Steadfast® (Novus Nutrition Brands, LLC, St. Charles, MO). We evaluated the ingredient for acceptance, safety, and short-term efficacy. To evaluate efficacy, we collected goniometric measures to determine range of motion in each tarsal joint before and after a 5-week experimental period where NEM® was offered in pelleted feed. We also determined time spent in locomotion from estimates of activity once per week. The ingredient was determined to be both acceptable as offered and apparently was safe for the cranes. There were no significant changes in the birds' weights or body condition scores during any period of the trial. There was a significant increase in overall tarsal flexion measurements in the 5 birds' affected legs ($P = 0.04$), and 1 bird showed +14 degrees of improvement in flexion. No changes were seen in measures of tarsal extension or in either measure in unaffected legs. The behavioral data was inconclusive due to the small sample size and large variation in the weekly estimates within individuals. Though there was evidence of increased joint mobility in all birds in this small pilot study, further study is needed to determine if NEM® is efficacious for managing osteoarthritis in cranes.

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Musculoskeletal abnormalities are prevalent in cranes. A retrospective survey at the International Crane Foundation (ICF), Baraboo, Wisconsin, showed musculoskeletal problems were the second leading cause of morbidity in whooping cranes (*Grus americana*) (Hartup et al. 2010). Known etiologies for avian lameness include infection, endocrine imbalance, developmental problem, nutritional deficiency, or trauma (Curro et al. 1992). Cranes have long life spans; if an injury occurs early in life, a crane may face chronic problems for many years with repercussions affecting individual welfare, reproduction, and conservation goals. Recurring mild lameness is often a sign of progressive osteoarthritis, also known as degenerative joint disease (Olsen et al. 1996). Osteoarthritis may lead to declines in joint function and captive breeding success; for example, by limiting proper incubation postures and lowering hatchability of naturally incubated eggs (Gabel and Mahan 1996).

Current therapy for degenerative arthropathies in cranes typically involves administration of non-steroidal anti-inflammatory drugs (NSAIDs) and changes in husbandry (Olsen et al. 1996, Cole et al. 2009). However, NSAIDs may not be suitable for long-term therapy. Mild hemorrhage of the proventricular mucosa has been documented in some cranes with short- and long-term exposure to meloxicam and piroxicam, respectively. Renal lesions have also been noted postmortem in cranes dosed with flunixin meglumine while at ICF. Siberian cranes (*Grus leucogeranus*) and whooping cranes administered varying levels of flunixin meglumine were diagnosed with visceral gout at necropsy (ICF unpublished data). Mortality occurred in 4 of 5 Siberian cranes and 1 of 4 whooping cranes given flunixin meglumine. No side effects or mortality were noted in other crane species administered flunixin meglumine, including blue cranes (*Anthropoides paradiseus*), hooded cranes (*G. monacha*), sandhill cranes (*G. canadensis*), and sarus cranes (*G. antigone*).

Steadfast® is a nutraceutical supplement currently marketed for dogs and horses to improve joint health by providing relief from discomfort and promoting mobility (Novus Nutrition Brands, LLC, St. Charles, MO). It is composed of eggshell membrane (NEM®, 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. 2010). The NEM® includes components such as collagen, hyaluronic acid, glucosamine, chondroitin sulfate, durmatan sulfate, desmosine, amino acids, and peptides. Dierenfeld et al. (2010) measured levels of the cartilage blood biomarker CTX-II to evaluate the efficacy of Steadfast® in camels. The decrease in levels of this cartilage marker has been shown to correlate with a decrease in inflammation and increased weight bearing in rats (Wedekind et al. 2010). However, it is unknown whether this biomarker is present in avian species since the composition of avian cartilage is different than that of mammals. Avian articular cartilage contains high levels of both collagen I and collagen II; the primary collagen in articular cartilage is collagen II in mammals (Eyre et al. 1978). Despite the differences in cartilage composition, there are many morphological and biochemical similarities between degenerative joint disease in mammalian and avian species (Anderson-Mackenzie et al. 1997). These similarities suggest that Steadfast® may also have some benefit in degenerative joint disease in birds.

This pilot study was conducted to determine the acceptance, safety, and potential efficacy of NEM®, and to determine if a larger trial is warranted. We expected birds to show improvements in joint health as evidenced by increased range of motion of the affected joint and increased locomotion behavior during the course of the study.

METHODS

Five cranes with chronic tarsal abnormalities were used in this study (Table 1). The birds were housed with mates in 15 × 18-m outdoor pens covered by flight netting with chain-link fencing along each side and grass covered soil as a substrate. Each pen included a 4.2 × 4.2-m indoor enclosure with a deep bedding of wood shavings over concrete substrate. Pelleted food and fresh water were provided ad libitum in buckets in the enclosures (Hartup and Schroeder 2006).

We used a cross-over study design where cranes received either the NEM® ingredient in their diet at 800 ppm (0.08% in reconstituted crane maintenance pellets, Zeigler Brothers Inc., Gardners, PA) or a placebo diet (original pellets without NEM®) for 5 weeks, followed by a 2-week washout period (placebo diet). The cranes then received the opposite treatment for an additional 5 weeks. Each crane served as its own control while on the placebo diet. Though the study was designed to be blinded, an unexpected food shortage in the experimental diet caused the researcher to become un-blinded. Since all cranes in the study were part of a breeding pair and food was shared, both pen mates received NEM®. Food was weighed before and after each feeding to determine the amount removed. Given that we could not determine the exact amount eaten by each member of the pair, we assumed that each crane ate half of the food removed from the feed bucket. The first trial period took place from 12 July 2010 to 15 August 2010; the second from 30 August 2010 to 3 October 2010.

On day 0 and day 35 of each 5-week period, specific data were collected. A physical examination was performed (by B. Hartup), with special attention paid to the hind limb joints. Each crane was weighed and assigned a body condition index (BCI) score (1-5 scale, 1 = minimal pectoral muscle mass with prominent sternum, 5 = robust, well rounded pectoral muscle mass, sternum palpated with difficulty) (Olsen et al. 1996). Also, the range of motion of each crane's tarsal joints was measured with a goniometer (Fig. 1). This measurement was taken by either K. Bauer or B. Hartup. A goniometer measurement of 180° was defined as a full flexion while a measurement of 0° was defined as full extension. A total of 4 measurements were taken: full flexion of the right leg, full extension of the right leg, full flexion of the left leg, and full extension of the left leg. Measurements were taken by aligning the fulcrum of the goniometer with the center of the tarsal joint. Gentle pressure was applied to the joint to achieve either full extension or full flexion. One arm of the goniometer was aligned parallel with the tibia and the other with the tarsometatarsus. To our knowledge, this is the first documentation of this methodology to determine range of motion in avian tarsal joints.

In addition, digital video cameras already present at ICF were used to monitor the movements of the

Table 1. Summary of the cranes used in the nutraceutical trial.

ID	Species	Age (years)	Sex	Musculoskeletal problem
Bubba	*Grus americana*	26	M	Slipped tendon (left tarsus)
Rattler	*G. americana*	42	M	Degenerative arthritis (left tarsus)
Dushenka	*G. leucogeranus*	29	M	Slipped tendon (left tarsus)
Kavir	*G. leucogeranus*	16	F	Previous injury to lateral collateral ligament (right tarsus)
Ranjit	*G. leucogeranus*	24	F	Slipped tendon (left tarsus)

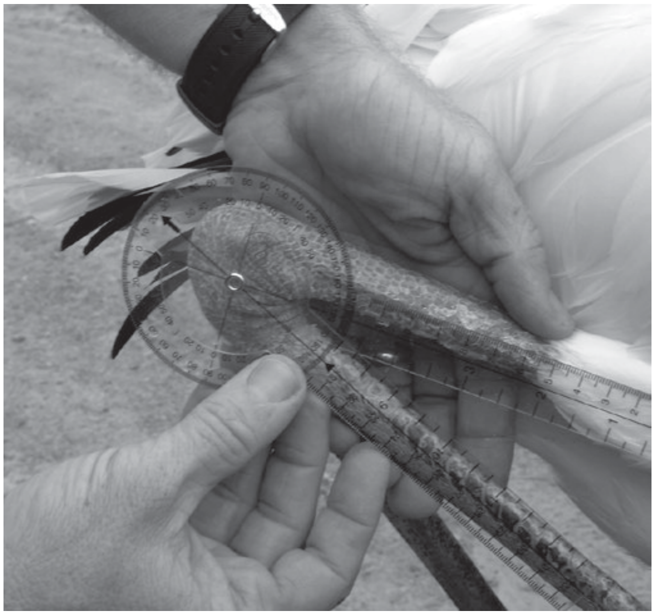

Figure 1. Goniometry measurement of tarsal flexion in a crane.

birds for 50 minutes per week during each 5-week period on a randomly selected day and time between 0700 and 1000 hours. This footage was assessed by 1 of 2 individuals, either K. Bauer or an intern aviculturist trained by K. Bauer, in order to record an activity budget of each crane, accounting for what percentage of time each crane spent in daily activities such as walking or resting. To decrease inter-observer variability, both observers participated in practice observation sessions. Behaviors for this activity budget were placed into 1 of 5 categories: foraging, locomotion, comfort, resting, or social interaction. If the crane spent time in its house, which was outside of the view of the camera, the behavior was recorded as out of sight. A behavior was recorded every 30 seconds during the 50 minute period for each crane, for a total of 100 data points. If the crane was recorded as out of sight for more than 40 of those data points, the data was discarded and the crane was given a new observation time for the week. If insufficient data points were again recorded, the 2 sets of data from the 2 observations periods were combined.

A food shortage shortened 1 crane's experimental period. The crane received the diet through day 16 of the 5-week period, and its final evaluation took place on day 17 of the trial period. Behavior data was collected for the first 3 weeks of this period.

The primary author was blinded to the treatment assigned each crane during the 2 5-week periods. We used unpaired *t*-tests to compare food consumption between periods among the cranes. Normal distributions were confirmed by visual inspection and review of skewness and kurtosis calculations on each set of treatment and control data and deemed acceptable. To analyze the goniometry data, BCI scores, and weights, we used a nonparametric Wilcoxon signed-rank test for paired samples (Statview 5.0.1, SAS Institute, Inc, Cary, NC). The Wilcoxon test is an alternative to the paired *t*-test applicable to small datasets and uses a ranking of differences between each pair of observations. The resultant *Z*-statistic tests the hypothesis that the sum of the ranks is equal to 0, assuming the distribution of ranks is symmetric around 0. The larger positive or negative number reflects greater differences between paired values. Statistical significance was established at $P < 0.05$.

RESULTS

Acceptance and Safety

Four of 5 individuals consumed significantly less of the experimental diet than the placebo diet, while the fifth individual consumed more of the experimental diet than the placebo diet (all $P < 0.01$, Table 2). Because the experimental diet was reconstituted in order to incorporate the nutraceutical product, the resulting feed did not maintain its cylindrical shape well. Each crane pair regularly reduced their experimental diet to a fine dust, but the placebo pellets always remained intact. This led to more frequent replacement and/or sifting of the experimental diet pellets, and ultimately led to the depletion of the experimental diet supply.

During the physical examinations at the end of the experimental diet period, 2 birds were diagnosed with additional abnormalities. One bird had developed pododermatitis (bumblefoot), and another had increased lateral instability in the tarsus, possibly due to an acute lateral collateral ligament injury. Both abnormalities occurred in the previously unaffected leg. No significant differences were observed in weights ($P = 0.58$) or BCI scores ($P = 0.29$) taken at day 35 of each period (Table 3).

Efficacy

We detected significant differences in baseline flexion and extension measurements between the

Table 2. Mean ± SD daily pelleted feed intake (g) of individual birds during 2 trial periods.

ID	Placebo diet	Experimental diet
Bubba	165 ± 38	126 ± 33
Rattler	138 ± 27	173 ± 36
Dushenka	200 ± 38	180 ± 29
Kavir	185 ± 44	151 ± 31
Ranjit	202 ± 64	164 ± 46

Table 3. Weights (kg) and body condition scores (1-5 scale) of individuals following 35 days consumption of either a placebo or experimental diet containing a nutraceutical joint supplement.

ID	Weight, placebo diet	Weight, experimental diet	BCI, placebo diet	BCI, experimental diet
Bubba	4.9	5.2	2	2.5
Rattler	5.6	5.6	2	2
Dushenka	6.6	6.4	4	2.5
Kavir	4.9	4.8	4	3
Ranjit	5.8	5.5	3	3

cranes' affected and unaffected legs taken at days 0 and 35 of the placebo diet period and day 0 of the experimental diet period (both $P < 0.01$, Table 4). After 35 days of consumption of the experimental diet, there was a significant change in the flexion measurements of the affected leg among the 5 cranes ($P = 0.04$), but not the extension measurements ($P = 0.72$, Table 5). No significant changes were observed in the measurements for the unaffected leg of any crane post-treatment.

Behavior varied greatly from week to week for each crane both on the experimental and the control diet. No identifiable trends were observed for the amount of time the cranes spent in locomotion during the experimental diet period.

Table 4. Control goniometry (in degrees, mean ± SD) based on 3 measurements from unaffected and affected legs of 5 cranes with chronic tarsal abnormalities. An overall decreased range of motion in the affected legs of the birds is reflected in lower flexion and greater extension measurements.

	Unaffected leg	Affected leg
Flexion	169.3 ± 6.8	152.8 ± 18.4
Extension	6.5 ± 2.6	9.6 ± 4.8

DISCUSSION

Acceptance and Safety

Overall, we believe that the diet containing NEM® was well accepted by the cranes used in this trial and appeared safe. The addition of the NEM® altered the normal delivery of the pellets, i.e., pellets containing NEM® were quickly reduced to crumbles. It may also have altered the palatability of the pellets. Overall, the amount of experimental diet consumed by the cranes was lower compared to placebo diet for 4 of the 5 cranes. This could have been due to modest rejection based on taste, or more likely, the reconstitution process and poor binding resulted in the cranes' inability to handle a more fragile pellet. We believe the food consumption results are best explained, however, by what we believe is an increase in food consumption in response to seasonal change. The 4 cranes with greater placebo diet intake did so in the second 5-week period of the study (early fall), and the 1 crane that consumed more experimental diet was the only bird to receive it in the latter period as well, all perhaps reflecting the first stages of migration readiness behavior. Regardless, no cranes experienced significant change in weight or body condition. Additional monitoring, including hematology and blood chemistry analysis, would be of benefit in future trials.

Table 5. Goniometry measurements (in degrees) from each bird's affected leg following 35 days consumption of either a placebo or experimental diet containing a nutraceutical joint supplement.

ID	Leg flexion, placebo diet	Leg flexion, experimental diet	Leg extension, placebo diet	Leg extension, experimental diet
Bubba	145	150	11	8
Rattler	158	163	7	4
Dushenka	157	166	15	15
Kavir	173	175	2	0
Ranjit	120	135	6	11

The fragility of the reconstituted diet was a problem in this study because 2 of the cranes had pre-existing beak malocclusions requiring greater food depths for successful consumption from buckets. A well-bound pellet would have mitigated this issue, but the cranes still appeared to meet their nutritional needs. Feeding the cranes tablets hidden in treats was not an option due to the malocclusions, as well as the overall lack of prior training of these subjects. In well-trained birds without complicating beak injuries, direct dosing using treats may be a viable option (Dierenfeld et al. 2010). Including the NEM® in the original extrusion and pelleting would likely result in a more durable product at delivery.

We noted no acute or systemic adverse effects at the concentration and route of NEM® administered. However, the safety of NEM® cannot be definitely determined due to a lack of hematologic and biochemical data. We are uncertain whether NEM® supplementation was associated with new musculoskeletal conditions diagnosed in 2 of the cranes during the experimental period, though it seems unlikely. The conditions emerged in the contralateral leg to the original, chronically affected leg in each crane. Normally these types of conditions occur secondarily when cranes place undue weight on a good leg while minimizing weight bearing on an affected leg. We hypothesized just the opposite: birds with improved joint function from NEM® supplementation would begin to bear weight in a more balanced manner and be less susceptible to injury or development of secondary musculoskeletal disease. Unfortunately, weight bearing distribution was not assessed in this study. If the birds were in fact still compensating for their chronically injured leg, it is less likely that they had truly improved joint health as a result of the NEM® added to their diet. A full treatment effect also seems less likely with the short duration of the treatment period.

Efficacy

We observed modest improvement in goniometry measurements relative to joint flexion, but not extension, in cranes with an abnormal leg that were provided a nutraceutical joint supplement for 5 weeks. The improvement in abnormal tarsal joint range of motion was approximately 5%. A more thorough comparison to younger individuals of the studied species would provide useful reference data. A longer trial with the same individuals or additional cases would serve to validate this product's potential for mitigating the progression and complications of osteoarthritis and musculoskeletal injuries common to cranes in captive environments.

We did not discern any semi-quantitative change in locomotion behavior in the cranes. The behavior data collected was likely insufficient in frequency and duration to accurately determine the locomotion budget for a given crane and determine an effect of the nutraceutical supplement. Activity budget data is notoriously variable day-to-day, and often highly biased by lack of observation when birds are out of sight. In addition, our observations coincided with a seasonal change that might also have affected activity levels (early fall). We suggest that any future trials include a long-term behavioral assessment as well as direct range of motion assessments within a single prolonged season (e.g., immediately post-breeding to fall) or across an annual cycle.

A primary challenge for further studies will be production of a processed diet containing the nutraceutical that can withstand normal delivery and field conditions encountered when feeding captive cranes. Additionally, full assessment of the therapeutic impact of NEM® will need assessment using multiple methods and over a longer time period. Other options for future trials include testing a therapeutic approach combining the use of NEM® with another product such as an NSAID.

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