Efficacy of an equine joint supplement, and the synergistic effect of its active ingredients (chelated trace minerals and natural eggshell membrane), as demonstrated in equine, swine, and an osteoarthritis rat model

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Purpose: To determine the efficacy of an equine joint supplement STEADFAST® and/or its active components (Natural Eggshell Membrane [NEM®] and chelated trace minerals [CTM]) in horses with naturally occurring osteoarthritis or in a chemically induced osteoarthritis rat model. In addition, the efficacy of CTM vs inorganic trace minerals (ITM) (Zn, Mn, and Cu) in reducing culling rates in swine was evaluated.

Methods: Horse trial: 16 mature horses with existing lameness were fed test joint supplement or placebo for 42 d. Lameness (American Association Equine Practitioner scoring system), serum biomarkers (C-terminal cross-linked telopeptide type II collagen [CTXII] and N-propeptide type IIA collagen [PIIANP]), and synovial fluid WBC were assessed biweekly. Rat trial: A chemically induced (monoiodoacetate [MIA]) osteoarthritis rat model was utilized. Rats were fed either negative control or joint supplement at 1% or 2% inclusion in Exp 1 (n=54) for 56 d. In Exp 2 (n=48), rats were fed control, NEM, CTM, or NEM + CTM for 42 d (28 d prefeed + Exp period). Rats were injected with MIA d29. Pain, knee swelling, and CTXII were measured post-MIA injection. Sow trial: Two farms with 6,400 sows each were fed ITM or 50:50 blend of ITM:CTM at equal TM levels for ∼3 yr. Treatments were initiated at weaning through entry into the breeding herd. Sows remained on treatment until culled. Sow retention rate and reasons for removal were measured.

Results: In horse and rat trials, chondromodulating effects of the joint supplement were observed: increased cartilage synthesis (PIIANP) or decreased cartilage degradation (CTXII). CTM + NEM decreased pain, swelling, and CTXII, compared to control and/or CTM or NEM alone (P<0.05). Gilt and sow culling rates were reduced >30% with CTM supplementation (P<0.001).

Conclusion: CTM, NEM, and the joint supplement improved skeletal and joint health. Our studies demonstrate the importance of CTM for the prevention and treatment of lameness. Keywords: lameness, monoiodoacetate, trace minerals, CTXII, PIIANP, rat, equine, swine

Introduction

Musculoskeletal diseases and lameness are major health issues in horses and livestock. Lameness is a common cause of reduced work and early retirement of horses, and the major cause of lameness is osteoarthritis (OA).1 Similarly in swine, premature culling of sows has a major impact on profitability, estimated to represent around 16% of farm income.2 Reproductive failure and lameness are the two main causes for the removal of young sows, accounting for about 42% and 17% of first and 35% and 16% of second...
parity culls, respectively.²,³ According to Pigchamp,⁴ the average annual replacement rate in North America is 49%. The average parity in the farm is only 2.5–3.⁴ In today’s economy, a major challenge for sow farms is to keep sows in the farms longer and productive. In a typical US farm, the economics are such that sows in farrow-to-finish operations need to reach their third parity in order to break even; that benchmark is the fourth parity in breed-to-wean situations.⁴ Poor sow longevity requires larger replacement gilt pools, regardless of whether a pork production system raises or purchases these gilts.

The role of nutrition in decreasing the incidence of lameness and OA is controversial because of inconsistent findings. Often, dietary supplements such as Ca, P, and vitamin D that affect mineralization are used to improve the inorganic matrix of bone, whereas less attention has been paid to the integrity and characteristics of organic matrix constituents. Trace minerals play an important role in bone formation and maintaining skeletal integrity.⁵ Zinc and copper are critical for proper formation of collagen, a structural protein that increases bone strength.⁵ While synthesis of collagen is Zn-dependent, the enzyme that cross-links collagen subunits into mature protein forms (lysyl oxidase) is Cu-dependent.⁶ Manganese also plays a role in bone development. The extracellular matrix of developing bone, particularly the proteoglycan matrix, requires Mn for proper development.⁷

In poultry and swine, increased growth rates have been accompanied by a variety of skeletal and other structural problems. Tibial dyschondroplasia (TD), a common developmental defect in fast-growing birds, is similar to osteochondrosis in mammals.⁸⁹ TD was an insignificant health concern 30 years ago, but currently it affects 50% of broiler chickens.⁹ Likewise, in swine, growth rate has been implicated in lameness, and osteochondrosis prevalence in pigs is estimated to occur in 85%–90% of all pigs.¹⁰ In horses, the role of fast growth rate is not as clear. For example, several studies have shown no relationship between weight gain and osteochondrosis.¹¹–¹⁴ However, preventing growth fluctuations in horses may be a critical factor. Horses kept under practical management situations are often fed diets that fail to meet known nutritional requirements.¹⁵ According to Gibbs and Cohen,¹⁶ 44% of farms fed unbalanced diets to young horses, particularly weanlings. The most common nutrient imbalances identified included excess energy intake, and excesses or deficiencies in protein, macro- and micromineral content, as well as calcium:phosphorus imbalances.¹⁷ As a result of lameness issues, trace minerals often are fed at levels that far exceed published NRC requirements for poultry, swine, and equine.¹⁵,¹⁷,¹⁸

Despite higher feeding rates, these structural problems persist, likely due to poor mineral bioavailability.⁵ Feeding higher mineral concentrations in an attempt to overcome low bioavailability may result in nutrient imbalances or antagonisms among minerals and other nutrients.⁷ Feeding chelated trace minerals (CTM) potentially would provide more bioavailable minerals and resist antagonisms, thereby offering advantages over inorganic trace minerals (ITM). Interestingly, chelated minerals (Zn, Mn, and Cu) have been shown to significantly alleviate structural defects in turkeys fed commercial diets.¹⁹,²⁰ Improvements included increased bone strength and width, improved foot pad score, and reduced incidence of TD and reduced incidence of synovitis.²⁰,²¹

OA is a complex disease process of articular cartilage that is associated with a variable degree of synovitis from natural aging, trauma, and/or disease.²²–²³ Degenerative joint disease (DJD) or OA is characterized by cartilage degradation and loss, leading to joint space narrowing, hypertrophy and hyperplasia of the synovial capsule, loss of synovial fluid, and, eventually, calcification of the articular cartilage and osteophyte formation.²⁴,²⁵ The disease is characterized by a loss of balance between synthesis and degradation of the articular cartilage.²⁵

Currently, there is no cure for DJD or OA, and most interventions target alleviating pain and retarding disease progression.²⁶–²⁷ Common interventions for both human and veterinary medicine range from weight loss and physical therapy to palliative pharmaceuticals such as nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids.²⁸–²⁹ Nutritional therapies potentially offer safe and effective treatment alternatives that could be used for extended periods of time without adverse health effects.²⁶–²⁸,²⁹ However, nutrition is more effective when used as a preventative measure or in slowing disease development, highlighting the need for practical and reliable detection techniques for DJD onset and progression.²⁶,²⁸ Serum biomarkers that indicate rates of tissue turnover in the joint have shown promise for the diagnosis and prognosis of joint ailments in both human and veterinary medicine.³⁰–³³ With specific regard to horses, DJD is one of the most common musculoskeletal disorders, with 60% of equine lameness caused by or related to DJD.³⁴

There are numerous animal models of DJD or OA; however, no consensus currently exists regarding which model and species is the most relevant for naturally occurring OA.³⁵ This lack of consensus originates from a poor understanding of the disease etiology and from the absence of clearly effective structure-modifying drugs that could be used to evaluate the relevance of the existing animal models.³⁵
Spontaneous models best mimic the slow progression of OA, but they are time-consuming and the progression of the disease is highly variable between individual animals (as in humans).\textsuperscript{35} Surgically and enzymatically induced models develop rapid and reproducible damage, but might be more relevant to the traumatic forms of OA than to the classical degenerative form of OA.\textsuperscript{35} Early-onset and severe models such as chemically induced OA are most economical and probably result in a lower number of false-positives, but could also result in a high number of false-negatives, especially in detecting structure-modifying effects of nutraceuticals.\textsuperscript{35}

Nutritional supplements may offer advantages over drugs in the treatment and intervention of OA.\textsuperscript{36} Because glucosamine and chondroitin sulfate are precursors to glycosaminoglycans (GAGs) and GAGs are a major component of joint cartilage, supplementation with these components might help rebuild cartilage.\textsuperscript{36,38} Despite their widespread use, there is only limited and conflicting data regarding the efficacy of these supplements.\textsuperscript{36,37} Furthermore, some research has shown these ingredients are poorly bioavailable and of inconsistent quality.\textsuperscript{36,38–41} Objective in vivo evaluation of nutraceutical-type products in horses is lacking. Natural Eggshell Membrane (ie, NEM, a product of ESM Technologies, LLC, Carthage, MO, USA) is a sustainable by-product of the poultry industry and represents an alternative novel natural source of collagen, GAGs, and proteins. In individuals with OA, NEM has been shown to be efficacious in a randomized, multicenter, double-blind, placebo-controlled study and in two open-label pilot clinical trials.\textsuperscript{42,43}

The efficacy of a joint health nutraceutical supplement (supplt), containing both CTM and NEM, was evaluated in horses with naturally occurring OA and a chemically induced OA rat model. In addition, a second OA rat trial was conducted to determine the efficacy of the active ingredients contained in the joint supplt (CTM and NEM), singly and in combination. Lastly, the benefits of CTM for skeletal health were evaluated in sows. It was our hypothesis that the addition of CTM would enhance the efficacy of a joint supplt and have beneficial effects in both the prevention and treatment of OA or lameness. The collective findings from our three different animal models demonstrate the importance of providing highly bioavailable trace minerals to support joint health.

**Materials and methods**

**Horse trial**

**Experimental design**

Following approval of the Texas A&M Animal Care and Use Committee, 16 mature (average, 18.8 yr; 3–25 yr of age) arthritic horses (Quarter x draft) were maintained at the Texas A&M University Horse Center. Prior to being admitted into the study, lameness examination was performed by an American College of Veterinary Surgeons (ACVS) board-certified equine surgeon and an equine nutritionist with 20+ years’ equine experience. Horses were blocked by lameness grade (AAEP [American Association of Equine Practitioners] scoring system) and affected joint. Affected joints included knees (11), hocks (3), stifle (1), and pastern (1). Within block (pair), horses were randomly assigned to either placebo or joint supplt (a product of Arenus, an Altera International LCD Company, Fort Collins, CO, USA). The composition of the STEADFAST® joint supplt is shown in Table 1. The placebo treatment consisted of alfalfa and molasses. The oral supplements were added to an existing diet consisting of pelleted concentrate and coastal Bermuda grass hay offered at 2% of body weight (as fed) with a 60:40 hay-to-concentrate ratio. All horses were treated twice daily with their respective treatment at 50 g/head/d, starting on d0 and ending on d42. Horses were housed on pasture daily and fed twice daily concentrate and treatment in individual stalls. The investigator was blinded as to treatment assignment to ensure that all observations were recorded in an unbiased manner. Product labels were identical except for lot number and color code.

**Exclusion/inclusion criteria**

For inclusion into the study, horses were required to have a diagnosis of naturally occurring OA and a swollen or affected joint based on clinical examination (Table 2). AAEP lameness scores (based on AAEP grade) between 0 (normal) and 5 (severe lameness) were targeted. If bilateral lameness was evident, the more severely affected limb was declared the affected limb. Suitable horses were excluded if they had joint surgery in the previous 120 d, intra-articular injections in the

**Table 1 Composition of STEADFAST® equine joint supplement**

| Active ingredients per 50 g serving | Amount |
|-----------------------------------|--------|
| NEM® joint mobility matrix         | 3.000 g |
| Chelated trace minerals (TELAfIRM)
| Calcium                           | 2.281 g |
| Phosphorus                        | 1.891 mg|
| Ascorbic acid                     | 1.000 mg|
| Biotin                            | 20 mg   |
| Vitamin D₃                        | 1.000 IU |

*Notes: *TELAfIRM includes a proprietary blend of chelated Zn, Cu, and Mn HMTBAs. The amount of Zn, Cu, and Mn provided by STEADFAST approximates 30% of the National Research Council (USA) recommendation for the horse. **Abbreviations:** HMTBAs, hydroxy-methylthiobutyric acid; NEM, Natural Eggshell Membrane.
Table 2. Characterization and identification of lame horses

| ID  | Treatment | Age | Sex | Lame limb | Joint | Joint sampled | Pair | Initial AAEP score |
|-----|-----------|-----|-----|-----------|-------|---------------|------|--------------------|
| B1  | Supplt    | 23  | M   | LF        | Knee  | L knee        | 1    | 4.5                |
| B2  | Supplt    | 21  | F   | LF        | Knee  | L knee        | 2    | 0                  |
| B3  | Supplt    | 23  | F   | LF        | Knee  | L knee        | 3    | 2                  |
| B4  | Supplt    | 15  | M   | RF        | Knee  | R knee        | 4    | 1                  |
| B5  | Supplt    | 22  | M   | RF        | Knee  | R knee        | 5    | 1                  |
| B6  | Supplt    | 23  | F   | RF        | Knee  | R knee        | 6    | 1                  |
| B7  | Supplt    | 3   | M   | LH        | Hock  | L hock        | 7    | 5                  |
| B8  | Supplt    | 16  | F   | RH        | Stifle| L knee        | 8    | 3                  |
| G1  | Control   | 25  | F   | LF        | Knee  | L knee        | 1    | 4                  |
| G2  | Control   | 24  | M   | LF        | Knee  | L knee        | 2    | 0                  |
| G3  | Control   | 25  | F   | LF        | Knee  | L knee        | 3    | 2                  |
| G4  | Control   | 19  | F   | RF        | Knee  | R knee        | 4    | 1                  |
| G5  | Control   | 11  | M   | RF        | Knee  | R knee        | 5    | 2                  |
| G7  | Control   | 18  | F   | RH        | Hock  | R hock        | 6    | 2                  |
| G8  | Control   | 10  | F   | LH        | Hock  | L knee        | 7    | 2                  |
| G9  | Control   | 22  | F   | RF        | Pastern| R knee        | 8    | 3                  |
| Average |          | 18.2|     |           |       |               | 2.2  | 2.0                |
| Control |          | 19.2|     |           |       |               |      |                    |

Notes: Data show baseline information for all 18 horses. Initial age and AAEP score were similar between treatment groups.

Abbreviations: AAEP, American Association of Equine Practitioners; LF, left front; LH, left hind; RF, right front; RH, right hind; Supplt, supplement.

previous 90 d, systemic GAGs in the previous 30 d, steroids or NSAIDs in the previous 7 d, or dietary supplements in the previous 14 d. In addition, no changes in shoeing or trimming occurred within 14 d of enrollment or during study. Age and AAEP score were similar between treatments.

Clinical variables
For each horse, clinical examinations were performed at 2 wk intervals from d0 to d42 by an investigator who was blinded to the treatment regimen. Horses were evaluated for lameness according to procedures described by the AAEP. In addition to AAEP lameness score, other parameters were measured using a visual analog scale (VAS) by marking on a horizontal line from 0 to 10, 0 being no response or no lameness and 10 being extreme response or maximum possible lameness (non-weight-bearing). VAS parameters included lameness at a walk (VAS walk), lameness at a trot (VAS trot), pain to manual joint flexion (VAS flex), and lameness after a 1-minute flexion test (VAS flex response). Passive joint flexion was performed by manually manipulating the involved joint into a position of maximum passive flexion and then trying to force the joint to flex a little more. Lameness after flexion (VAS LF) was evaluated immediately after assessment of pain to manual joint flexion.

Serum analysis
A blood sample (20 mL) was collected at baseline and d14, 28, and 42. All samples were frozen at −80°C until analysis. Assessment of cartilage biomarkers was performed using the following commercial ELISA (enzyme-linked immunosorbent assay) kits: CTXII (C-terminal cross-linked telopeptide type II collagen; Nordic Bioscience Diagnostics, serum pre-clinical Cartilaps ELISA, catalog No 3CAL4000, Fountain Hills, AZ, USA), PIIANP (N-propeptide type IIA collagen; Linco PIIANP ELISA, catalog No EZPIIANP-53K, Billerica, MA, USA), and osteocalcin (Osteocalcin, Quidel Metra Osteocalcin, ELISA reference No 8002, San Diego, CA, USA). Each ELISA kit was previously validated for use in horses.

Synovial fluid analysis
Synovial fluid (~1–3 mL) was aseptically aspirated from the affected joint at baseline and d14, 28, and 42. If the veterinarian was unsuccessful in obtaining synovial fluid from the lame joint on d0, a corresponding joint was chosen and served as the joint of interest throughout the remainder of the trial. The collected fluid was placed in a tube containing EDTA and assayed immediately for WBC.

Statistical analysis
The experiment was designed as a 2 treatment randomized block design, using 16 horses as experimental units paired into eight blocks based on lameness grade and affected joint. Data were collected at d0, 14, 28, and 42, resulting in a repeated measures design within the randomized complete blocks. SAS (v.9.1, SAS Institute Inc., Cary, NC, USA) PROC MIXED was used to perform repeated measures analysis of variance on d14, 28, and 42; data were expressed
as differences from d0. The model included baseline (d0) values as a covariate. One-sided treatment comparison test, in the direction of joint suppl improvement over the control diet, was conducted overall and at each collection time. P-values \( \leq 0.05 \) were considered statistically significant. Correlation analysis was performed (SAS PROC CORR) using Pearson correlation coefficients to explore the relationship among the various response variables (v.9.1, SAS).

**Rat trials**

**Induction of osteoarthritis**

The procedures used in this study were in accordance with the animal care standard operating procedure of Novus International Inc. Male Wistar rats (220 g; Charles River) were housed in solid-bottom cages with corncob bedding. Rats were fed AIN-93G diets, and water was available ad libitum, starting 28 d prior to knee injections. For monooiodoacetate (MIA)-induced arthritis, rats were anesthetized with isoflurane and given a single intra-articular injection of 0.6 mg MIA (trial 1) and 1 mg MIA (trial 2) through the infrapatellar ligament of the right or left knee. Site of injection (left vs right) was randomly assigned and equally balanced among left and right knees. MIA (Sigma-Aldrich, catalog No 12512, Saint Louis, MO, USA) was dissolved in physiologic saline and administered in a volume of 50 \( \mu \)L using a 26-guage, 0.5-inch needle. A Hamilton PB 600-1 repeating dispenser (model 750; Hamilton Company, Reno, NV, USA) with a 700 series luer tip microliter syringe was used for precise injection of an automated volume. The control knees were not injected. The weights of the rats averaged 350 g (trial 1) and 330 g (trial 2) at the time of MIA injection.

**Treatments (experiments 1 and 2)**

The treatments evaluated in Exp 1 and 2 were products (at the time of writing of this paper) of Novus International Inc., ESM Technologies, LLC, and Arenus (now owned by Altera International, LTD) and included an equine joint suppl (Exp 1), NEM\(^+\) (Exp 2), and chelated minerals (ZnHMTBa, CuHMTBa, MnHMTBa [trade names MINTREX\(^+\) and TELAFIRM\(^+\)]; Exp 2). In Exp 1, treatments were fed for 28 d (prefeed) prior to MIA injection and were continued for an additional 28 d. Fifty-four rats were assigned to one of three treatments: 1) AIN-93G diet control, 2) As 1% + 0.6% NEM, 3) As 1% + 0.75% CTM (blend of chelated minerals: MINTREX Zn, Mn, and Cu), and 4) As 1% + 0.6% NEM + 0.75% CTM (for each treatment, n=2 rats).

**Assessment of change in hind paw weight shift**

Changes in hind paw weight distribution between the right and left limbs were utilized as an index of joint discomfort. An incapacitance tester (IITC Life Science, Woodland Hills, CA, USA) was used to measure weight distribution. This indirect measure of pain has been shown to be a reproducible and sensitive method to assess the efficacy of anti-inflammatory and analgesic agents for OA.\(^{45}\) Rats were placed in an angled plexiglass chamber positioned so that each hind paw rested on a separate force plate. The force exerted by each hind limb (g) was averaged over 3 individual 5-second intervals. Results are presented as the difference in grams between the control and the arthritic limb. Thus, the higher the value, the more weight placed on the control knee, suggestive of a painful arthritic knee. A negative value indicates that more weight was placed on the arthritic than on the control knee. Measurements were taken on d1, 4, 7, 14, 21, and 28 post-MIA injection (trial 1) and d1, 3, 7, and 14 post-MIA injection (trial 2).

**Assessment of change in knee swelling**

An Ames spring-loaded caliper was used to measure knee swelling. Rats were lightly anesthetized with isoflurane before taking the measurement. Measurements were taken on d1, 4, 7, 14, 21, and 28 post-MIA injection (trial 1) and d1, 3, 7, and 14 post-MIA injection (trial 2).

**Serum biomarker analyses**

Rats were lightly anesthetized with isoflurane before collection of blood samples. A blood sample was taken via cardiac puncture. Serum samples were collected on d7, 14, and 28 (trial 1 only) post-MIA injection. All samples were frozen at –80°C until analysis. ELISA kits were used for measurement of CTXII (Nordic Bioscience Diagnostics, serum pre-clinical Cartilaps ELISA, catalog No 3CAL4000) and COMP (cartilage oligomeric matrix protein; MD Biosciences, COMP ELISA, catalog No A-COMP 96, St Paul, MN, USA) (Exp 2 only).

**Statistical analyses**

Analysis of variance was performed using the GLM (v.9.1, SAS Institute Inc., Cary, NC, USA) appropriate for a completely randomized design. Probability of type I error less
than 0.05 was considered significant; \( P < 0.10 \) was considered a trend.

**Sow trials**

**Experimental design**

The procedures used in the sow study were in accordance with the animal care standard operating procedure of Novus International Inc. Two sister farms (same production manager and location about 3 miles from each other) with 6,400 sows each were used in this study.46 These farms were evaluated from April 2007 and continued until March 2010, and production was considered above the industry average during this time. Both farms were stable for porcine reproductive and respiratory syndrome (PRRS) virus when the trial was started and PRRS negative at completion of the trial. Over 18,000 replacement gilt and sows (Pig Improvement Company [PIC C22 or PIC C29], Hendersonville, TN, USA) were involved in this study. Gilts from a single source parent farm were moved to one of the two farms (control vs CTM). Treatments were initiated upon arrival (weaning) and continued through growing and entry into the breeding herd. Each month, about 300 weanling gilts entered the farm with a target of 50% selection rate. Sows remained on treatment until culled from the herd. Replacement gilts were blocked by group based on the monthly supply of weaned gilts (cohorts and blocks). Cohorts were formed based on sow entry date. Sows that entered the farms within a certain month (first day to last day of the month) were assigned to one cohort. Only sows within groups that were old enough to produce at least four parities were included in the data analyses. The treatments at both farms started approximately at the same time. Gilts and sows remained on treatment until culled. All feed was made at the same feed mill. Feed samples were collected periodically to confirm mineral supplementation levels. One farm was fed the inorganic mineral control (ZnO, CuSO\(_4\), and MnO) and the other used CTM (MINTREX Cu, Mn, and Zn; Novus International, Inc., Saint Charles, MO, USA) to replace 50% of the inorganic minerals, except Se, I, and Fe. Both farms received a total of 0.3 mg/kg Se in the final diet, with 50% of Se as inorganic and 50% organic (ZORIEN® SeY, Novus International). Total mineral level in both farms was equal: target supplemental levels were Zn, 165 mg/kg; Cu, 16 mg/kg; and Mn, 38 mg/kg in the final diet. NRC swine recommendations are 50, 5, and 10 mg/kg diet for Zn, Cu, and Mn, respectively, for gestation/lactation diets; 80, 5, and 3 mg/kg for nursery pigs (10–20 kg); 60, 4, and 2 mg/kg for grower pigs; and 50, 3, and 2 mg/kg for finisher pigs, respectively.18

**Statistical analysis**

Only gilts with at least one service date were included in the analysis. Sow data included 15 cohorts and 4 parities. For gilts, CTM and control were compared on the basis of removal rate (%), relative removal rate due to locomotion (%), and mortality (%). For sows, removal rate (%) for parity 1–2, 1–3, and 1–4, relative removal rate due to locomotion (%), and mortality (%) were used to compare CTM vs control. Removal rate for sows was defined as the percentage of sows that farrowed compared to total number of sows in parity 1. Relative removal rate for sows was defined as the percentage of sows removed for a particular reason compared to the total number of sows removed. All treatment percentage comparisons were based on chi-square analysis using SAS (v.9.1, SAS Institute Inc.) PROC FREQ. Differences in CTM and control percentages were considered significant at \( P < 0.05 \); trends at \( P < 0.10 \). Additionally for each variable, percent reduction with CTM over control was calculated.

**Results**

**Horse trial**

**Clinical examinations**

There was a significant reduction in VAS flexion score between baseline and wk 2 (Tables 3 and 4) for horses fed the joint suppl (\( P < 0.05 \)). This variable showed improvement with the joint suppl (relative to placebo) only at wk 2. Although the investigators were blinded to the treatments, it was their impression that visual improvement was observed on two of the horses fed the joint suppl (the two most lame horses as assessed by AAEP scoring) as early as wk 2 of the study. One horse was able to put weight on a limb that was previously not utilized, and the second horse was moving more freely. These visual observations were consistent with the improvements observed in joint flexion.

**Serum analysis**

There was no effect of joint suppl observed for CTXII (Tables 4 and 5). This biomarker was highly variable in this horse population, with baseline values ranging from 0 to 218 pg/mL. Normal CTXII reference ranges for healthy horses 5 years of age and older are from 0 to 80 pg/mL according to Nordic Biosciences, manufacturer of CTXII. It is worth noting that for the two horses on the joint suppl with CTXII above normal reference range, the percent reduction in CTXII from baseline to wk 2 was 97% and 84%, decreasing to normal range by wk 2 and staying within this range throughout the study duration. CTXII was, however, significantly correlated to AAEP lameness scores (Table 6) \( (r=0.35, \ P=0.020) \). CTXII was also correlated to multiple VAS measurements,
including flexion ($r=0.33, P=0.026$), trot ($r=0.42, P=0.005$), walk ($r=0.42, P=0.005$), and FR ($r=0.40, P=0.005$).

Overall, serum PIIANP (Tables 4 and 5) was increased in horses fed the joint suppl t relative to baseline, whereas horses on placebo showed no change ($P<0.05$), and at d28, also tended to be increased ($P=0.07$) for suppl t vs placebo relative to baseline. Serum PIIANP was also correlated (Table 6) to AAEP lameness scores ($r=0.37, P=0.0125$) and osteocalcin ($r=0.64, P<0.0001$). Serum osteocalcin tended to increase relative to baseline (d42, $P=0.06$) for horses fed

### Table 3 Treatment means for clinical measurements as affected by treatment and time (equine)

| Variable       | Treatment | Day 0     | Day 14    | Day 28    | Day 42    |
|----------------|-----------|-----------|-----------|-----------|-----------|
| Lameness (AAEP)| Suppl t   | 2.19±0.64 | 2.44±0.50 | 2.13±0.71 | 2.31±0.49 |
|                | Placebo   | 2.00±0.42 | 1.63±0.46 | 1.75±0.59 | 2.25±0.45 |
| VAS walk       | Suppl t   | 2.75±1.05 | 2.75±1.31 | 2.63±1.31 | 2.75±1.10 |
|                | Placebo   | 1.50±0.82 | 1.50±0.82 | 1.50±1.00 | 1.75±1.05 |
| VAS trot       | Suppl t   | 3.69±1.11 | 4.13±1.32 | 3.81±1.34 | 3.75±1.10 |
|                | Placebo   | 3.50±0.87 | 2.63±0.94 | 3.38±1.08 | 3.63±0.96 |
| VAS flex       | Suppl t   | 4.88±1.11 | 3.83±0.84*| 4.29±1.11 | 4.14±1.09 |
|                | Placebo   | 2.88±0.97 | 2.88±0.88 | 2.50±0.85 | 2.88±1.04 |
| VAS flex response | Suppl t | 5.50±1.21 | 4.00±1.13 | 5.00±1.14 | 4.57±1.21 |
|                | Placebo   | 3.00±0.96 | 2.75±0.70 | 3.00±1.13 | 2.63±0.92 |

Notes: SAS PROC MIXED was used to perform a repeated measures analysis of variance on d14, 28, and 42 and evaluated as differences from d0. The model included baseline (d0) as a covariate. AAEP lameness scores were on a scale between 0 and 5, 5 being severe lameness. Other variables were scored using a VAS, by marking on a horizontal line from 0 to 10, 0 being no lameness and 10 being extreme lameness or non-weight-bearing. *$P<0.05$ suppl t vs placebo difference at d14 vs d0 for VAS flexion. **$P<0.05$ suppl t vs placebo difference at d14 vs d0 for VAS flexion. "AAEP, American Association of Equine Practitioners; Suppl t, supplement; VAS, visual analog score.

### Table 4 Analysis of change from baseline over time with baseline covariate for serum biomarkers and lameness measurements (equine)

| Variable      | Day | Suppl t mean Δ (std error) | Placebo mean Δ (std error) | Difference | Std error of difference | P-value |
|---------------|-----|---------------------------|----------------------------|------------|------------------------|---------|
| Serum PIIANP (ng/mL) | Overall | 137.1 (63.7) | 28.8 (63.7) | 108.3 | 54.5 | 0.0471** |
|                | 14   | 135.9 (83.7) | 52.3 (83.7) | 83.5 | 94.0 | 0.1910 |
|                | 28   | 138.6 (83.7) | -4.6 (83.7) | 143.2 | 94.0 | 0.0696* |
|                | 42   | 136.7 (83.7) | 38.6 (83.7) | 98.1 | 94.0 | 0.1528 |
| Serum osteocalcin (ng/mL) | Overall | 0.79 (1.1) | -0.72 (1.1) | 1.51 | 1.54 | 0.1822 |
|                | 14   | 2.37 (1.5) | 0.26 (1.5) | 2.12 | 2.05 | 0.1556 |
|                | 28   | -1.73 (1.5) | -0.87 (1.5) | -0.86 | 2.05 | 0.6602 |
|                | 42   | 1.73 (1.5) | -1.5 (1.5) | 3.26 | 2.05 | 0.0616* |
| Serum CTXII (pg/mL) | Overall | -28.1 (4.1) | -28.3 (4.1) | 0.2 | 5.1 | 0.5184 |
|                | 14   | -33.8 (6.3) | -24.8 (6.3) | -9.0 | 8.6 | 0.1527 |
|                | 28   | -27.0 (6.3) | -35.6 (6.3) | 8.6 | 8.6 | 0.8376 |
|                | 42   | -23.5 (6.3) | -24.6 (6.3) | 1.1 | 8.6 | 0.5505 |
| VAS flexion    | Overall | -0.58 (0.34) | -0.28 (0.32) | -0.31 | 0.45 | 0.2622 |
|                | 14   | -1.28 (0.45) | -0.15 (0.44) | -1.13 | 0.62 | 0.0397** |
|                | 28   | -0.16 (0.47) | -0.53 (0.44) | 0.37 | 0.63 | 0.4068 |
|                | 42   | -0.30 (0.47) | -0.15 (0.44) | -0.15 | 0.63 | 0.3481 |
| VAS flex response | Overall | -0.81 (0.61) | -0.45 (0.59) | -0.36 | 0.89 | 0.3481 |
|                | 14   | -1.17 (0.66) | -0.49 (0.64) | -0.68 | 0.95 | 0.2409 |
|                | 28   | -0.42 (0.67) | -0.24 (0.64) | -0.18 | 0.95 | 0.4256 |
|                | 42   | -0.85 (0.67) | -0.61 (0.64) | -0.23 | 0.95 | 0.4040 |
| AAEP lameness  | Overall | 0.13 (0.26) | -0.15 (0.26) | 0.28 | 0.21 | 0.8880 |
|                | 14   | 0.28 (0.29) | -0.40 (0.29) | 0.68 | 0.29 | 0.9870 |
|                | 28   | -0.04 (0.29) | -0.28 (0.29) | 0.24 | 0.29 | 0.7950 |
|                | 42   | 0.15 (0.29) | 0.22 (0.29) | -0.07 | 0.29 | 0.9401 |

Notes: SAS PROC MIXED was used to perform a repeated measures analysis of variance on d14, 28, and 42 and evaluated as differences from d0. The model included baseline (d0) as a covariate. AAEP lameness scores were on a scale between 0 and 5, 5 being severe lameness. Other lameness variables were scored using a VAS, by marking on a horizontal line from 0 to 10, 0 being no lameness and 10 being extreme lameness or non-weight-bearing. PIIANP is a synthetic collagen type II marker; osteocalcin is a synthetic bone biomarker, and CTXII is a degradative collagen type II marker. **$P<0.05$ suppl t vs placebo difference overall for PIIANP; suppl t vs placebo difference at d14 vs d0 for VAS flexion. **$P<0.10$ suppl t vs placebo difference at d28 vs d0 for PIIANP; suppl t vs placebo difference at d42 vs d0 for osteocalcin. AAEP, American Association of Equine Practitioners; CTXII, C-terminal cross-linked telopeptide type II collagen; PIIANP, N-propeptide type IIA collagen; Suppl t, supplement; Std, standard; VAS, visual analog score; Δ, change.
Significant correlation coefficients between serum biomarkers and synovial fluid WBC as affected by treatment and time (equine) 

| Variable                          | Treatment | Day 0             | Day 14            | Day 28             | Day 42             |
|-----------------------------------|-----------|-------------------|-------------------|--------------------|--------------------|
| Serum CTXII (pg/mL)               | Supplt    | 54.72±26.8        | 5.11±2.74         | 11.96±6.65         | 15.41±4.05        |
|                                   | Placebo   | 22.53±9.81        | 13.48±7.19        | 2.75±1.83          | 13.71±10.51       |
| Serum PIIANP (ng/mL)              | Supplt    | 972.0±319.7       | 1,111.7±282.6     | 1,114.4±296.8      | 1,112.5±412.3     |
|                                   | Placebo   | 872.8±127.0       | 921.4±130.2       | 864.6±133.7        | 907.6±139.0       |
| Serum osteocalcin (ng/mL)         | Supplt    | 12.0±1.6          | 14.2±2.3          | 10.1±1.6           | 13.6±1.4a         |
|                                   | Placebo   | 11.2±1.8          | 11.4±2.6          | 9.7±1.8            | 10.7±1.6          |
| Synovial fluid WBC (cells/µL)     | Supplt    | 124.0±87          | 1,721±1,447       | 777.0±574          | 557.9±217         |
|                                   | Placebo   | 103.4±36          | 244.8±79          | 156.4±46           | 173.3±74          |

Notes: SAS PROC MIXED was used to perform a repeated measures analysis of variance on day 14, 28, and 42 and evaluated as differences from day 0. The model included baseline (d0) as a covariate. PIIANP is a synthetic collagen type II marker, osteocalcin is a synthetic bone marker, and CTXII is a degradative collagen type II marker.

Abbreviations: CTXII, C-terminal cross-linked telopeptide type II collagen; PIIANP, N-propeptide type IIA collagen; Supplt, joint supplement; WBC, white blood cells.

the joint supplt, compared to control (Tables 4 and 5). Serum osteocalcin was also correlated (Table 6) to AAEP lameness scores ($r=0.57$, $P<0.0001$), VAS flexion ($r=0.38$, $P=0.0125$), and VAS flex response ($r=0.50$, $P<0.001$).

Synovial fluid analysis
Although WBC in synovial fluid (Table 5) was not significantly affected by dietary treatment, this variable was correlated (Table 6) to AAEP lameness score ($r=0.41$, $P=0.005$).

Table 6 Significant correlation coefficients between serum biomarkers, synovial fluid WBC, and lameness measurements (equine)

| Biomarker                          | Biomarker or lameness variable | Correlation coefficient | P-value |
|------------------------------------|--------------------------------|-------------------------|---------|
| Serum PIIANP (ng/mL)               | AAEp                           | 0.37                    | 0.0125  |
|                                   | Osteocalcin                     | 0.64                    | $<0.0001$|
|                                   | SF WBC                          | 0.60                    | $<0.0001$|
| Serum osteocalcin (ng/mL)          | AAEp                           | 0.57                    | $<0.0001$|
|                                   | SF WBC                          | 0.76                    | $<0.0001$|
|                                   | VAS flexion                     | 0.38                    | 0.0125  |
|                                   | VAS FR                          | 0.50                    | 0.0010  |
| Synovial fluid WBC (cells/µL)      | AAEp                           | 0.41                    | 0.0050  |
|                                   | VAS LF                          | 0.63                    | $<0.0001$|
|                                   | VAS trot                        | 0.66                    | $<0.0001$|
|                                   | VAS walk                        | 0.61                    | $<0.0001$|
| Serum CTXII (pg/mL)               | VAS flexion                     | 0.33                    | 0.0260  |
|                                   | VAS FR                          | 0.42                    | 0.0050  |
|                                   | AAEp                            | 0.40                    | 0.0050  |

Notes: Pearson correlation coefficients and $P$-values between serum biomarkers, synovial fluid WBC, and lameness scores. PIIANP is a synthetic collagen type II marker; osteocalcin is a synthetic bone marker; and CTXII is a degradative collagen type II marker.

Abbreviations: AAEp, American Association of Equine Practitioners; PIIANP, N-propeptide type IIA collagen; SF WBC, visual analog scale; VAS flexion, pain to manual joint flexion; VAS FR, flex response (lameness after a 1-minute flexion test); VAS LF, lameness after flexion; VAS trot, lameness at a trot; VAS walk, lameness at a walk; CTXII, C-terminal cross-linked telopeptide type II collagen; SF WBC, synovial fluid white blood cells.

In addition, PIIANP ($r=0.60$, $P<0.0001$) and osteocalcin ($r=0.76$, $P<0.0001$) were also correlated to synovial fluid WBC. Synovial fluid WBC was also correlated to VAS LF ($r=0.63$, $P<0.0001$), VAS trot ($r=0.56$, $P<0.0001$), and VAS walk ($r=0.61$, $P<0.0001$). Synovial fluid cartilage biomarker analyses were more variable than serum biomarker analyses, and owing to difficulties in synovial fluid collection, baseline samples were missing for many of the horses.

Rat trial

Experiment 1
Relative to the other treatments, rats fed 2% joint suppl were able to bear more weight on arthritic limb on d14 post-MIA injection ($P<0.05$) (Figure 1A) and were numerically better than control at all but one time point. CTXII (Figure 1B) was significantly decreased in rats fed 2% joint suppl at d7, 14, and 28 ($P<0.05$) relative to control and decreased in rats fed 1% joint suppl at d28 ($P<0.05$).

Experiment 2
Significant differences in hind paw weight distribution (Figure 2A) were observed on d1 between rats fed NEM + CTM vs rats fed the negative control ($P<0.05$) and a trend on d3 and 7 ($P<0.10$) for rats fed the combination of NEM + CTM relative to rats fed the negative control (AIN-93G). Injection of MIA resulted in a time-dependent change in joint swelling as measured by calipers (Figure 2B). Swelling was highest for all treatments at d1 and decreased thereafter. Significant differences were observed on d3 post-MIA injection for rats fed the combination of NEM + CTM ($P<0.05$) relative to NEM or CTM fed singly ($P<0.05$). Reductions in CTXII (Figure 2C) were observed at d7 ($P<0.10$) and d14 ($P<0.05$) for rats fed NEM only. Reductions in CTXII were also observed for rats fed the combination of NEM + CTM, at d7 ($P<0.10$). Reductions in COMP (Figure 2D), also a
Chelated trace minerals and joint supplement for OA and lameness

Figure 1 Hind paw weight shift (A) and CTXII (B) in rats with osteoarthritis induced with MIA (mean ± SE for Exp 1).

Notes: Data represent mean values from 18 rats/treatment for rats fed control (white bars), 1% joint suppl (light gray bar, SF1) and 2% joint suppl (dark gray bars, SF2). (A) Change in hind paw weight distribution is an indirect measure of joint discomfort. The values presented are the difference between the control (uninjected) and the test knee. A lower value indicates the rat is able to bear more weight on its arthritic knee. A reduction in hind paw weight shift was observed for rats fed 2% joint suppl at d14 (P<0.05) relative to rats fed 1% joint suppl and was numerically better than control at all but one time point. (B) Relative to the control, a reduction in CTXII, a measure of cartilage degradation, was observed for rats fed 2% joint suppl at d7, 14, and 28 (P<0.05) as well as for rats fed 1% joint suppl at d28 (P<0.05). a,b Values are significantly different (P<0.05).

Abbreviations: Ctrl, control; CTXII, C-terminal cross-linked telopeptide type II collagen; SF1, joint suppl at 1% dietary inclusion; SF2, joint suppl at 2% dietary inclusion; MIA, monooiodoacetate; SE, standard error.

degradative marker, were observed for rats fed CTM and the combination of CTM + NEM, at d14 (P<0.05).

Sow trial

Gilt data

Gilt is defined as replacement female that was selected from first service to first farrowing date. Gilt removal rate was reduced 9.1% with CTM supplementation, with removal rates of 8.0% vs 8.8% for CTM and control, respectively (P=0.04, Table 7). A 34.8% reduction in relative removal rate due to locomotion (9.0% vs 13.8%, P<0.001) was observed in gilts fed CTM compared to those fed ITM. Similarly, a 28.6% reduction in mortality was observed for CTM vs control (1.5% vs 2.1%, P=0.001).

Sow data

In parity 1–2, the group of sows fed CTM experienced an 11.5% reduction in removal rate compared to control (10.0% vs 11.3%, P=0.06, Table 7). A similar pattern of reduction in removal rate with CTM over control was observed in parity 1–3 and 1–4. In parity 1–3, the CTM group displayed a 20.2% reduction in removal rate compared to control (17.8% vs 22.3%, respectively, P<0.001). In parity 1–4, the reduction in removal rate with CTM over control was 23.6% (27.9% vs
Relative concentrations of biomarkers can yield information about disease onset, expected rate of progression, and effects of therapy. As the most abundant protein component of cartilage, type II collagen molecules have been extensively investigated as biomarkers of DJD. A critical review to assess the usefulness of different type II collagen biomarkers found that CTXII concentration was beneficial for the purpose of diagnosing DJD, evaluating the burden of disease, determining a prognosis, and quantifying treatment effects. No other type II collagen biomarker was found to be as ubiquitously useful. Other studies have shown that high concentrations of CTXII correspond to an increased risk of DJD and faster progression of joint space narrowing (as assessed by radiography), and cartilage loss (as assessed by magnetic resonance imaging [MRI]). COMP, a noncollagenous degradative marker, has likewise been shown to be a useful biomarker of OA.

The ideal nutritional therapy for DJD would decrease cartilage degradative markers (eg, CTXII, COMP) and/or increase cartilage synthetic markers (PIIANP) or bone synthetic markers (osteocalcin). Serum PIIANP has been shown in previous studies to be a reliable indicator of disease prognosis. In patients with both low levels of PIIANP and high levels of CTXII, the progression of OA was eightfold more rapid (as assessed by radiography and arthroscopy).
Table 7 Chelated trace minerals reduced gilt and sow removal rate due to locomotion

| Variable       | CTM* | Control | % Reduction with CTM | P-value |
|----------------|------|---------|----------------------|---------|
| Gilt           | (n=10,725) | (n=10,729) | 8.0**   | 8.8**   | 9.1   | 0.04 |
| Removal rate (%) |       |         |                     |         |
| Relative removal rate by reason† (%) |       |         |                     |         |
| Locomotion     | 9.0** | 13.8**  | 34.8                | <0.001  |
| Mortality (%)  | 1.5** | 2.1***  | 28.6                | 0.001   |
| Sow            | (n=3,994) | (n=4,418) | 10.4**  | 16.1**  | 35.4  | <0.001 |
| Removal rate (%) |       |         |                     |         |
| Parity 1–2     | 10.0** | 11.3%   | 11.5                | 0.06    |
| Parity 1–3     | 17.8** | 22.3**  | 20.2                | <0.001  |
| Parity 1–4     | 27.9** | 36.5**  | 23.6                | <0.001  |
| Relative removal rate by reason† (%) |       |         |                     |         |
| Locomotion     | 10.4** | 16.1**  | 35.4                | <0.001  |
| Mortality (%)  | 8.6*  | 10.4*   | 17.3                | 0.08    |

Notes: *Gilt is defined as replacement female from first service to first farrowing. Only gilts with at least one service date were included in the analysis. Sow data included 15 cohorts and up to 4 parities. Percentage comparisons based on chi-square analyses using SAS PROC FREQ. CTM’s trade name is MINTREX and includes Zn, Cu, and Mn HMTBa. Relative removal rate for gilts/sows was defined as the percentage of gilts/sows removed for a particular reason compared to the total number of gilts/sows removed. Removal rate for sows was defined as the percentage of sows that farrowed in a particular parity compared to the total number of sows removed in parity 1. †P < 0.10; **P < 0.05.

Abbreviations: CTM, chelated trace minerals; HMTBAs, hydroxy-methylthiobutyric acid.

than in other patients. Similar results in another turkey trial indicated increased bone-breaking strength with CTM, as determined by four-point bending and torsional assays. Interestingly, in the control treatments, ITM levels were formulated at commercial levels, far exceeding published requirements. Thus, these animals were not deficient in trace minerals. Trace mineral requirements for optimal bone development may be greater than for maximization of growth rate, and requirements are probably increased in faster-growing animals. Furthermore, the chelated form of trace minerals appears to be more effective for joint and skeletal health than inorganic forms. These structural and tissue improvements make sense, given that these trace minerals play fundamental roles in collagen and keratin synthesis and cross-linking, bone development, and immune response. Bone-breaking strength is a function of collagen cross-linking. Lysyl oxidase, the enzyme that cross-links collagen, is Cu-dependent, and lysyl oxidase also cross-links elastin, which is found in connective tissues, primarily in the cardiovascular system and intestines. Thus, Cu promotes skin, bone, tendon, and intestinal strength. Use of CTM not only improves structural integrity of cartilage and bone, but may improve the integrity of other connective tissues such as tendons and ligaments.
Micromineral needs are presumably increased in today’s high-producing maternal sow lines with improved reproduction performance. Sow mineral reserves are depleted over a period of three parities, and body mineral loss (macro and micro) is greater in higher-producing sows compared to lower-producing sows. The lower mortality and removal rate in both gilts and sows with CTM supplementation may be due to better body mineral status and consequently better immune function. Peters and Mahan indicated that lactation sow mineral daily intake was reduced as sow aged in parity from 1–6, suggesting that mineral intake in old lactation sows may be insufficient to maintain offspring and body maintenance. CTM lowered removal rates and removals due to locomotion problems. Gill suggested that poor locomotion is one of the most costly reasons for removal. Sows with leg problems may have zero value because they may not be able to make it to the processing plant. Locomotion removals were reduced with CTM supplementation. Relative locomotion removal rates were 10.4% vs 16.1% for CTM and control, respectively. The reduction in locomotion removal rates gives the farm manager more flexibility to cull sows and make decisions based on reproduction targets. Our findings suggest that supplying a more bioavailable trace mineral is beneficial for skeletal and joint health in production sows and may provide important managerial and economic benefits to the sow farm.

It is difficult to demonstrate therapeutic efficacy of a nutraceutical in an arthritic animal population. The progression of OA is highly variable and heterogeneous. Some biomarkers increase initially because of the remodeling of bone and cartilage that is taking place, but then decrease at later stages; so within a population, these biomarkers may be moving in opposite directions. In our horse population, the horses averaged 18 yr in age, but only five of the 16 horses had an AAEP lameness score of 3 or higher and only 4 had CTX concentrations that were outside of normal reference range. It is difficult to show improvement if biomarkers in the population being measured are in normal reference range. It is equally, if not more, difficult to demonstrate prophylactic effects for nutrition in the prevention of lameness or arthritis. Large animal numbers, repeated parities, and long study durations are necessary in order to show statistical differences and are expensive studies to conduct. The use of an early-onset chemically induced OA model, on the one hand, produces a more homogenous model (i.e., the severity and progression of OA are more consistent within the animal population), but the severity and rapid progression may set a high hurdle for nutraceuticals to overcome. Previous studies have, however, demonstrated the efficacy of certain nutraceuticals such as glucosamine and/or chondroitin sulfate using chemically or surgically induced rheumatoid or OA models.

Our studies had several limitations. In our horse study, there were small numbers, resulting in a lack of statistical sensitivity. The AAEP and VAS measurements used to assess lameness were subjective, and more objective measurements such as radiography and histology were not evaluated. While CTXII, COMP, and PIIANP appear to be promising for early detection of joint degeneration, none are sufficient to serve as a surrogate marker of disease for either individuals or population. Ideally, these biomarkers would be used in combination with other measurements of lameness or disease progression, including radiography, histology, MRI, and/or objective and functional measures of limb/joint function. It should be noted that CTXII has not been widely used as a biomarker in equine. Nonetheless, recent studies in humans and other species continue to show CTXII to be one of the more promising biomarkers, useful for early detection of joint degeneration, and correlated to symptoms or functional measurements of OA and should be explored more in equine.

Despite the hurdles, collectively, our studies in horses, sows, and rats demonstrated a beneficial effect of CTM both in the prevention and in the treatment of lameness and OA. Use of CTM decreased the culling rates in sows, and in combination with NEM, CTM was beneficial in reducing pain, inflammation, and cartilage degradation. Furthermore, supplementation with our joint supplement was shown in our chemically induced rat model to have anti-inflammatory and chondromodulating effects and, in horses, chondromodulating effects. Additionally, beneficial effects of our joint supplement have been demonstrated in camels, and NEM benefits have been demonstrated in geriatric cranes. These results further establish that the beneficial effects of our joint supplement and NEM translate across multiple species.

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Disclosure

The authors report no conflicts of interest in this work.

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