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ORIGINAL RESEARCH

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

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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.[8,9] 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.[15] 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.[5] 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 synovitus.[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).[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.[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.[35]

Nutritional supplements may offer advantages over drugs in the treatment and intervention of OA.[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.[36,38] Despite their widespread use, there is only limited and conflicting data regarding the efficacy of these supplements.[36,37] Furthermore, some research has shown these ingredients are poorly bioavailable and of inconsistent quality.[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.[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	
NEM® joint mobility matrix	3,000 mg
Chelated trace minerals (TELAFIRM®)*	2,281 mg
Calcium	2,239 mg
Phosphorus	1,891 mg
Ascorbic acid	1,000 mg
Biotin	20 mg
Vitamin D_3	1,000 IU

Notes: *TELAFIRM includes a proprietary blend of chelated Zn, Cu, and Mn HMTBa. The amount of Zn, Cu, and Mn provided by STEADFAST approximates 30% of the National Research Council (USA) recommendation for the horse.
Abbreviations: HMTBa, 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	Supplt	18.2						2.2
	Control	19.2						2.0

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 supplt improvement over the control diet, was conducted overall and at each collection time. *P*-values ≤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,[44] and water was available ad libitum, starting 28 d prior to knee injections. For monoiodoacetate (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 µL 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 supplt (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% + 1% equine joint supplt, and 3) As 1% + 2% equine joint supplt (18 rats/treatment group). In Exp 2, 48 rats were fed treatments for 28 d (prefeed). Following MIA injection (d29), rats continued on experimental treatments an additional 14 d. Treatments in Exp 2 included 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 gilts 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 supplt ($P<0.05$). This variable showed improvement with the joint supplt (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 supplt (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 supplt 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 supplt 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,

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)	Supplt	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	Supplt	2.75±1.05	2.75±1.31	2.63±1.31	2.75±1.10
	Placebo	1.50±0.98	1.50±0.82	1.50±1.00	1.75±1.05
VAS trot	Supplt	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 flexion	Supplt	4.88±1.11	3.38±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	Supplt	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. The 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. *$P<0.05$ supplt vs placebo difference at d14 vs d0 for VAS flexion.
Abbreviations: AAEP, American Association of Equine Practitioners; Supplt, supplement; VAS, visual analog score.

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

Variable	Day	Supplt 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	0.4041

Notes: SAS PROC MIXED was used to perform a repeated measures analysis of variance on d14, 28, and 42 and was 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. The 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. **$P<0.05$ supplt vs placebo difference overall for PIIANP; supplt vs placebo difference at d14 vs d0 for VAS flexion. *$P<0.10$ supplt vs placebo difference at d28 vs d0 for PIIANP; supplt vs placebo difference at d42 vs d0 for osteocalcin.
Abbreviations: AAEP, American Association of Equine Practitioners; CTXII, C-terminal cross-linked telopeptide type II collagen; PIIANP, N-propeptide type IIA collagen; Supplt, supplement; Std, standard; VAS, visual analog score; Δ, change.

Table 5 Treatment means for 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	864.4±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±1.4*
	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±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 d14, 28, and 42 and evaluated as differences from d0. The model included baseline (d0) as a covariate. PIIANP is a synthetic collagen type II marker, osteocalcin is a synthetic bone biomarker, and CTXII is a degradative collagen type II marker. *$P<0.10$ supplt vs placebo difference at d28 vs d0 for PIIANP; supplt vs placebo difference at d42 vs d0 for osteocalcin.
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	0.56	<0.0001
	VAS walk	0.61	<0.0001
Serum CTXII (pg/mL)	VAS flexion	0.33	0.0260
	VAS trot	0.42	0.0050
	VAS walk	0.42	0.0050
	VAS FR	0.40	0.0050
	AAEP	0.35	0.0200

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 biomarker, and CTXII is a degradative collagen type II marker.
Abbreviations: AAEP, American Association of Equine Practitioners; PIIANP, N-propeptide type IIA collagen; VAS, 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 supplt 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 supplt at d7, 14, and 28 ($P<0.05$) relative to control and decreased in rats fed 1% joint supplt 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

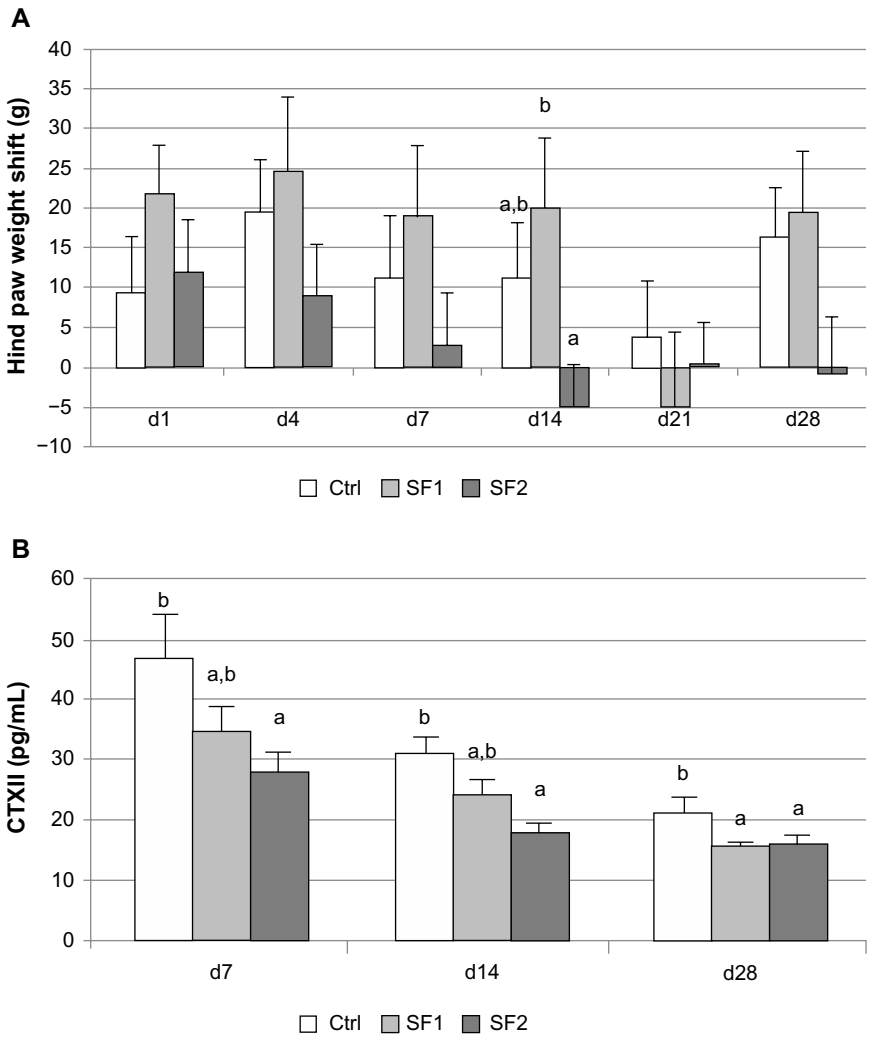

Figure 1 Hind paw weight shift (**A**) and CTXII (**B**) in rats with osteoarthritis induced with MIA (mean ± SE for Exp 1).
Notes: Data represent mean values from 18 rats/treatment for rats fed control (white bars), 1% joint supplt (light gray bar, SF1) and 2% joint supplt (dark gray bars, SF2). (**A**) 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 supplt at d14 ($P<0.05$) relative to rats fed 1% joint supplt 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 supplt at d7, 14, and 28 ($P<0.05$) as well as for rats fed 1% joint supplt 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 supplt at 1% dietary inclusion; SF2, joint supplt at 2% dietary inclusion; MIA, monoiodoacetate; 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

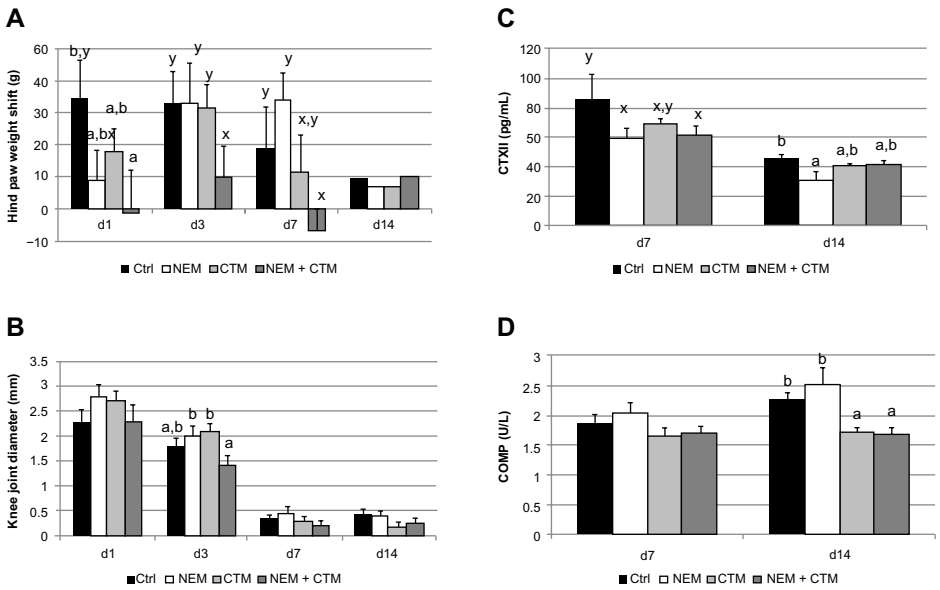

Figure 2 Hind paw weight shift (**A**), knee joint diameter (**B**), CTXII (**C**), and COMP (**D**) in rats with osteoarthritis induced with MIA (mean ± SE for Exp 2).
Notes: Data represent mean values from 12 rats/trt for rats fed control (black bars), NEM (white bars), CTM (light gray bars), and NEM + CTM (dark gray bars). (**A**) Time course pattern of MIA-induced joint pain (g; control minus arthritic knee). Significant differences were observed on d1 post-MIA injection for rats fed NEM + CTM ($P<0.05$) relative to control and a trend ($P<0.10$) for rats fed NEM only to bear more weight on arthritic limb vs rats fed the control. On d3 and7, there was a trend for rats fed NEM + CTM to be lower ($P<0.10$) relative to control. (**B**) Time course pattern of MIA-induced swelling (mm; arthritic knee minus control knee). Significant differences were observed on d3 post-MIA injection for rats fed NEM + CTM ($P<0.05$) relative to rats fed NEM or CTM only. (**C**) Reductions in serum CTXII (pg/mL) occurred on d7 in rats fed NEM alone or the combination of NEM + CTM ($P<0.10$). Reductions in CTXII also occurred on d14 for rats fed NEM singly ($P<0.05$). (**D**) Reductions in COMP (U/L) occurred on d14 in rats fed CTM alone or the combination of NEM + CTM ($P<0.05$). ^{a,b}Values are significantly different ($P<0.05$); $^{x,y}P<0.10$.
Abbreviations: NEM, Natural Eggshell Membrane; COMP, cartilage oligomeric matrix protein; CTM, chelated trace minerals; Ctrl, control; CTXII, C-terminal cross-linked telopeptide type II collagen; MIA, monoiodoacetate; SE, standard error.

36.5%, respectively, $P<0.001$). Further, sows fed CTM had lower relative removal rate due to locomotion compared to the control group (10.4% vs 16.1%, respectively, $P<0.001$, Table 7). The difference in percentages between treatment and control represents a 35.4% reduction in the locomotion culling rate for sows fed CTM. Additionally, mortality was 8.6% and 10.4% for sows fed CTM and ITM, respectively ($P=0.08$). Both numbers are within industry average in North America according to Pigchamp.[4]

Discussion

The hallmark of OA or DJD is loss of articular cartilage.[24,47] Inflammation of the synovium, or synovitis, appears to be one of the most important features of early DJD and correlates well with joint pain and function.[22] DJD leads to a higher rate of cartilage degradation than synthesis due to excessive catabolic activity, loss of the structural integrity of the extracellular matrix, and a high rate of apoptosis, causing a decrease in chondrocyte concentration, which, in turn, reduces the ability of cartilage to repair itself.[22]

Measuring concentrations of biomarkers associated with the synthesis and degradation of joint tissues has shown promise for the diagnosis and prognosis of joint ailments.[32,48] Relative concentrations of biomarkers can yield information about disease onset, expected rate of progression, and effects of therapy.[49] As the most abundant protein component of cartilage, type II collagen molecules have been extensively investigated as biomarkers of DJD.[50] 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.[50] No other type II collagen biomarker was found to be as ubiquitously useful.[50] 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]).[51–54] COMP, a noncollagenous degradative marker, has likewise been shown to be a useful biomarker of OA.[55–59]

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.[30,60] 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[a]

Variable	CTM[b]	Control	% Reduction with CTM[b]	*P*-value
Gilt	**(n=10,725)**	**(n=10,729)**		
Removal rate (%)	8.0**	8.8**	9.1	0.04
Relative removal rate by reason[c] (%)				
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)**		
Removal rate[d] (%)				
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[c] (%)				
Locomotion	10.4**	16.1**	35.4	<0.001
Mortality (%)	8.6*	10.4*	17.3	0.08

Notes: [a]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. [b]CTM's trade name is MINTREX and includes Zn, Cu, and Mn HMTBa. [c]Relative removal rate for gilts/sows was defined as percentage of gilts/sows removed for a particular reason compared to the total number of gilts/sows removed. [d]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; HMTBa, hydroxy-methylthiobutyric acid.

than in other patients.[60] Use of a combination of markers appears to improve the prediction of disease progression. The findings from our horse trial demonstrated significant correlations between AAEP lameness scoring, synovial fluid WBC counts, and serum CTXII, PIIANP, and osteocalcin, suggesting a link between synovitis and OA, which has been confirmed by other studies.[23] The significant correlations found in our horse study between biomarkers and lameness measurements further support that these biomarkers may be useful for early detection of joint degeneration, reliable indicators of disease progress, and correlated to symptoms or functional measurements of OA/lameness, which is also supported by other studies.[50–54] The numerical decrease in serum biomarker CTXII and increase in serum PIIANP and osteocalcin observed in our study suggest that our joint supplt had chondromodulating effects. The combination of these findings suggests slowing of cartilage loss and rebuilding of cartilage and bone tissue.

In agreement with our horse trial, the rat OA studies also showed a reduction in CTXII and/or COMP with the joint supplt or the combination of NEM and CTM, the active ingredients in the joint supplt. Furthermore, reductions in knee swelling and pain were also observed in rats fed the combination of NEM and CTM. Collectively, results from both the horse and rat studies suggest that the joint supplt has both anti-inflammatory and chondromodulating effects. CTXII was shown in both the horse and rat model to be a sensitive and consistent biomarker, and this is supported by literature.[30,60,61] Synergistic effects were demonstrated with the combination of NEM and CTM: CTM + NEM decreased pain, swelling, CTXII, and COMP compared to control and/or CTM or NEM alone.

It is our belief that CTM, as opposed to microminerals per se, is a critical component of our joint supplt efficacy. Cu bioavailability (as measured by intestinal breaking strength in poultry) was significantly greater with Cu-HMTBa compared to other organic Cu sources (Cu-lysine, Cu-proteinate) and inorganic Cu sulfate.[62] Data in cows also indicate increased bioavailability of Cu-HMTBa relative to other Cu sources.[63] Similarly, different biomarkers of Zn bioavailability (intestinal breaking strength, metallothionein, and bone Zn) demonstrated significantly greater bioavailability with Zn-HMTBa than with Zn-methionine, Zn-proteinate, Zn amino acid complex, and Zn sulfate.[63–66] These advantages in bioavailability translate to numerous benefits in animal health. A recent trial in turkeys evaluated the impact of CTM supplementation on leg development, synovitis, and bone strength.[21] Adding CTM to the diet significantly reduced the incidence of TD, synovitis, and footpad lesions. Interestingly, synovitis was positively correlated with lameness.[21] Similar results in another turkey trial indicated increased bone-breaking strength with CTM, as determined by four-point bending and torsional assays.[20] 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.[20] Furthermore, the chelated form of trace minerals appears to be more effective for joint and skeletal health than inorganic forms.[19–20] 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.[5] Bone-breaking strength is a function of collagen cross-linking.[67] Lysyl oxidase, the enzyme that cross-links collagen, is Cu-dependent,[6] 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.[62] 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.[62]

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,[68] 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[69] 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[70] 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 (ie, 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.[35] Previous studies[71–73] 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.[74] 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.[1,31] 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.[50,75–77]

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,[78] and NEM benefits have been demonstrated in geriatric cranes.[79] 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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