Evaluation of the Safety and Efficacy of the Dietary Supplement Actistatin® on Established Glucosamine and Chondroitin Therapy in the Horse

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ABSTRACT

Seventy five equine subjects were selected from a large population of high-performance, chronic joint pain, and/or limited ROM sufferers. Inclusion criteria for the study required AAEP recognized equine lameness scores of at least 2 or higher, for a minimum of 6 months prior to the start date of this study. Blood, urine and synovial fluid samples were collected on day 1 of the study and evaluated to establish baselines. One week later, the horses were randomized into two groups of subjects; Group-A live product and Group-B control. Group A consisted of 50-subjects that were provided with a total average daily intake of 11 grams of a commercial oral glucosamine and chondroitin(Glu/Chon) product (GLC® 5500) with the addition of 260 mg of a proprietary nutritional supplement blend including potassium citrate, licorice root extract, chlorella, and N-acetyl D-glucosamine, hereby referred to by the trademark name Actistatin®. Daily administration protocol required 5.63 grams of the composition BID with regular feedings (one full scoop of products in the morning and one full scoop in the evening of grain rations). Subjects in Control Group B were fed normal rations without the addition of any secondary nutritional supplements or placebo to establish and maintain control. Data included, pain frequency, as determined by lameness response to joint flexion. Lameness grade and range of motion (ROM) were collected at baseline, week-1, week-2, week-4, and at 4-week intervals thereafter (4, 8, 12, 16, 20, and 24 weeks). Blood and urine samples were collected and evaluated at baseline and at 4-week intervals thereafter (4, 8, 12, 16, 20, and 24 weeks). Synovial fluid samples were collected at baseline, week 4, and week 24. Data related to pain frequency, lameness, joint flexion, ROM, as well as gross and hematological serum, and urine biochemical data, were compared using ANOVA and pair-wise t-testing between treatment groups to identify effects and safety of treatment. General equine physical examinations were conducted, including hematological and biochemical evaluations using a patented computer-based software program, Optimal Equine Analysis™ (OEA) that tests blood and urine samples for 39 hematological, biochemical and histological indicators. Blood samples were assayed using HPLC and DMMB to determine serum bioavailability for glucosamine and chondroitin sulfate as measured in total Gly-

cosaminoglycan (GAG) content. Bioavailability data from both groups was compared to baseline and that of equivalent dosing of GLC® 5500 without Actistatin® using standard statistic mathematical calculations to identify effects of treatment. Synovial fluids were taken from the tarsus of 40 equines: (30 in Group A, and 10 in Group B) and tested using 10 specific biochemical markers including: total protein, Hyaluronan, Albumin, Glucose, Sigma, LDH, Sibley-Lehiniger, Sigma-Frankelunits (units/ml and Sigma-Frankelunits /ml) and Glutamic pyruvic transaminaseactivity. Cytologic and mucinous precipitate quality. Testing was compared using standard deviation to individual baseline to identify effects of treatment.

Results

Statistical data analysis of equine joint pain frequency as scored using AAEP lameness scores. Flexion and range of motion indicated significant differences between groups: Group A - joint pain frequency was significantly reduced from 4.520 to 0.125 as compared to a statistically insignificant reduction of 4.690 to 4.114 in Group B. AAEP Lameness Scores were significantly reduced from an average score of 2.190 on week 1 to <.5 at week-24 for Group A, while Group B remained statistically unchanged (p=<.002). Group A also showed improvement in Flexion Scores from 2.276 on week 1 to 0.148 at week-24 (p=<.002), while Group B remained statistically unchanged. ROM scores showed significant improvement in Group A from 5.592 on week 1 to 9.844 at week 24, (p=<.002), while Group B remained mostly unchanged. Statistical data analysis of hematological and biochemical data indicated significant differences between groups: The measurements that showed significant in-group change from baseline to finish were limited to Group A. Improvement in urine ORP $p<0.01$, ph $p<0.01$ and ST $p<0.01$ were all observed in Group A as compared to Control Group B. Blood serum analysis indicated improved ORP<0.01 and ph, $p<0.01$. Changes in blood ST were not significant as compared to baseline. Bioavailability as determined by serum analysis for Glu/Chon proved to be >60% in the Actistatin® Group A, compared to <30% detectable Glu/Chon in GLC® 5500 without Actistatin® and control. The relevant value ($p<0.02$) showed significant passage of the active ingredients through the digestive system and availability in serum. Significant improvement in all parameters of synovial fluid quality, volume, gross appearance, relative viscosity, and mucinous precipitate quality were observed $p<0.006$. Most notably, a steady increase in available hyaluronan was detected base=0.5N, week 4=1.5N (2.0-4.0mg/ml.), week 24=3.8 N(2.0-4.0mg/ml.)

Conclusion and Clinical Relevance

Oral supplementation of Glu/Chon as found in the commercial product GLC® 5500, with the addition of the facilitating agent Actistatin® to the equine subjects in this study, showed improvement in several specific areas of testing… pain frequency as determined by lameness scores, flexion, and ROM testing improved significantly in live product Group A as compared to control Group B. Biochemical and hematological improvement was observed using established wellness parameters in Group A as compared to Group B. Significant increases in serum bioavailability of exogenous Glu/Chon were observed in Group A as compared to either baseline or Glu/Chon (GLC® 5500) without Actistatin®. Significant improvements were also observed in multiple synovial fluid markers in Group A as compared to Group B. In addition no adverse side effects of supplementation were recorded or reported throughout the span of the study.

The resultant reduction in lameness scores and improvement in flexion and ROM indicate efficacy for the commercial product GLC® 5500 with Actistatin® as an effective oral treatment for equine lameness. Significant wellness changes in overall ORP and pH were seen at the cellular level indicating that supplemental Glu/Chon (GLC® 5500)

with the addition of Actistatin® may help to improve the production and utilization of energy, nutrient uptake and minimization of damaging free radicals in the horse. The addition of Actistatin® to supplemental Glu/Chon (GLC® 5500) to the daily feed regimen of the horses in control group A showed significant improvement in absorption and delivery to serum of both glucosamine and chondroitin. Bioavailability was determined using accepted HPLC and DMMB serum analysis as providing >60% of the total milligrams of the embodiment delivered to serum as compared to baseline and Glu/Chon (GLC® 5500) without Actistatin®. The resulting increase from 0, ≤30% and ≥60% indicate a significant improvement in Glu/Chon survivability through the digestive system with the addition of Actistatin®. The resultant increase in serum Glu/Chon content demonstrates Actistatin® to be an effective facilitator for improved oral GAG delivery in the horse. Significant improvements in synovial fluid markers throughout the study indicate that the addition of Glu/Chon (GLC® 5500) with Actistatin® to the subject's diet has a direct effect on synovial tissues. Specific increases in hyaluronan count, volume, and relative viscosity suggest that this protocol may provide a viable, non-invasive method for improving the health of equine synovial tissues.

The combined findings indicate that Actistatin® is an improvement to prior Glu/Chon delivery methods by delivering significantly higher levels of Glu/Chon to serum than previously observed. The findings suggest that Glu/Chon (GLC® 5500) with Actistatin® is safe for use in horses and may act to moderate the severity and progression of factors contributing to equine joint lameness through multiple biochemical pathways. It is also indicated that Glu/Chon (GLC® 5500) with Actistatin® provides an effective non-invasive method for improving synovial fluid concentrations and hyaluronan volume. The objective findings indicate that increases in serum Glu/Chon to the extent observed in this study may have a direct disease-modifying effect on multiple biochemical and physiological mechanisms involved in equine lameness and may prove to be an effective treatment for such conditions.

INTRODUCTION

Equine lameness is one of the primary contributors to reduced volume and frequency of training in equine athleticism. Horses in training are subject to musculoskeletal stresses that may contribute to tendon, muscle, and osteoarticular injury. Many equines involved in the performance horse industry are often subject to continued training protocol even when signs of injury are evident. This continued exposure to dynamic and concussive forces is one of the primary contributors to degenerative joint disease in performance and pleasure horses.[1] Progressive degenerative changes in bone and articular cartilage are categorized as osteoarthritis, and have been determined to contribute to 60% of clinically diagnosed cases of lameness.[2] The majority of articular cartilage is comprised of collagen, proteoglycans, and primary manufacturing cells called chondrocytes that are responsible for cellular matrix maintenance and hylauronan production. Degeneration of the cartilage occurs as physical and biochemical changes within the matrix prevent the normal repair and maintenance of the tissues, resulting in accelerated cellular death and cartilage thinning. Symptomatic pain responses to cartilage thinning are manifested in tactile signs of equine lameness, reduced flexion and limited range of motion.

Many therapies have been successfully implemented to treat and manage this condition. Invasive methods that include intra-articular medications (corticosteroids, hyaluronic acid, and polysulfated GAG's), as well as protein and gene expression products, help mediate and suppress symptoms, regulate inflammatory pathways, and provide integral regenerative components to help treat equine lameness.[3-7] While intra-articular injections are statistically considered safe and effective, the cost is quite great, and there are inherent risks associated with these invasive therapies, including septicemia and

nerve damage.[8] For these reasons, the introduction of oral alternatives that work within similar therapeutic pathways have gained popularity.

Oral nutritional supplements termed "nutraceuticals" are natural dietary supplements that supply necessary precursors, building blocks, and catalysts that modify equine lameness by influencing cartilage biosynthesis, mediating inflammatory responses and slowing cartilage degradation.[9-11] Two of the most well known and studied nutraceuticals for equine musculoskeletal related lameness are glucosamine and chondroitin sulfate (Glu/Chon). There have been several in-vitro studies that demonstrate the beneficial effects of Glu/Chon on equine cartilage explants in stimulating chondrocytes to repair cartilage, produce hylauronan, and reduce degenerative enzymes.[12-16] Likewise, many in-vivo trials demonstrate the oral application of Glu/Chon have beneficial effects on equine lameness related to osteoarthritis. Numerous veterinary and industry studies have validated the efficacy of this application through radiological monitoring of the disease process and symptomatic relief of numerous clinical indicators including lameness, stride length, joint effusion, and heat.[17-26] Favorable outcomes within the body of work has resulted in widespread parental use of Glu/Chon for the treatment of equine lameness as well as their use as a preventative in working horses within the equine populace.

Pure forms of glucosamine and chondroitin are recommended and used by the medical community as therapies for equine lameness as related to osteoarthritis. However, until recently, information on the absorption, serum pharmacokinetics, and synovial fluid concentrations of these products when taken orally has been limited. Within the last few years, multiple species trials have validated the bioavailability of oral applications of Glu/Chon; their ability to enter serum concentrations;[27,28,29] as well as delivery to synovial fluids.[30,31,32] In all the cited cases, the metabolic loss of Glu/Chon through the digestive systems proves significant, although varied, depending on the species of animal studied. It is observed that relatively small quantities of the ingested compounds are subsequently detectable in serum: 19% in rats, 12% in dogs, and as little as 5% in horses.

It is further evident that of the detectable serum Glu/Chon, a relatively small amount ≤10% is incorporated and into synovial fluids. This metabolic loss is best illustrated by comparing serum analysis with previous radio marker testing of Glu/Chon, in which systemic absorption rates of up to 90% for glucosamine,[33,34,35] and up to 70% for chondroitin sulfates[36] have been recorded. The contrast in the findings clearly demonstrates significant metabolic loss of orally administered Glu/Chon by gastrointestinal tissues and processes prior to delivery to serum or synovial fluid concentrations.

This metabolic loss is further evident in the most recent evaluation of oral glucosamine bioavailability in the horse performed at the University of Montreal.[37] The study demonstrated significant differences in the bioavailability and delivery to serum of oral glucosamine in direct correlation to both the solubility of the exogenous salt form and complexity of molecular bond. Within the study, glucosamine sulfate outperformed glucosamine hydrochloride by delivering 9.4% of the ingested compound to serum as compared to 6.1% for the hydrochloride form, (a 35% improvement). It was again demonstrated that of the available glucosamine delivered to serum, ≤10% was detectable in synovial fluids. As a result, glucosamine sulfate delivered 39% more glucosamine to synovial fluid concentrations than the hydrochloride form.

The findings proved to be consistent with the same10 to 1 ratio between available serum levels of glucosamine and resultant passage through to synovial fluids found in previous studies.[38] The therapeutic implications are also significant as this study demonstrates a 6-fold increase in synovial glucosamine concentrations over baseline

levels, validating oral glucosamine as a possible disease modifying agent. Similar data is available for orally administered chondroitin sulfates in the horse, demonstrating molecular weight to be the primary determining factor in passage to serum concentrations and availability for synovial incorporation.[39] It is therefore vital for any orally administered Glu/Chon supplement to provide sufficient quantity of the medium to serum for it to be considered of therapeutic value.

The Glu/Chon chosen for the study is a patented combination of glucosamine and chondroitin that has been tested in a non-fasting oral administrationin order to provide higher levels of Glu/Chon to serum in bioequivalency than other forms tested by this laboratory. Serum testing using HPLC and DMMB, indicates that the combination of all four orally accepted glucosamine forms found in the commercial product GLC® 5500 including glucosamine HCl, glucosamine sulfate NaCl, glucosamine sulfate 2KCl, and N-Acetyl D-Glucosamine along with low molecular weight chondroitin sulfate, ascorbate, and manganese delivered ≤30% of the embodiment to serum in the horse. Previous testing by this laboratory showed similar findings with ≥43% of the same orally administered compound being delivered to serum in canine.40 According to the company, documented improvement in Glu/Chon delivery to serum as compared to isolate forms may be attributed to the complementary ionic bonds of the salt carriers as well as the addition of N-Acetyl D-Glucosamine. Previous clinical studies indicate that nearly 100% of ingested N-Acetyl D-Glucosamine is metabolized in stomach, duodenum, and small intestine.41-44 This is theorized to satisfy metabolic glycoprotein necessities of the epithelium and may be one of the mechanism that allows for greater Glu/Chon passage to serum for this embodiment. This combination of Glu/Chon has also been well studied for its long term therapeutic value in treating equine lameness. An independent, 8-year study demonstrated the addition of GLC® 5500 to the diet of performance horses significantly reducing clinical lameness, and reduced the need for intra-articular injections as related to equine osteoarthritis.45 For these reasons, the product GLC® 5500 was chosen for incorporation in the current study.

The current study was designed to evaluate the biological effect of the nutritional supplement Actistatin® on Glu/Chon therapy in the horse. According to the manufacturer, Actistatin® works as a facilitating agent to improve Glu/Chon and other target protein fractions (TPF) survivability through the digestive system, resulting in higher percentages of Glu/Chon delivered to the bloodstream than otherwise possible. It is reported that Actistatin® potentiates the therapeutic effects of supplemental Glu/Chon by improving the zeta potential for Glu/Chon, improving the bodies uptake, intracellular absorption, utilization, and overall bioavailability of exogenous Glu/Chon and other TPF.

According to the patent, the embodiment works through multiple modes of action to achieve this effect through direct and indirect interaction with primary cellular and digestive sites including stomach, duodenum, and small intestine. While the methods of action are not the focus of this investigation it is helpful to have a basic understanding of how improvement in Glu/Chon delivery to serum may be achieved.

Actistatin® is reported to work through multiple specific pathologies to improve Glu/Chon survivabilityincluding:

- Satisfying specific gastric cellular metabolic energy requirements for glycosaminoglycan metabolism; reducing parietal, goblet and epithelial mitochondria metabolic requirements
- Prostoglandin production
- Facilitating physiological and biochemical changes in cell signaling, hormone secretion, digestive juice secretion, and stabilizing digestive pH.

Actistatin® is also reported to develop synergistic relationships between the embodiments that protect

Glu/Chonintegrity, prevent oxidation, slow proteolysis, and balance biochemical relationships necessary for optimal delivery of TPF through the digestive system and overall utilization of Glu/Chon systemically.

The structure of this multi-center study is four-tiered, and designed to evaluate the therapeutic potential of the combination of Actistatin® and an established Glu/Chon supplement. The criteria for determining therapeutic potential within this body of work includes:

- Monitoring and comparing changes in lameness evaluations, reduction of tactile pain frequency, changes in joint flexion, and changes in range of motion between Active Group A and Control Group B.
- Monitoring and comparing biochemical and hematological changes using established wellness parameters for overall safety Active Group A and Control Group B.
- Monitoring and comparing the percentage of detectable serum Glu/Chon found in the bloodstream after oral supplementation of active ingredients in Group A, as compared to baseline and previous testing.
- Monitoring and comparing multiple established synovial fluid markers in Active Group A as compared to Group B.

MATERIALS AND METHODS:

Study Outline, Animals, and Supplement Regimen

This was a 6-month, randomized, multi-center equine study evaluating lameness, biochemical safety, Glu/Chon oral bioavailability and synovial fluid concentrations. This 75-subject study used equines drawn from a large population of high performance working animals diagnosed with clinical joint pain and resultant symptomatic lameness. All subjects were categorized as chronic joint pain/limited ROM sufferers with an AAEP Lameness score of 2 or higher. The subjects were randomized into two groups of subjects. The total group was divided into 50 subjects in Active Group A and 25 subjects in Control Group B. The 50 subjects in Active Group A were provided supplemental glucosamine and chondroitin in the commercial product GLC® 5500 in conjunction with the nutritional supplement Actistatin® Subjects in Group A received the supplements both in the morning and in the evening with normal grain rations. The 25 subjects remained the control group. In order to establish and maintain control, they were fed normal rations without the addition of any secondary nutritional supplements. As they are equines and no placebo effect is present, they were not given a placebo product. No other intramuscular, intravenous, or topical therapies directed at joint pain were administered during the course of the study.

Supplement Dosing

Equines in Active Group A were administered the Glu/Chon product GLC® 5500 at the rate of 1 gms per 100 pounds of body weight per day. This dose was divided equally between morning and evening feedings and top dressed on normal rations. The average horse in the study received one scoop of GLC® 5500 twice per day. Each scoop contained 1,200 mg glucosamine sulfate NaCl, 1,200 mg glucosamine sulfate 2KCl, 1,200 mg glucosamine HCl, 300 mg N-Acetyl D-Glucosamine, and 1,200 mg chondroitin sulfate, 300 mg ascorbate, and 100 mg manganese for an average total of 5.5 gms of active ingredient BID. The average horse in this trial received 11 gms of GLC® 5500 per day. In addition, each horse received 130 mg of the nutritional supplement Actistatin® BID, mixed with each administration of GLC® 5500. This dose was given twice a day for the duration of the study, providing a total of 260 mg of Actistatin® per day. Actistatin® consists of a proprietary mixture of potassium citrate, licorice root extract, chlorella, and N-acetyl D-glucosamine. Owners were instructed to add one full scoop of the combined product in the morning and one full scoop of the combined product in the evening and to increase or decrease the dose based on the manufacturers recommendations. Equines

in Control Group B were not given any supplements for the duration of the study.

Inclusion Criteria

1. Subjects whose owners have signed a written informed consent consistent with required guidelines prior to participation in the trial.
2. Subjects 1 year of age or older, mare/gelding/stallion.
3. Joint pain/stiffness/limiting ROM symptoms must have been presented for a minimum of the last consecutive 6 months at a frequency of at least 10 times each month.
4. Prior to this study subjects must have been diagnosed with chronic lameness and exhibiting joint pain and/ or inflammation based on typical symptomology, ie, associated pain in joints/pasterns/hocks/fetlocks/shoulders, difficulty standing or moving, or changing positions or reductions in ROM as determined by veterinarian.
5. Subjects who were able to follow the protocol as designed by Fenestra Research labs.
6. Subjects who are currently in performance training in either reining/jumping/dressage/cutting.
7. Subjects who are exercised for a minimum of 1 hour daily 4-times weekly.
8. Generally good health.

Exclusion Criteria

1. History of serious diseases or illness diagnosed at this time.
2. Known moderate to severe renal insufficiency.
3. Recent history (<6 months prior to Visit 1) of myocardial infarction.
4. Subjects with a history of cancer within the last 5 years.
5. Subjects with treated viruses, bacteria, or any communicable diseases within the last 6 months.
6. Subjects who are not currently in performance training or active showing.
7. Subjects who are currently in a pulmonary rehabilitation program or who have completed a pulmonary rehabilitation program in the 12 weeks prior to the screening visit (Visit 1).
8. Subjects currently prescribed diuretic medications, cardiac stimulants, or any other prescribed or non-prescribed medication that may, in the opinion of the Fenestra Research staff, alter testing results.
9. Use of opiate analgesics prescribed or otherwise obtained for any treatment reason including joint pain, limited movement, show performance, or to increase comfort levels.
10. Mares who are pregnant, lactating, or nursing or who may become pregnant during the course of the study.
11. Subjects with uncontrolled ulcers.
12. Subjects with any condition not previously named that, in the opinion of the investigators or intake staff, would jeopardize the safety of the subject or affect the validity of the data collected in this study.

Evaluations

Following an initial screening at Visit 1 (week 0), subjects entered a 1-week baseline period (subjects were not to be given any OTC's, prescription drugs, or natural products for the remainder of the study other than the test product). Subjects who met all inclusion criteria and none of the exclusion criteria during the intake at Visit 2 (week 1) were then randomized into either the active product Group A, or Control Group B. Participants were provided instructions along with a protocol describing daily dosing to follow for the duration of the study as well as a patient log for recording administration of product, date, time, and daily observations. Site evaluations and data used in the study were conducted by the author and collected on week 1, week 4, week 8, week 12, week 16, week 20, and week24 from both Group A and Group B. Site evaluations included complete physical examinations, lameness evaluations, flex test results, range of motion, and joint pain frequency/intensity

evaluations for both groups. Blood, saliva, and urine samples were collected from both Group A and Group B on day 1 to establish baseline, and then again at week 2, week 4, week 8, week 12, week 16, week 20, and week 24. Synovial fluid samples were drawn from 37 horses within group A and 3 horses within Group B that chose to participate in the synovial fluid monitoring portion of the trial. Synovial fluid samples were collected at week 1, week 4 and week 24 respectively. All clinical evaluations adhered to strict study procedures, and at each site evaluation the study's protocol was again gone over with each subject's owner, trainer, or veterinarian on an individual basis. Final evaluations of test subjects were completed on visit 8 (week 24) of this study.

Physical Examination

All subjects were given a complete physical examination in order qualify as being in general good health. Overall condition including visual observation of the subject's body and limbs for conformation, symmetry, swellings, stance, and other musculoskeletal abnormalities were evaluated. Additionally, the condition of the subject's haircoat/skin and eyes, as well as neurologic assessment, abdominal/GI system, lymph system, behavior, and attitude, along with temperature, respiratory rate, and heart rate/rhythm, were recorded.

Lameness Evaluation

In order to stay within the standards of the industry, lameness evaluations were conducted using the accepted protocol found in the publication: Musculoskeletal Causes of Lameness and Poor Performance in Horses. Each horse was evaluated at a walk to assess and evaluate the gait abnormality or lameness at baseline on week 1, week 4, week 8, week 12, week 16, week 20, and week 24. The more lame horses exhibited a more noticeable lameness at a walk. The horse was then evaluated at a trot, which is the optimal gait for detection of lameness. The horse was observed from the front and back while moving to and from the examiner, as well as from both sides. The amount of weight bearing, the length of stride, the flight and landing of the feet, and the carriage of the head and neck were evaluated. This was further facilitated by careful palpation of the limb(s) both with the horse bearing weight on the leg and with the leg held off the ground. Palpation for fluctuant swellings (effusion in joints, tendon sheaths, and/or bursa, subcutaneous swellings, etc.), firm swellings (such as bony exostoses, mineralized, or fibrotic soft tissue, etc.), heat, and pain on manipulation were also assessed. Effusion in a joint or tendon sheath typically suggests an inflammatory response such as synovitis or tenosynovitis associated with trauma, infection, or other type of condition. Flexing and extending the joint, and comparing this with findings on the opposite limb, is used to assess range of motion (ROM). Reductions in comparative mobility, unwillingness to perform testing procedure during manipulation of the joints or palpation of tendons, ligaments, or bone may suggest involvement of those structures.

Hoof testers were applied to different areas along the entire circumference of the foot to assess sensitivity to pressure. A positive response to hoof tester suggesting asubsolar abscess, bruising, laminitis (founder), a fracture of the third phalanx (coffin bone), or navicular bone, navicular disease, or other abnormalities of soft tissue and bony structures within the hoof were reasons for exclusion. During the examination, conformational faults, angular or flexural limb deformities or abnormalities of the horse's stance are noted.

Degree or grade of lameness was interpreted using the standardized grading scale, developed and adopted by the American Association of Equine Practitioners as listed below:

Grade 0: Lameness not perceptible under any circumstances.

Grade 1: Lameness is difficult to observe, and is not consistently apparent, regardless of circumstances (weight carrying, circling, inclines, hard surface, etc).

Grade 2: Lameness is difficult to observe at

a walk or when trotting in a straight line, but consistently apparent under certain circumstances (weight carrying, circling, inclines, hard surface, etc).

Grade 3: Lameness is consistently observable at a trot under all circumstances.

Grade 4: Lameness is obvious at a walk.

Grade 5: Lameness produces minimal weight-bearing in motion and/or at rest or a complete inability to move.

Once the lameness has been graded based upon baseline walking and jogging, then the horse's limbs may be stressed by lunging the horse or jogging it in circles to both the left and right, flexing individual joints (usually for 60 seconds), re-applying hoof testers, or other perturbations to try to exacerbate the lameness in order to help localize the source of the pain (lameness).

Flexion Test

Standard flexion tests were performed at baseline on week 1, week 4, week 8, week 12, week 16, week 20, and week 24 and recorded using a descending, 4-point semi-quantative scale:

- 0= walks or trots off with no detectable signs of lameness or restrictions.
- 3= Trots off seven strides or more with detectable signs of lameness and restriction.

To perform this exam, the horse's leg is elevated, bent at a specific joint, held for approximately 60 seconds, then returned to the ground. We then observe the movement of the horse as it is jogged down a hard level surface. Observations are made and recorded for at least 1 minute. Each joint including stifle, knee, hock, etc was evaluated in this matter at each visit. There was no influence of weight, height, or ROM on the score of the flexion test as in sound horses.

Range of Motion (ROM) Test

Range of Motion Tests were performed at baseline on week 1, week 4, week 8, week 12, week 16, week 20, and week 24 using an ascending 10-point semi-quantitative scale...1= Severe restrictions in limb flexion angles and 10= No abnormal restrictions in limb flexion angles. The maximum flexion angles of multiple joints were measured and the range of motion (ROM) of the joints were calculated and scored using comparative limb values. Within this study, it should be noted that there is no relationship between ROM and the outcome of the flexion test.

Pain Evaluation

Joint pain was assessed using the combined data collected using the widely accepted procedures of flex testing ROM testing and lameness scores. Onset and disappearance times were recorded using a standard in-take charting procedure. Daily records were kept by each owner and/or trainer and reviewed by the Fenestra Research staff interviewer during on-site follow-ups on week 1, week 2, week 4, week 8, week 12, week 16, week 20, and week 24 as well.

Blood Draw Procedure

Blood samples were collected from each horse 45-60 minutes after ingestion of feed rations containing supplement in Group A and non-supplemented feed rations in Group B. Each horse had blood draws done at the Baseline, week 2, week 4, week 8, week 12, week 16, week 20, and week 24. Tools used to collect samples included 18-20 gauge needle, needle holder, and Vacutainer tube. All samples were collected from the contralateral jugular vein and collected in red tubes. Each blood draw was collected as three separate vials of blood from the same site at the same time. Each tube contained 3cc's of blood at the time of collection. The total average on each horse is the overall average of these three samples. Blood samples were drawn on testing and re-testing days through out this study. Blood samples were drawn at approximately the same time each test day, labeled, and recorded for testing.

Blood Draw Testing

All blood samples were collected and refrigerated at 42 degrees within 15-minutes of being drawn, and were warmed to 68 degrees prior to testing. In addition, all samples were tested within 10 hours of collection. Serum plasma was obtained by

centrifugation at 1,500 rpm at 74 F for 15 minutes, and was stored at 70 F to 75 F until analyzed. Three separate analytical methods were used to process the samples collected. Optimal Equine Wellness (OEW) Analysis was carried out as follows:

- Whole blood samples were analyzed using established panels without secondary preparation.
- Serum Glucosamine Analysis: Plasma was obtained by centrifugation prior to HPLC.
- Serum Chondroitin Analysis: Plasma was obtained by centrifugation prior to DMMB reagent testing.

Glucosamine Testing

Blood samples were centrifuged and plasma samples extracted. Plasma glucosamine concentrations were determined using accepted guidelines and methods.47Specifically, phenylisothiocyanate-derivatization, reverse-phase high-performance liquid chromatography (HPLC) Analyses were performed with an internal standard (20 µl galactosamine [100 µmol/l]) added to 200 µl plasma. Proteins were precipitated with acetonitrile and centrifuged for 15 minutes for 1,500 rpm at 74 F. Supernatant was removed and mixed with 50 µl N-2-hydroxyethylpiperazine-N′-2-ethanesulfonate and 125 µl of 5% phenylisothiocyanate in acetonitrile. Derivatization reactions were completed in 10 minutes: solvent and unreacted phenylisothiocyanate were removed and reconstituted in 200 µl of 20% acetonitrile. HPLC separations were isocratically performed for 15 minutes at a flow rate of 0.7 ml/min. Glucosamine percentages were recorded and determination of glucosamine percentiles in blood were calculated against total blood volume, 100% ≤.029355ug/1ml each 3-cc sample was compared using .088066ug/ml glucosamine p=<0.002.

Chondroitin Testing

Blood samples were centrifuged and plasma samples extracted. Chondroitin sulfate levels in plasma were determined following ANVISA's validated guidelines. Percentages of chondroitin sulfate delivered to serum were established using the 1,9,dimethyl- dimethylene blue (DMMB) method for determining sulfated glycosaminoglycans (GAG) in plasma. Liquid extraction (using papain in an aphosphate:cysteine buffer) and spectrophotometry quantification were applied. Samples were incubated and agitated for 16 hours. After incubation, 500 L of NaCL, 4 M were added and agitated to derive supernatant. The supernatant was precipitated, vacuum-dried, and re-suspended in 100 mL of water. One milliliter of the DMMB reagent was added to the water-diluted samples, followed by a careful agitation. Reading was performed in a spectrophotometer at 525 nm, within 3 minutes counting from the addition of DMMB. The standard curve was performed using a chondroitin sulfate solution. Chondroitin absorbance values were recorded and determination of GAG percentiles in blood were calculated against total amount of GAG in each calibrator (.03522ug/ml). A linear equation was applied to straight- line absorbance to calculate the amount of GAG in each sample p=<0.002.

Optimal Equine Analysis

Whole blood samples and urine samples were warmed to room temperature and tested using Optimal Equine Analysis™ (OEA) protocol. Blood samples were tested using chemical hematology for all OEA data points. Urine samples were tested for all OEA data points. All of the data was then evaluated using the patented software based analytical system with the trademark name Optimal Equine Analysis.™ Multiple biochemical and physiological parameters that are representative of known healthy states were extracted, including pH, rH2-oxidation and reduction, R-resistivity, C-conductivity, Nitrate, Ammonia, Brix-refractometry, specific gravity, oxidative stress, cellular respiration, renal balance, hepatic balance, digestion, hydration, toxicity, adrenal balance, protein digestion, carbohydrate digestion, blood glucose balance, anabolic and catabolic status, and surface tension.. Objective testing procedures are the basis for this

analysis, so there is no subjective input from the tester. This analytical method indicates cellular imbalances based on established and theoretical ideals to evaluate health at the cellular level. The broad panel provides an objective overview of health status from baseline to study completion (full OEA methodology and analysis measurements attached). Due to the comprehensive nature of this testing method and that 39 specific tests were performed, this study will focus on three specific tests to determine statistical significance as compared to optimal levels: ORP (Oxidation-Reduction Potential), pH and ST (Surface Tension).

Synovial Fluid Draw

Synovial fluid collection occurred at Baseline, week 4, and week 24, the final day of this study. Analyses of synovial fluid was obtained by arthrocentesis from a total of 40 horses in the study. All Synovial fluid was excavated from the hocks. Sites for arthrocentesis were prepared aseptically. Synovial fluid (2 to 4 mL) was directly aspirated from the joints by use of a 20-gauge needle and syringe. Following aspiration of the fluid into a syringe, the fluid was transferred to both plain and EDTA Vacutainer tubes, refrigerated at 5°C, transferred to laboratory, and tested within 10 hours of collection. All standard arthrocentesis procedures were strictly adhered to. All horses were wrapped with clean bandages after procedure and monitored for infection after these procedures. No side effects and no lameness was reported or observed after testing on any of the horse.

Synovial Fluid Testing

Physical, biochemical, and cytologic properties of synovial fluid from the test subjects were evaluated at baseline, week 4, and week 24 the final day of this study. The volume and gross appearance of synovial fluid from all tarsal joints were recorded at the time of arthrocentesis. Volume of synovial fluid varied in direct proportion to individual tarsal joint size as would be expected. Gross appearance of tarsal synovial fluid was homogeneously pale yellow and free of flocculent debris. Samples did not clot at room temperature or when they were stored at 5°C. All standard procedures were performed to determine relative viscosity, mean degree of hyaluronic acid polymerization, protein concentration, and volume of synovial fluid.

- Protein Concentration: The differential protein fractions in synovial fluid were evaluated using paper electrophoresis following treatment of the sample with hyaluronidase.
- Viscosity and Hyaluronan Polymerization: The viscosity of the synovial fluid is directly related to the hyaluronan content and it is a measure of the quantity and quality or degree of polymerization of the hyaluronan. The viscosity of the synovial fluid was measured by rotation viscometry with four different shear rates.

  Mucinous precipitate quality was evaluated by adding 0.5 ml of synovial fluid to 2 ml of 2% acetic acid and mixing it rapidly with a glass rod. After mucin clot formation the samples were centrifuged at room temperature for 15 minutes. The supernatant was carefully decanted and dialysed to remove acetate prior to HPLC evaluation.
- Cytologic Examination: Cells were collected in EDTA vials and preserved in them as they offer the highest standard of accuracy. Total white blood cell counts were performed on synovial fluid using hemocytometers. A physiologic saline diluent was used and not the usual white cell diluent containing acetic acid, for the latter precipitates the hyaluronate-protein complex. Red blood cells were preferentially lysed by hypotonic saline. Smears for differential cell counts were prepared in the standard way for peripheral blood smears. The sample was centrifuged and the sediment resuspended in 0.5 ml of supernatant. A smear was subsequently completed. All smears were air dried and stained with Wright's stain as is the standard. Total WBC count

was determined via refractometry and an automated cell counter.

• Biochemical Markers: Biochemical markers for identification of proteoglycan fragments and glycosaminoglycans (GAGs) and synovial fluid include the dimethyl methylene blue (DMMB) assay using conjugation of 1.9-dimethylmethylene blue to GAGs and comparing the spectrophotometric absorption with that of a chondroitin sulfate standard. The DMMB assay identifies all GAGs present in synovial fluid regardless of origin.

Daily and Exercise Routine

Daily routines (feeding schedule, turnout times) remained the same as before the study.

No suggestion or injunction for or against any specific activity related to these sports was given or implied to trainers. This activity-related decision was completely at the discretion of the trainer. There was no requirement that any given horse be exercised on any given day. This too was completely at the discretion of the trainer/owner.

Compliance to the protocol was monitored and maintained through bi-weekly visits and phone calls with Fenestra Labs Clinical Studies personnel in addition to the clinical evaluations.

Clinical Data Analysis

Statistical Methods: The group responses were tabulated as the number, or frequency, of joint pain/discomfort over the 6-month test period, and the intensity of these attacks were averaged for an overall intensity score for the test period. All averages are reported as mean ± standard error. The statistical model is a two factor repeated measures experiment with a grouping factor and one repeated factor (Normal, Test). Data for frequency and intensity were analyzed separately at baseline, comparing the normal discomfort experience among groups, and over the 6-month experience, comparing the test discomfort experience among groups using a One-Way Analysis of Variance (ANOVA). The comparison of the test experience to the normal experience among groups was compared using the appropriate repeated measures ANOVA.

All overall comparisons among groups were followed by pair-wise Student t-tests between groups. To assess the changes between the normal and test experience the difference between normal and test measures was compared in a One-Way ANOVA followed by pair-wise t-tests. The frequency and intensity responses by day over the 6 months of the study were tabulated for each group and fit separately to an exponential decay model with a common intercept and different slopes for each group using the model Response=exp(a-(b1*Dum1 + b2*Dum2 + b3)*Day)). This model expresses the decay constants b1 and b2 as deviations from b3 by using the dummy variable method where Dum1=1 for the Placebo group and Dum1=0 otherwise, and Dum2=1 for the Live Product group and Dum2=0 otherwise. If the statistical tests for b1 and b2 are different from zero, this indicates that the rate of decay in the control and live product group. In order to express the relative change over time from Day 1, the data were tabulated as a percent of Day 1 values and fit to the exponential decay model.

RESULTS

Lameness Evaluation

Statistical data analysis of joint pain frequency, lameness scores, flexion, and range of motion indicated significant differences between groups A and B: Joint Pain Fre-

Table 1

	Normal	Test	Difference
Control	11.0 ±0.81	10.7 ± 0.58	0.24 ± 0.51
Live Product	12.6 ± 0.93	10.8 ± 0.65	1.8 ± 0.62

quency: The statistical analyses support the following discussion of the results. The overall joint pain frequency responses as compared to pre-test frequency by group are shown in Table 1.

Following the study, the average number of attacks in the Live product group was significantly lower than that of the Control group ($p<0.01$) and lower than that of the pre-test normal frequencies of the Live group (<0.01). The relationship between normal (pre-test) frequencies of onset of pain within the joints while on study is shown in Figure 1. It will be noted that the frequency of joint pain attacks in the Control group was significantly correlated with pre-test normal frequencies ($r= 0.97$, $p < 0.01$) and in the Live Product group ($r= 0.97$, $p < 0.001$).

By the Analysis of Variance, the normal (pre-test) intensity scores were all significantly different from each other, with the Live Product group having significantly higher intensity at baseline than did the Control group. The intensity of painful attacks during the time on study was significantly lower in the Live Product group than the control group (see study conclusions for this data).

Statistical comparison of Joint Pain Frequency, lameness scores, flexion and range of motion as recorded on day one-baseline, on week-2, week-4, week-8, week-12, week-16, week-20, and week-24 showed significant improvement in Group A as compared to Group B. Statistical analysis demonstrates the following changes in figures 1 through 4.

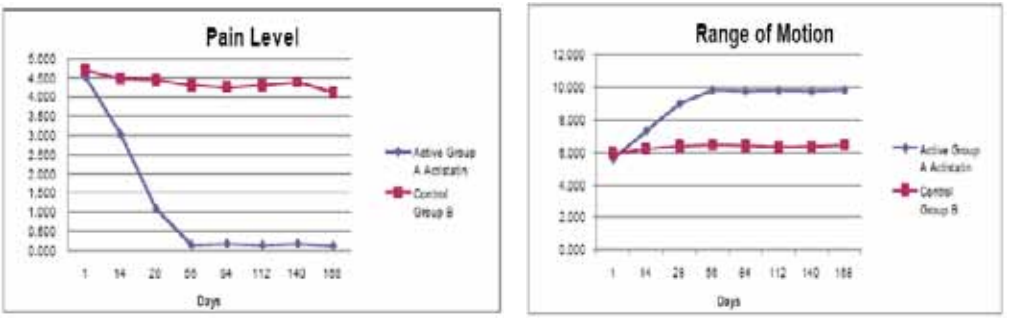
Optimal Equine Analysis Evaluation

Statistical data analysis of histological and biochemical data indicated significant differences between groups; the measurements that showed significant in-group change from baseline to finish were limited to Group A. Improvement in urine ORP $p<0.01$, ph $p<0.01$, and ST $p<0.01$ were all observed in Group A as compared to Control Group B. Blood serum analysis indicated improved ORP<0.01 and ph, $p<0.01$ changes in blood ST were not significant as compared to baseline. Certain physiologic parameters indicative of various states of electrolyte imbalances and cellular pH were measured during this study using OEA apparatus and calculation algorithms. OEA apparatus and calculation algorithms are proprietary and were developed by Fenestra Research Labs. All measurements were taken at baseline and at 4-week intervals thereafter. There was no statistically significant change in any parameter measured for the control group. The data listed below is the average overall change for subjects in the live product group. Each sample was drawn from each subject using standard

Figure 1

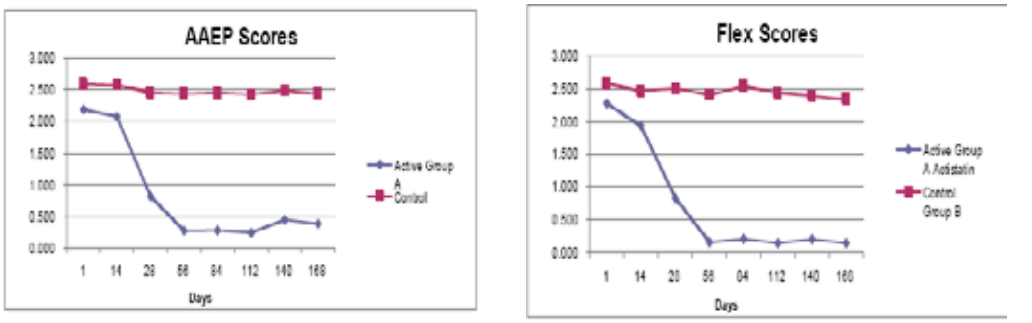
Actistatin Group A - 50 Horses						Control Group B - 25 Horses				
DAY	PAIN LEVEL	ROM	AAEP	FLEX		DAY	PAIN LEVEL	ROM	AAEP	FLEX
1	4.520	5.592	2.190	2.276		1	4.690	5.929	2.603	2.579
14	3.025	7.339	2.075	1.929		14	4.468	6.198	2.579	2.464
28	1.080	9.024	0.817	0.832		28	4.437	6.365	2.460	2.508
56	0.141	9.828	0.275	0.156		56	4.306	6.462	2.437	2.405
84	0.172	9.781	0.278	0.204		84	4.246	6.413	2.458	2.539
112	0.141	9.813	0.242	0.149		112	4.302	6.325	2.433	2.433
140	0.172	9.781	0.444	0.203		140	4.381	6.342	2.491	2.389
168	0.125	9.844	0.382	0.148		168	4.114	6.436	2.439	2.333

Figure 2



Group A - Joint pain frequency was reduced from 4.520 on week 1 to 0.125 at week-24. (p=<.002)
Group B - Joint pain frequency remained relatively constant at 4.690 on week 1 to 4.114 week-24. (p=<.002)
Group A - ROM scores were improved from 5.592 on week-1 to 9.844 at week-24. (p=<.002)
Group B - ROM remained relatively constant at 5.929 on week-1 to 6.436 at week-24.(p=<.002)

Figure 3



Group A - AAEP Lameness Scores reduced from 2.190 on week 1 to 0.382 at week-24. (p=<.002)
Group B - AAEP Lameness Scores remained relatively constant at 2.603 on week 1 to 2.439 week-24. (p=<.002)
Group A - Flexion Scores were improved from 2.276 on week-1 to 0.148 at week-24. (p=<.002)
Group B - Flexion Scores remained relatively constant at 2.508 on week-1 to 2.333 at week-24.(p=<.002)

sample collection procedures. The measurements that showed significant in-group change from baseline to finish were limited to the Live Product Group and are shown in Table 2.

A significant change from baseline to finish in urinary sample testing for ORP, pH, and ST were seen in the Live Product group for this study. Also a significant change from baseline to finish in blood sample testing for ORP and pH were measured. The change seen in the blood samples ST was not a significant change compared to baseline.

Bioavailability Evaluation

Centrifuged plasma samples were subjected to HPLC and DMMB testing to determine total serum analysis for glucosamine and chondroitin. Analysis of the combined data indicate a bioavailability as delivery to serum for the combined embodiment for Glu/Chon plus Actistatin® to be ≥60% in the active Group A as calculated from established control. Direct comparison to combined bioavailability of GLC® 5500 ≤30% showed a significant increase (≥100%) in detectable Glu/Chon in the bloodstream. The relevant value ($p<0.002$) showed significant passage of the active ingredients through the digestive system and availability in serum.

Glucosamine percentages were recorded and determination of glucosamine percentiles in blood were calculated against total blood volume, 100% ≤.029355ug/1ml

each 3-cc sample was established using .088066ug/ml as the standard for 100% delivery of glucosamine to serum. p=<0.002. Cromatogram spikes recorded at 8.25-8.30 min and 9.15-9.20min for glucosamine reflect serum glucosamine levels of 63% (.00548ug/ml) for the Glu/Chon product GLC® 5500 with Actistatin® in Active Group A as compared to 30% (.0264ug/ml) for equivalent Glu/Chon levels (GLC® 5500) without the additive. Resultant improvement in passage of glucosamine to serum with the additive Actistatin® should be considered significant.

Chondroitin absorbance values were recorded and determination of GAG percentiles in blood were calculated against total amount of GAG in each calibrator (.03522ug/ml). A linear equation was applied to straight line absorbance to calculate the amount of GAG in each sample p=<0.002. Absorbance rates were monitored using a spectrophotometer, and results collected and recorded and added to calculations. Rates indicated detectable GAG levels of .02113ug/ml for Active Group A to be ≤60% of the embodiment delivered to serum as compared to calibration and ≤26% (.00915ug/ml) for equivalent GAG levels (GLC® 5500) recorded without the additive. Resultant improvement in passage of chondroitin as GAG content to serum with the additive Actistatin® should be considered significant.

Synovial Fluid Evaluation

The volume and gross appearance of synovial fluid from all tarsal joints were recorded at the time of arthrocentesis. Volume of synovial fluid varied in direct proportion to individual tarsal joint size as would be expected. Gross appearance of tarsal synovial fluid was homogeneously pale yellow and free of flocculent debris samples did not clot at room temperature or when they were stored at 5°C.

All standard procedures were performed to determine whether hyaluronan count, relative viscosity, mean degree of hyaluronic acid polymerization, protein concentration, volume of synovial fluid from each joint was affected by the oral ingestion of GLC® 5500 and Actistatin® in Active Group A as compared to Control Group B.

Significant improvement in all parameters of synovial fluid quality, volume, gross appearance, relative viscosity and mucinous precipitate quality were observed p<0.006. Most notably, a steady increase in available hyaluronan was detected base=0.5N, week 4=1.5N(2.0-4.0mg/ml.), week 24=3.8 N(2.0-4.0mg/ml.)

White and red blood cell count was tested at Baseline, week-4 and week-24

1. The average for overall for the 40-horses was on Baseline, 87 cells/mm3 respectively. Neutrophils, lymphocytes, and large mononuclear cells were

Table 2

Group	Test	*Diff of Means	Wilcoxon Paired Sample Test Significance
A	U-ORP	-11.5%	p<0.01
A	B-ORP	+10.6%	p<0.01
B	U-pH	+18.5%	p<0.01
C	B-pH	+15.9%	p<0.01
B	U- ST	-15.2%	p<0.01
C	B-ST	-6.8%	P<0.01

**% Change in means Base vs Finish*

observed, the percentage of neutrophils was approximately 6%.

2. The average for overall for the 40-horses was on week-4, 87 cells/mm3 respectively. Neutrophils, lymphocytes, and large mononuclear cells were observed, the percentage of neutrophils was approximately 8%.

3. The average for overall for the 40-horses was on week-24, 77 cells/mm3 respectively. Neutrophils, lymphocytes, and large mononuclear cells were observed, the percentage of neutrophils was approximately 7%.

No significant changes to white or red blood cells were apparent for this study.

Results of Examination of Physical

Volume (ml./joint) .....14 ml	.....	N(6.25 - 21)
Gross appear ance*.....14	.....	N-14
Relative viscosity (at 37 C.) 4.6	.....	N(1.94 - 6.99)
Mucinous precipitate quality**....3.77 ± 0.16		N-11(2-4) F-1

Properties of Tarsal Synovial Fluid

Volume (ml./joint) .....20 ml	.....	N(6.25 - 21)
Gross appear ance*.....14	.....	N-14
Relative viscosity (at 37 C.) 3.76	.....	N(1.94 - 6.99)
Mucinous precipitate quality**....3.24 ± 0.13		N-11(2-4) F-1

Synovial Mean±4 s.e.* P<0.006

Baseline

Week-24

CONCLUSION AND CLINICAL RELEVANCE

The use of glucosamine and chondroitin for the treatment of equine lameness has been widely accepted and implemented over the last 20 years. The development of this study was multi-tiered to evaluate whether bioavailability of these ingredients Glu/Chon as found in the commercial product, GLC® 5500, could be improved with the addition of a facilitating agent (Actistatin®).Whether the changes in bioavailability produced physiological changes in relation to equine lameness. Within the scope of this study, it was demonstrated that the oral administration of GLC® 5500 and Actistatin® to the diet of equine subjects in this study did in fact demonstrate multiple modalities for improving equine lameness. The subjects in this study showed improvement in four specific areas of testing.

Pain frequency as determined by lameness scores, flexion, and range of motion testing improved significantly in live product Group A as compared to control Group B. Group A joint pain's frequency was significantly reduced from 4.520 to 0.125 as compared to a statistically insignificant reduction of 4.690 to 4.114 in Group B. AAEP Lameness Scores were significantly reduced from an average score of 2.190 on week-1 to <.5 at week-24 for Group A while Group B remained statistically unchanged, (p=<.002). Group A also showed improvement in Flexion Scores from 2.276 on week 1 to 0.148 at week 24, (p=<.002) while Group B remained statistically unchanged. ROM scores showed significant improvement in Group A from 5.592 on week 1 to 9.844 at week 24, (p=<.002) while Group B remained mostly unchanged. The resultant reduction in joint pain frequency, lameness scores and improvement in flexion and range of motion indicate efficacy for the commercial product GLC® 5500 with Actistatin® as an effective oral treatment for equine lameness.

Biochemical and hematological improvement was observed using established wellness parameters in Group A as Compared to Group B. Significant wellness changes in overall ORP and PH were seen at the cellular level, indicating the product GLC® 5500 with the addition of Actistatin® may help to improve the production and utilization of energy, nutrient uptake, and minimization of damaging free radicals in the horse. The addition of GLC® 5500 with Actistatin® to the daily feed regimen of the horses in control group A resulted in significant increases in serum bioavailability of exogenous glucosamine and chondroitin as compared to baseline, Glu/Chon without Actistatin® and Control Group B. Non-fasting blood analysis using HPLC and DMMB indicate that Glu/Chon as found in GLC® 5500 with the addition of Actistatin® provided ≥60% of the total mgs of the embodiment delivered to serum as compared to ≤30% GLC® 5500 without Actistatin® and 0,

Significant Data from Samples at Baseline
Total protein (Gm./100 ml.) …….. 9 2.18 N(6.53 - 7.93) (0.83 - 2.17)
Hyaluronan……..... 0.5 N(2.0-4.0mg/ml.)
Albumin: globulin ratio ..... 4.5 5.8 N(0.84 2.76) (0.31 - 8.06)
Sugar (mg./100 ml.) ..... 148 102 N(57 - 138) (33 - 105)
Sigma (units/ml.) …….. 0.046 0.039 N(0.04 - 0.52) (0.00 -0.27)
LDH (units/ml)……..…….. 794 92 N(100 - 760) (0 - 94)
Sibley-Lehninger (units/ml.)……..…….. 17 3 N(2 -19) (1 -29)
Sigma-Frankelunits(ml.)…… 100 9 N(90 - 190) (8 - 60)
Glutamic pyruvic transaminaseactivity)….. 3 15 N(5 -14) (2-10)
(Sigma-Frankelunits/ml.)…….…… 11 24 N(O 50) (0-23)
Significantly (P < 0.001) higher than corresponding synovial fluid value.

Significant Data from Samples at Week-4
Total protein (Gm./100 ml.) ..... 7.6 1.84 N(6.53 - 7.93) (0.83 - 2.17)
Hyaluronan……..... 1.5N(2.0-4.0mg/ml.)
Albumin: globulin ratio ..... 4.0 5.5 N(0.84 2.76) (0.31 - 8.06)
Sugar (mg./100 ml.) ..... 79 100 N(57 - 138) (33 - 105)
Sigma (units/ml.) …… 0.05 0.11 N(0.04 - 0.52) (0.00 -0.27)
LDH (units/ml)……. 788 90 N(100 - 760) (0 - 94)
Sibley-Lehninger (units/ml.)…… 18 3 N(2 -19) (1 -29)
Sigma-Frankelunits(ml.)……..…… 116 22 N(90 - 190) (8 - 60)
Glutamic pyruvic transaminaseactivity)……. 5 4N(5 -14) (2-10)
(Sigma-Frankelunits/ml.)…….…… 25 12N(O 50) (0-23)
Significantly (P < 0.001) higher than corresponding synovial fluid value.

Significant Data from Samples at Week-24
Total protein (Gm./100 ml.) ……..... 7.5 1.78N(6.53 - 7.93) (0.83 - 2.17)
Hyaluronan……... 3.8 N(2.0-4.0mg/ml.)
Albumin: globulin ratio ..... 2.06 5.66N(0.84 2.76) (0.31 - 8.06)
Sugar (mg./100 ml.) ..... 52 36N(57 - 138) (33 - 105)
Sigma (units/ml.) ……..…. 0.05 0.21 N(0.04 - 0.52) (0.00 -0.27)
LDH (units/ml)……... 206 55N(100 - 760) (0 - 94)
Sibley-Lehninger (units/ml.)……..…… 18 22 N(2 -19) (1 -29)
Sigma-Frankelunits(ml.)……..…. 94 42N(90 - 190) (8 - 60)
Glutamic pyruvic transaminaseactivity)…… 14 10N(5 -14) (2-10)
(Sigma-Frankelunits/ml.)…….…… 26 18N(O 50) (0-23)
Significantly (P < 0.001) higher than corresponding synovial fluid value.

control. The resulting increase from 0, ≤30% and ≥60% indicate a significant improvement in Glu/Chon survivability through the digestive system with the addition of Actistatin®. The resultant increase in serum Glu/Chon content demonstrates Actistatin® to be an effective facilitator for improved oral GAG delivery in the horse. Significant improvement in multiple synovial fluid markers were evident in Group A as compared to Group B.

These findings indicate that the addition of GLC® 5500 with Actistatin® to the subject's diet has a direct effect on synovial tissues. Specific increases in hyaluronan count, volume, and relative viscosity suggest that this protocol may provide a viable, non-invasive method for improving the health of equine synovial tissues. Further, these findings indicate that higher circulating

GAG levels as Glu/Chon influence endogenous hyaluronan production. In addition, no adverse side effects were recorded or reported throughout the span of the study, indicating the application of Actistatin® to oral Glu/Chon supplementation is regarded as safe. The combined findings indicate that Actistatin® is an improvement to prior Glu/Chon delivery methods by delivering significantly higher levels of Glu/Chon to serum than previously observed. The findings suggest that GLC® 5500 with Actistatin® is safe for use in horses and may act to moderate the severity and progression of factors contributing to equine joint lameness through multiple biochemical pathways. It is also indicated that GLC® 5500 with Actistatin® provides an effective non-invasive method for improving synovial fluid concentrations and hyaluronan volume. The objective findings indicate that increases in Glu/Chon to the extent observed in this study may have a direct disease-modifying effect on multiple biochemical and physiological mechanisms involved in equine lameness and may prove to be an effective treatment for such conditions.

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