Objective: The purpose of this study was to examine the effectiveness of Glyco-Flex® III using ground reaction force measurements and select synovial biomarkers of OA on normal dogs with an induced stable stifle OA model.

Summary: Previous *in vitro* analysis of inflammation induced canine chondrocyte treated with Glyco-Flex® III strongly support anti-inflammatory and antioxidant properties of the product. In this *in vivo* study, the anti-inflammatory properties of Glyco-Flex® III was evaluated using purpose bred dogs and a new model of an osteoarthritis.

Background: Treatment of clinical osteoarthritis (OA) in dogs has included the use of non-steroidal anti-inflammatory drugs. A limited number of studies have been reported investigating the effects of oral nutraceuticals (e.g. chondroitin sulfate, glucosamine, methylsulfonylmethane, etc.) administered to dogs with clinical OA. The results of these studies have been based on either subjective lameness evaluations or limited ground reaction force analysis involving clinical subjects after they have developed OA. This model of OA was developed to specifically mirror actual OA conditions rather than destabilized inflamed joints.

Methods: Eight purpose bred hound cross dogs were selected for a randomized, double blind experimental study with a crossover design. OA was induced in the stifle joint of all 8 dogs by creating an arthroscopically-assisted full thickness cartilage defect on the medial femoral condyle of a unilateral stifle. Following surgery intra-articular injections of a proteolytic enzyme (chymopapain) were given to increase inflammation and limit healing of the defect. OA was confirmed radiographically after 21 days. The dogs were randomized into 2 groups: a treatment group received 1.5 tablets of Glyco-Flex® III every 12 hours for 6 weeks; an OA control group receiving no treatment. After a washout period of four weeks the groups were reversed. Bi-weekly force plate analysis was performed. An increase of >15% in peak vertical force (PVF) and vertical impulse (VI) at any stage during treatment was considered a significant response to treatment. Synovial fluid was collected from the affected stifle joint every 2 weeks and analyzed for prostaglandin E2 (PGE2), nitric oxide (NO), interleukin-6 (IL-6), matrix metalloproteinase-3 (MMP3), soluble collagen (SC), tumor necrosis factor-α (TNFα) and sulfated glycosaminoglycans (sGAG) levels using commercially available test kits. Ground reaction force and synovial OA marker data over time was statistically analyzed using repeated measures ANOVA and a Tukey's test post-hoc. Significance was set at P < 0.05 for all statistical testing. Results are reported as mean +/- SEM.

Results: All dogs had a marked lameness and radiographic changes associated with OA 21 days after surgery. One dog remained non weight-bearing lame for a period of time and was excluded from analysis. The remaining dogs underwent a treatment were then crossed-over for a control phase. In the treatment group 43% (3/7) of dogs had a significant improvement in lameness ("responders"). This response was maximal after 4 weeks of treatment, with 41±10% increase in PVF and 44±13% increase in VI over the pretreatment values in the responder treatment group. There was a significant difference between the combined mean percent changes (positive) of PVF and VI for weeks 4 and 6 of the responder treatment group compared to responder control group with regard to baseline. After 6 weeks of treatment, the mean synovial PGE2 levels were significantly lower in the responder treatment group (8.5±4.0 pg/ul) compared to the responder control group (26.3±4.0 pg/ul). Soluble collagen was significantly lower in the responder treatment group (849.8±53.2 μg/ml) compared to the responder control group (1106.4±53.2 μg/ml) over the same period. Other synovial markers in the responder group had trends for lower levels compared to the control group.
Conclusion: In this model of stifle OA, 43% of patients responded positively to Glyco-Flex® III supplementation as measured by significant increases in PVF and VI during treatment. Ground reaction forces did not improve in the untreated animals. Synovial PGE and SC levels were significantly lower in the responder treatment group supporting clinical anti-inflammatory and collagen stabilizing activities of the product. PGE and SC level results correspond to the in vitro analysis of Glyco-Flex® III. The results of the study suggest that Glyco-Flex® III may be an effective non-pharmaceutical treatment that can reverse the clinical signs of OA in dogs and reduce the severity of cartilage breakdown and synovitis.

Clinical Relevance: The results of the study suggest that Glyco-Flex® III may be an effective non-pharmaceutical treatment that can reverse the clinical signs of OA in dogs. Future studies are needed and should include larger animal numbers and pharmacokinetic analysis of Glyco-Flex® III to investigate the responder-nonresponder phenomenon.