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Therapeutic Efficacy and Safety Evaluation of Flex Choice™ in Moderately Osteoarthritic Dogs*

Research Article

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Abstract

Osteoarthritis (OA) is a chronic inflammatory degenerative joint disease that affects humans and animals alike. Currently, >20% of the adult and 80% of the geriatric dog population in the US (>90 million) suffer from OA. The pathophysiology of OA is very complex as it involves multiple mechanisms and molecular pathways. Currently, there are several choices to manage OA, but many veterinarians choose nutraceuticals because of lesser or no side effects compared to pharmaceuticals. The present investigation was undertaken to assess anti-arthritic efficacy and safety of Flex Choice™ chews (Clinics Choice, LLC) in dogs with moderate OA. Flex Choice™ chews are composed of krill oil, hyaluronic acid, astaxanthin, *Boswellia serrata* extract, green-lipped mussel, and iron transport tocopheryl polyethylene glycol succinate (ITPGS). Dogs with OA received Flex Choice™ chews b.i.d. for 150 days. Each month, dogs were given a full exam and were evaluated for arthritic pain (overall pain, pain upon limb manipulation, and pain after physical exertion) using the Glasgow scoring system, CBC, and serum biomarkers of liver (bilirubin, ALT, and AST), kidney (BUN and creatinine), and heart and skeletal muscle (CK) functions. Dogs receiving Flex Choice™ showed marked reductions in overall pain (52%), pain upon limb manipulation (35%), and pain after physical exertion (40%). The active ingredients in Flex Choice™ exert antioxidative, anti-inflammatory, immunomodulatory, analgesic, cartilage repair and anti-osteoarthritic effects. ITPGS, in addition to being a bioenhancer, exerts its effects such as antioxidative and anti-inflammatory. No significant (P>0.01) change occurred in physical parameters, CBC, and serum biomarkers of liver, kidney, and heart functions. Findings revealed that Flex Choice™ significantly ameliorated OA-associated pain, and it was well tolerated by dogs with moderate OA.

Keywords: Osteoarthritis; Canine; Flex Choice; Krill Oil; Hyaluronic Acid; Astaxanthin; *Boswellia Serrata* Extract; Green-Lipped Mussel; ITPGS.

Abbreviations: ACLT: Anterior Cruciate Ligament Transection; ADAMTS-4: A Disintegrin And Metalloproteinase Thrombospondin Motifs 4; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ATX: Astaxanthin; BSE; *Boswellia Serrata* Extract; BUN; Blood Urea Nitrogen; CBC; Complete Blood Count; CK: Creatine Kinase; COX: Cyclooxygenase; CT scan: Computed Tomography Scan; DBIL: Direct Bilirubin; DHA: Docosahexaenoic Acid; ECM: Extra Cellular Matrix; EDTA: Ethylene Diamine Tetra Acetic Acid; EPA: Eicosapentaenoic Acid; ESR: Erythrocyte Sedimentation Rate; ETA: Eicosatetraenoic Acid; GAG: Glycosaminoglycans; GLM: Green-Lipped Mussel; HA: Hyaluronic Acid; HCT: Hematocrit; HGB: Hemoglobin; IACUC: Institutional Animal Care and Use Committee; IL-1α: Interleukin-1α; IL-1β, iNOS: Inducible Nitric Oxide Synthase; Interleukin-1β; ITPGS: Iron Transport Tocopheryl Polyethylene Glycol Succinate; LPO: Lipoxygenase; MDR: Multidrug Resistance; MMP: Matrix Metalloproteinase; MRI: Magnetic Resonance Imaging; mTOR: Mammalian Targets Of Rapamycin; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; OA: Osteoarthritis; PGE: Prostaglandin E; P-gp: P-Glycoprotein; PUFA: Polyunsaturated Fatty Acids; RA: Rheumatoid Arthritis; RBC: Red Blood Cell; RNS: Reactive Nitrogen Species; ROS: Reactive Oxygen Species; TBIL: Total Bilirubin; TNF-α: Tumor Necrosis Factor-α; WBC: White Blood Cell.

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Introduction

Currently, the world's canine population is more than 900 million, of which 93 million reside in the US. In the US, the majority of dogs are companions while some others are used for service or special needs. Every fifth adult dog suffers from some form of arthritis. The two most common types of arthritis are osteoarthritis (OA) and rheumatoid arthritis (RA). OA occurs with greater frequency than RA, and large breed dogs are prone to develop OA (>45%) as compared to smaller breeds [34, 35]. OA is an inflammatory joint disease characterized by chronic and progressive cartilage degeneration, osteophyte formation, subchondral sclerosis, bone marrow lesions, hypertrophy of bone at the margin, and changes in the synovial membrane. OA not only affects cartilage, but the entire joint including bones, ligaments, nerves and surrounding muscles [58, 89]. Common clinical signs and symptoms associated with OA in dogs include limping, immobility, stiffness of joints, crepitus, periarticular swelling, palpable effusion, pain upon manipulation of the joint, and lameness [33, 81, 49, 29, 20, 67, 35, 41].

The pathophysiology of OA is very complex [80, 58, 22, 32, 61, 107, 34, 35] due to the involvement of multiple etiologies (aging, injury, excessive or lack of exercise, nutritional deficiency, obesity, genetic predisposition, infection, etc.) in this disease [60, 103, 54, 34, 35], and as a result, treatment is also complicated [32-35]. Degradation of articular cartilage is a hallmark of OA, which occurs in two phases: an anabolic phase, in which chondrocytes attempt to repair the damaged extra cellular matrix (ECM); and a catabolic phase, in which enzymes produced by different cells (including chondrocytes) digest the ECM [101]. This catabolic phase also includes inhibition of matrix synthesis, thereby ensuing accelerated erosion of the cartilage [15, 44]. In OA cartilage, a decrease in the number of chondrocytes and in their ability to regenerate the ECM in response to stress has been described [75]. Cell death of chondrocytes is a combination between apoptosis and autophagy during the pathogenesis of OA [2]. During cartilage degeneration, the inflammatory processes cause excess production of ROS, RNS, oxygen, and PGE2 levels, and as a result, their increased levels can be found within the joint [8, 109, 18]. Activity of pro-inflammatory cytokine IL-1β can be detected in synovial fluid of OA joints where it induces gene expression of matrix-degrading enzymes in the chondrocytes. In addition to oxidative/nitrosative stress and inflammation, multiple molecular pathways are involved in structural and functional damage to cartilage, progression of OA, and OA associated pain [51, 83, 32, 110, 44, 34, 35].

In clinical veterinary settings, diagnosis of OA is based on observational [33, 49, 29, 67, 34, 35] and radiographic findings [67, 78]. Even today, CT scan and/or MRI findings reveal changes of the joint and cartilage degeneration, which are consistent with OA, but are limited to humans and experimental studies [74, 46, 27]. In recent years, a large number of biomarkers of OA have been recognized that play key roles in diagnosis, prognosis and therapeutic intervention [10, 31, 34, 35].

Since there is no cure for OA, management of OA in canines is usually aimed at minimizing joint pain by reducing inflammation, slowing the progression of cartilage degeneration and improving cartilage repair, thereby increasing the joint's flexibility and quality of life for the animal. In the past, among various choices (such as NSAIDS, therapeutic drugs, physiotherapy, surgery, acupuncture, and lifestyle changes), veterinarians have used NSAIDs [26, 40, 48, 108, 71, 52, 25]. However, due to severe side effects such as gastrointestinal bleeding, cardiac, hepatic, and renal dysfunction, reduced appetite, vomiting, and inhibition of bone healing, many veterinarians are currently using nutraceuticals [63, 12, 66, 93, 76, 42, 87, 20, 1, 34, 35].

In a series of clinical trials, we have evaluated several nutraceuticals singly or in combination for their efficacy and safety in moderately OA dogs [24, 23, 72, 33, 49, 29, 67]. Some of these nutraceuticals provided remarkable reduction in pain and osteophyte formation and enhanced cartilage repair associated with OA in dogs. The present investigation was undertaken to evaluate the efficacy and safety of Flex Choice™ in moderately OA dogs. Flex Choice™ chews are composed of krill oil, hyaluronic acid, astaxanthin, *Boswellia serrata* extract, green-lipped mussel, and iron transport tocopheryl polyethylene glycol succinate (ITPGS). By having several ingredients, a novel nutraceutical product Flex Choice™ exerted an anti-arthritic effect via multiple mechanisms exerting antioxidative, anti-inflammatory, immunomodulatory, and analgesic effects.

Materials and Methods

Animals and their Treatment with Flex Choice™

Five client-owned moderately osteoarthritic dogs, weighting between 35-64 pounds each, were used in this investigation. A brief description of each dog is provided below in Table 1.

Dogs having any disease related to heart, liver, kidney or any other organ or cancer were not included in this study. Institutional Animal Care and Use Committee (IACUC) approval and owner consents were obtained prior to the initiation of this investigation. In order to avoid five months of suffering, a placebo group was not included in this trial. Instead, prior to initiation of the study, base-line values were considered as control values. Moreover,

Table 1. Description of dogs used in this study.

Name	Breed	Gender	Age (years)	Body wt (pounds)
Lucchese	Australian cattle dog	Female (spayed)	9	36.4
Bailey	Chocolate Labrador retriever	Female (spayed)	9	63.4
Brutus	Black Labrador retriever	Male (neutered)	13	64.0
Reecey	Labrador retriever/Australian shepherd cross	Male (neutered)	7	56.8
Goblin	German shepherd/ boxer mix	Male	6	41.4

findings of our previous clinical trials indicated that OA dogs receiving placebo showed no improvement in OA associated pain [24, 23, 72, 33, 49, 29, 67]. Trial dogs did not receive any treatment or supplement for 3 to 4 weeks prior to initiation of this study or during the study period. All five dogs received a Flex Choice™ chew (provided by Vets Plus, Inc. of Menomonie, WI) twice daily (one chew before morning meal and one chew before evening meal) for a period of 150 days. Each Flex Choice™ chew weighed 560.3 mg and consisted of 249.9 mg krill oil, 25.8 mg sodium hyaluronate, 24.6 mg astaxanthin, 10 mg *Boswellia serrata* extract (BSE), 200 mg green-lipped mussel (GLM), and 50 mg iron transport tocopheryl polyethylene glycol succinate (ITPGS).

Astaxanthin (ATX) in Flex Choice™ was chemically a 3,3-dihydroxy-β, β-carotene-4, 4'-dione with a chemical formula $C_{40}H_{52}O_4$ and molecular weight 596.84 [CAS, 472-61-7]. The Structural formula of ATX is shown in Figure 1. ATX is a metabolite of zeaxanthin and/or canthaxanthin, containing both hydroxyl and ketone functional groups.

Hyaluronic acid (HA) used in this study was a low molecular weight sodium salt of hyaluronate, which is a glycosaminoglycan and long-chain polymer of disaccharide units of Na-glucuronate-*N*-acetylglucosamine [CAS, 9004-61-9]. Its structural formula is shown in Figure 2.

ITPGS is a novel formulation consisting of two molecules, the iron transport protein ovotransferrin (IT) and α-tocopheryl polyethylene glycol succinate (TPGS). Its structural formula is shown below in Figure 3. The exact molecular weight and CAS number for ITPGS have yet to be established.

Although the *Boswellia serrata* extract (BSE) contains many phytochemicals, 3-acetyl-11-keto-β-boswellic acid (AKBA) is the one that exerts anti-OA activity. It has a chemical formula $C_{32}H_{48}O_5$, with a molecular weight 512.7 [CAS, 67416-61-9]. Its structural formula is shown in Figure 4.

Figure 1. Structural formula of astaxanthin (ATX).

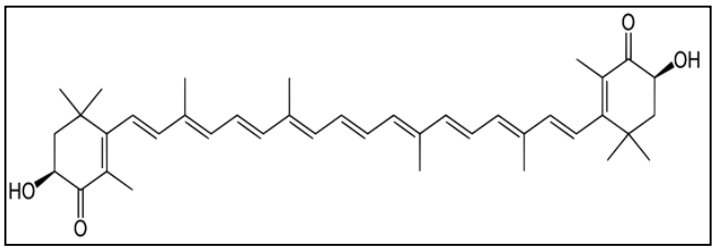


Figure 2. Structural formula of sodium hyaluronate.

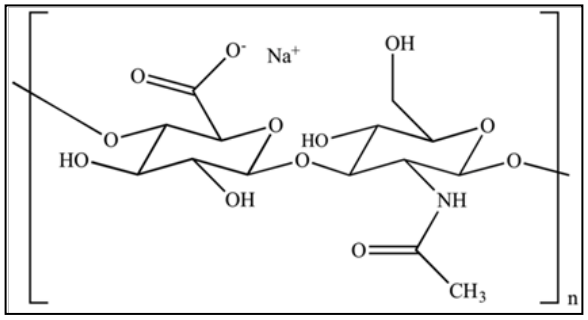


Figure 3. Structural formula of ITPGS (IT.TPGS).

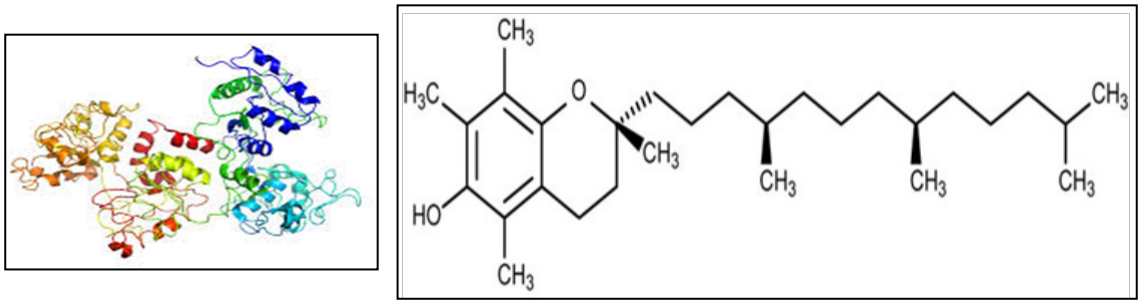
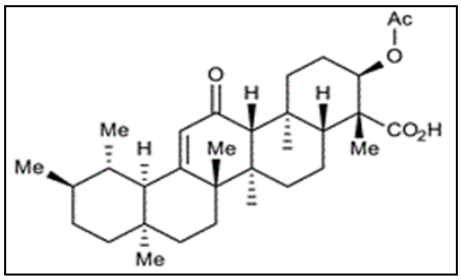


Figure 4. Structural formula of 3-acetyl-11-keto-β-boswellic acid (AKBA).



Both krill oil and green-lipped mussel contain omega-3 fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are of a great health significance. EPA has a chemical formula $C_{20}H_{30}O_2$, with a molecular weight 302.451 [CAS, 10417-94-4], and in biological/physiological literature, it is given the name 20:5 (n-3) or its trivial name timnodomic acid. Its structural formula is shown in Figure 5. It needs to be noted that EPA is precursor to DHA.

The chemical formula of DHA is $C_{22}H_{32}O_2$, molecular weight is 328.488, and CAS is [6217-54-5]. In biological/physiological literature, DHA is given the name 22:6 (n-3), and its trivial name is cervonic acid. The structural formula of DHA is shown below in Figure 6.

Physical Examination

On a monthly basis, each dog was given a full physical examination for body weight, body temperature and heart rate. Heart rate and respiration rate were observed using a stethoscope, and results were recorded in beats per minute. We were unable to measure respiratory rate because of panting during examination. Body temperature was recorded using a digital rectal thermometer set to °F. Body weight was measured using an electronic scale and recorded in pounds.

Blood/Serum Analysis

On a monthly basis, blood samples were collected from the jugular or cephalic vein using a 5 mL syringe and 20 gauge needle. Samples were then placed into a 3.5 ml serum separator tube and a 2 ml EDTA tube. Following centrifugation, serum samples were collected from the clot and analyzed for liver (bilirubin, AST, and ALT), kidney (BUN and creatinine), and heart and skeletal muscle (CK) functions, using a Beckman AU 480 serum analyzer. Whole blood was analyzed for an erythrocyte sedimentation rate (ESR) and complete blood count (CBC) using a Sysmex XT-2000iV. ESR was used to test for inflammatory biomarkers.

Pain Measurement

On a monthly basis, each dog was examined for osteoarthritis (OA) associated pain (overall pain, pain upon limb manipulation, and pain after physical exertion), using a Glasgow scoring system for a period of 150 days [33, 49, 29, 67]. In brief, overall pain on a scale of 0-10, was graded as: 0, no pain; 2.5, mild pain; 5, moderate pain; 7.5, severe pain; and 10, severe and constant pain. Overall pain was measured on a scale of 0-10 because it covered a broad range of daily activities, including gait quality, rising from a sitting or lying position, and lowering into a sitting or lying position. Pain upon limb manipulation was evaluated in each limb while the dog was in standing recumbency observing vocalization, body posture, stiffness/resistance, flexibility, integrity, crepitus in the joints, and range of motion using a scale of 0-4 as: 0, no pain; 1, mild pain; 2, moderate pain; 3, severe pain; and 4, severe and constant pain. Pain after physical exertion was evaluated based on vocalization, body posture, flexibility, resistance, and range of motion after 2 minutes of jogging. Canines were evaluated for evidence of lameness during and after exercise using the scale of 0-4 as: 0, no pain; 1, mild pain; 2, moderate pain; 3, severe pain; and 4, severe and constant pain.

Radiographic Evaluation

Arthritic joints (elbow and stifle) were evaluated radiographically using a DUOCON 1 VIDEX MACHLETT (125 KVP) equipped with digital imaging software on day 0 and day 150. Radiographs were taken on Days 0 and 150 to determine if administration of Flex Choice™ may have reversed some of the arthritic changes in joints.

Statistical Analysis

Data were statistically analyzed for Mean ± SEM and Analysis of Variance (ANOVA) coupled with Duncan's multiple-comparison test for significance (Alpha=0.05) using NCSS9 software.

Results

The effect of Flex Choice™ was assessed on body weight, rectal temperature, heart rate, and respiratory rate on Day 0 and each month for 150 days. The data are summarized in Table 2, and expressed in terms of Mean ± SEM.

Body weight for Day 0 was 52.4 ± 5.71 and Day 150 was 52.6 ± 6.57. No significant differences ($P > 0.05$) were observed in canine weight throughout study duration. Body temperature (rectal temperature measured in °F) on Day 0 was 101.9 ± 0.27 and on Day 150 was 101.4 ± 0.20. No significant difference ($P > 0.05$) in rectal temperature was noted in canines throughout the

Figure 5. Structural formula of eicosapentaenoic acid (EPA).

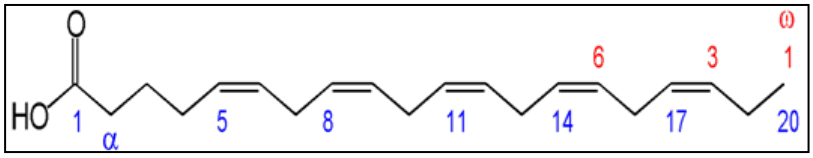
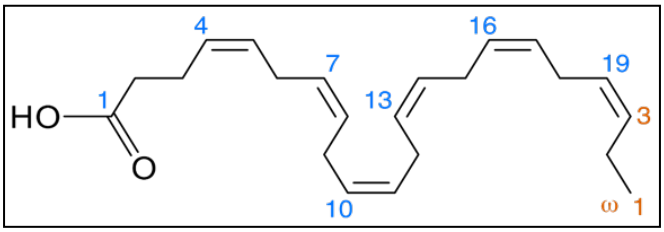


Figure 6. Structural formula of docosahexaenoic acid (DHA).



study duration. Heart rate (beats/minute) was observed using a stethoscope. Heart rate remained significantly unchanged during the period of this study. Respiration rate (breaths per min) was measured using a stethoscope, but panting interfered with valid measurement and values could not be obtained.

Data of overall pain, pain upon limb manipulation, and pain after physical exertion from dogs treated with Flex Choice™ are shown in Figures 7-9. Baseline value of overall pain on Day 0 was 4.6 ± 0.40, indicating the pain level of moderate osteoarthritis (Figure 7). The pain level was significantly reduced ($P < 0.05$) on Day 120 (3.2 ± 0.49) and the trend continued until the last observation on Day 150 (2.4 ± 0.40) with maximum (52%) pain reduction.

Figure 8 shows results of pain during limb manipulation from Day 0 to Day 150. Baseline value on Day 0 was 2.0 ± 0.00. A significant decline in pain was observed on Day 120 (1.3 ± 0.12). On Day 150, there was a marked reduction in pain, but this was statistically insignificant due to a slight increase in variation.

Figure 9 shows results of pain after physical exertion in dogs treated with Flex Choice™ for 150 days. The level of pain on Day 0 was 2.0 ± 0.00, which was significantly reduced by Day 120 (1.2 ± 0.12), with maximum reduction on Day 150 (1.2 ± 0.25).

Table 3 presents the data of serum chemistry parameters (AST, ALT, total bilirubin, direct bilirubin, BUN, creatinine, and creatine kinase) from dogs receiving Flex Choice™. Values of these parameters remained insignificant ($P > 0.05$) throughout study duration.

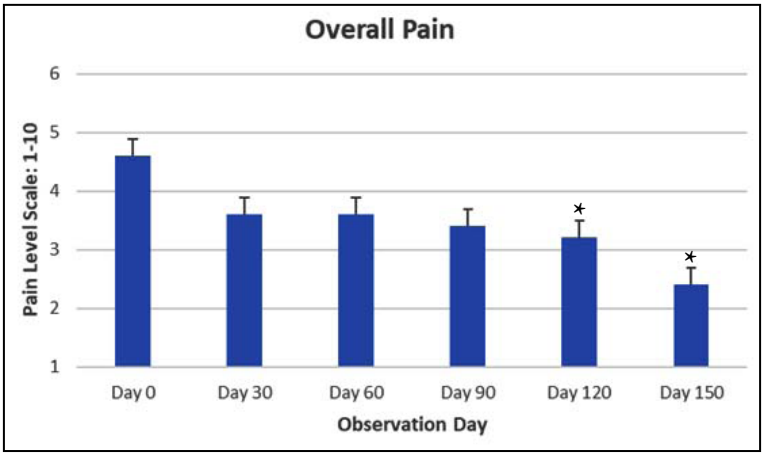
Complete blood count (CBC) parameters (RBC, WBC, HCT, and

Table 2. Effects of Flex Choice™ on body weight, rectal temperature and heart rate in dogs.

Parameter	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150
Body weight	52.4 ± 5.71	52.8 ± 5.89	53.0 ± 6.72	53.1 ± 6.71	52.9 ± 6.42	52.6 ± 6.57
Rectal temperature	101.9 ± 0.27	101.9 ± 0.62	101.5 ± 0.45	101.8 ± 0.36	101.6 ± 0.07	101.4 ±0.20
Heart rate	132.8 ± 11.55	120.8 ± 12.55	88.4 ± 7.33	109.6 ± 15.88	107.2 ± 10.84	112.8 ± 13.05

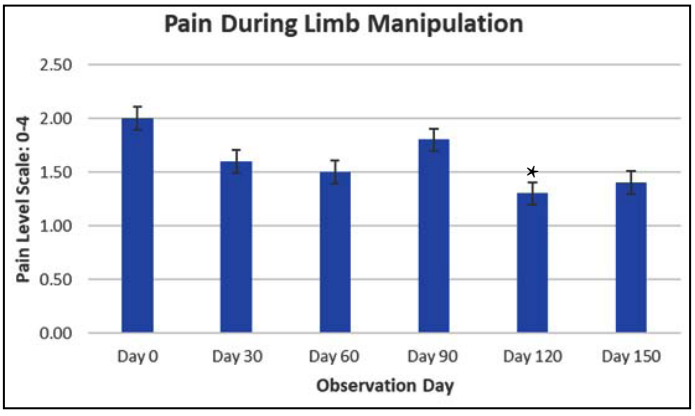
Each value represents Mean ± SEM (n=5).
*Statistically significant difference from value of Day 0 ($P < 0.05$).

Figure 7. Effect of Flex Choice™ on overall pain in dogs.



*Statistically significant difference from value of Day 0 ($P < 0.05$).

Figure 8. Effect of Flex Choice™ on pain during limb manipulation in dogs.



*Statistically significant difference from value of Day 0 ($P < 0.05$).

HGB) remained significantly unchanged (>0.05) throughout the study duration (Table 4).

Erythrocyte sedimentation (ESR) rates were performed, but values were not accurate due to delay in analysis from time of sample collection. Those results were discarded from this study.

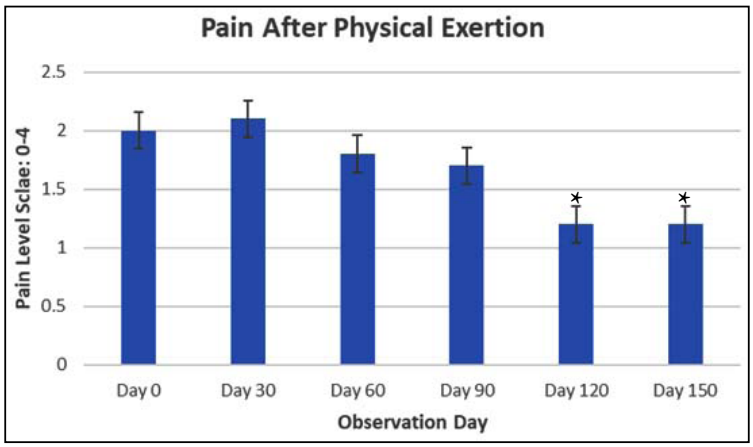
Radiographs were taken on Day 0 and Day 150 of all five canines. Findings revealed no appreciable differences between radiographs from Day 0 and Day 150. For proper comparison of joints on radiographs, positioning of each joint must be practically identical each time and this was not the case for this study (Figures not shown). However, attempts were made to keep technique and positioning the same each time for a better comparison. Canines were unable to be placed in exactly the same position for both radiograph sessions as different restrainers were used as well as expected movement from the animals. Technique used was as close to identical as possible, but some adjustments were made if the radiograph did not turn out appropriately on the first attempt.

Discussion

The use of nutraceuticals is becoming more and more common in veterinary medicine for prevention and treatment of common diseases, including OA [104, 33, 82, 65, 67, 32, 35, 36, 25]. New nutraceuticals or new use of old nutraceuticals are being evaluated for efficacy and safety in dogs with OA.

Even today, non-steroidal anti-inflammatory drugs (NSAIDs) are considered a mainstay in pain management associated with OA due to their analgesic and anti-inflammatory properties. NSAIDs act primarily to reduce the biosynthesis of prostaglandins by inhibiting cyclooxygenase (COX). There are two isoforms of COX (COX-1 and COX-2). COX-1 is found in most body tissues

Figure 9. Effect of Flex Choice™ on pain after physical exertion in dogs.



*Statistically significant difference from value of Day 0 ($P < 0.05$).

Table 3. Effects of Flex Choice™ on liver, kidney and heart function biomarkers in dogs.

Parameter	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150
AST (IU/L)	26.6 ± 3.61	25.6 ± 2.11	26.8 ± 5.30	26.6 ± 4.06	28.8 ± 6.86	23.4 ± 3.23
ALT (IU/L)	51.6 ± 14.61	48.2 ± 8.67	63.8 ± 29.92	67.0 ± 30.02	85.6 ± 44.55	62.8 ± 24.40
TBIL (mg/dl)	0.18 ± 0.04	0.14 ± 0.02	0.16 ± 0.24	0.16 ± 0.02	0.14 ± 0.02	0.12 ± 0.02
DBIL (mg/dl)	0.0 ± 0.00	0.0 ± 0.00	0.02 ± 0.02	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
BUN (mg/dl)	17.0 ± 3.59	17.4 ± 2.23	15.2 ± 1.98	13.2 ± 4.05	16.0 ± 3.05	18.2 ± 2.46
Creatinine (mg/dl)	0.90 ± 0.09	0.94 ± 0.06	0.92 ± 0.09	0.90 ± 0.10	0.90 ± 0.11	0.94 ± 0.10
CK (IU/L)	165.6 ± 18.17	106.0 ± 19.15	132.0 ± 38.09	150.6 ± 40.27	163.8 ± 56.54	112.8 ± 15.81

Each value represents Mean ± SEM (n=5).
*Statistically significant difference from value of Day 0 ($P < 0.05$).

Table 4. Effects of Flex Choice™ on complete blood count (CBC) parameters in dogs.

Parameter	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150
RBC	6.73 ± 0.26	6.93 ± 0.2	6.95 ± 0.36	6.82 ± 0.29	6.75 ± 0.32	6.43 ± 0.29
WBC	8.19 ± 0.62	8.28 ± 0.44	8.19 ± 0.62	8.71 ± 0.98	9.04 ± 1.81	8.26 ± 0.6
HCT	48.30 ± 2.02	49.02 ± 1.16	48.72 ± 2.43	47.68 ± 1.99	47.06 ± 1.89	45.60 ± 2.07
HGB	16.16 ± 0.69	16.64 ± 0.40	16.88 ± 0.90	16.50 ± 0.69	16.24 ± 0.76	15.72 ± 0.69

Each value represents Mean ± SEM (n=5).
*Statistically significant difference from value of Day 0 ($P < 0.05$).

and is responsible for mediating a variety of normal physiological effects, including hemostasis, gastrointestinal mucosal protection, and protection of the kidney from hypotensive insult. COX-2 is activated in damaged and inflammed tissues and it also catalyzes the formation of prostaglandin, which is associated with the inflammatory response. One downside to the use of some NSAIDs is that they inhibit both isoforms of COX, thereby causing side effects such as GI, liver, kidney and cardiovascular dysfunctions [66, 1].

Flex Choice™ (a novel formulation) was evaluated in the present investigation for its therapeutic efficacy and safety in moderately OA dogs. The working hypothesis was that Flex Choice™ would alleviate significant pain associated with OA without producing adverse effects in dogs. Data were collected for vital signs, pain measurement (overall pain, pain during limb manipulation, and pain after physical exertion), parameters of serum chemistry and complete blood count (CBC).

Dogs receiving Flex Choice™ showed significant alleviations in overall pain (52%), pain upon limb manipulation (35%), and pain after physical exertion (40%) (Figure 7-9). By having multiple ingredients (krill oil, hyaluronic acid, astaxanthin, *Boswellia serrata* extract, green-lipped mussel, and iron transport tocopheryl polyethylene glycol succinate), Flex Choice™ might have exerted anti-OA effect due to its antioxidative, anti-inflammatory, analgesic, and immunomodulatory properties. Each ingredient present in Flex Choice™ is discussed below in brief for mechanism of action, and safety and toxicity.

Krill oil is a source of omega-3 fatty acids, phospholipids and antioxidants. Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs), which are beneficial for the treatment of canine OA [30, 84, 85, 64, 100]. Krill oil is an excellent source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with enhanced oral absorption (14; 57; 94). Omega-3 fatty acids exert anti-OA effects by reducing IL-1α, IL-1β, PGE2, ADAMTS-4, COX-2, iNOS, TNF-α, MMP-3, MMP-13, and aggrecanase and collagenase activities, as well as by increasing collagen synthesis. EPA is the most effective type of omega-3, followed by DHA. PUFAs (EPA and DHA) protect activation of autophagy in chondrocytes by modulating mammalian targets of rapamycin (mTOR) signaling pathway, thereby exerting an anti-OA effect [106, 32, 35]. In a number of clinical studies, omega-3 fatty acids, singly or in combination with other anti-OA nutraceuticals, have been shown to improve OA in dogs and cats without side effects [21,104, 30, 84, 85, 64].

Hyaluronic acid (HA), produced by chondrocytes, synoviocytes (predominately type B), and fibroblasts, is a non-sulfated, naturally occurring non-protein glycosaminoglycan (GAG). HA is an important constituent of articular cartilage (as it coats each chondrocyte) present in the extracellular matrix (ECM), and it contributes to cell proliferation, migration, and morphogenesis. By having distinct rheological properties (excellent viscoelasticity, high moisture retention capacity, high biocompatibility, and hygroscopic activity), HA acts as a lubricant, shock absorber, joint structure stabilizer, and water balance - and flow resistance regulator at a concentration as low as 0.1% [36]. The molecular weight of HA can vary from 5,000 to 20,000,000 Da. With aging, HA size appears to decrease but its volume may increase.

Recently, Gupta et al., (2019c) reviewed physiological and pharmacological roles of HA in the body. For the treatment of OA, HA is usually administered intra-articularly (IA), but it can also be administered orally or intravenously. HA has been reported to reach joints following oral administration, making it a justifiable route of administration [9]. HA is effective in treating OA as it may have a role in regulating the synthesis of proteoglycans during maturation of articular cartilage. It may also play a role in the repair processes of articular cartilage. HA works by mitigating activities of pro-inflammatory mediators and pain-producing neuropeptides that are released by activated synovial cells. HA can also reduce nerve impulses and sensitivity that are associated with pain from OA [38]. In OA, HA has been shown to prevent degradation of cartilage and may even promote regeneration. Overall, HA is a slow acting anti-OA agent that is reported to exert antioxidative, anti-inflammatory, immunomodulatory, and analgesic effects via multiple mechanisms involving receptors, enzymes and metabolic pathways [32, 34-36]. Clinical trials in OA canines with HA have been carried out primarily in surgically (anterior cruciate ligament transection, ACLT)-induced OA [97, 59, 47]. The anti-OA effects of HA appear to be due to its unique rheological properties and pharmacological mechanisms [36].

Astaxanthin (ATX) is a xanthophyll carotenoid that is predominately produced by microalgae (*Haematococcus pluvialis*) and a number of bacteria and fungi. ATX possesses strong antioxidant activity because it neutralizes singlet oxygen [102], scavenges free radicals, inhibits lipid peroxidation, enhances immune system function, regulates gene expression, and maintains mitochondria in the reduced state [50, 43, 69, 111, 17, 68, 90, 28]. In addition to anti-oxidative and anti-nitrosative effects, ATX has been reported to exert anti-inflammatory and anti-apoptotic activities [28]. Although ATX does not have any direct link to managing or treating OA, it does have many health benefits that contribute to its use as a supplement. ATX in Flex Choice™ might have provided anti-OA effects in dogs by exerting anti-oxidative/anti-nitrosative, anti-inflammatory, immunomodulatory, analgesic, and cartilage repair effects [50, 69, 70, 19, 45, 16]. In a number of studies, ATX has been reported to be safe from a dietary supplementation point of view [98, 102, 105, 90, 91].

Boswellia serrata (Indian Frankincense) is a plant native to India. The extracts of this plant have been used for treating chronic inflammatory diseases in humans and animals for thousands of years. *B. serrata* extract contains many phytoconstituents, including four major pentacyclic triterpenic acids (β-boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic acid, and acetyl-11-keto-β-boswellic acid) [95]. *Boswellia serrata* extract (BSE), used for anti-OA effects in dogs and humans, contains high concentrations of active constituents 3-acetyl-11-keto-β-boswellic acid (AKBA) and 11-keto-β-boswellic acid (KBA). AKBA contributes most to BSE's health benefits and it is present in concentrations of 2-3%. Acetyl-boswellic acid exerts anti-inflammatory activity by inhibiting leukotriene synthesis and 5-lipoxygenase (an enzyme responsible for inflammation) activity [3, 88, 4, 32]. In some studies, 30% AKBA provided improvement in joint mobility and comfort within a week [32]. In a number of studies, *B. serrata* resin/extract/boswellic acids have been found effective in ameliorating OA associated clinical signs in dogs and humans [55, 79, 92, 6, 37, 62] mainly due to their anti-inflammatory and immunomodulatory properties [4, 5, 95, 86]. Multiple molecular targets have been identified for pharmacological actions of

boswellic acids [73, 5, 86]. Safety and toxicity studies suggest that BSE and boswellic acids are safe for use as nutraceuticals and are well tolerated in dogs and humans [7, 96, 53, 56, 62].

Green-lipped mussel (*Perna canaliculus*) is a nutraceutical that is rich in glycosaminoglycans (GAGs), omega-3 fatty acids, and eicosatetraenoic acid (ETA). GAGs play an important role in the treatment of OA as they exert anti-inflammatory activities and lubricate joints [35]. ETA is a dual inhibitor of arachidonic acid oxygenation by both COX and lipoxygenase (LPO) pathways. Green-lipped mussel (GLM) works through a similar mechanism of action as that of NSAIDs, but without producing adverse effects. GLM is a gastroprotective agent and does not affect platelet aggregation, suggesting that ETA may selectively block COX-2 without affecting COX-1 [77, 11]. Bui and Bierer (2003) evaluated the efficacy of GLM powder (0.3% of a dry diet) for alleviating clinical signs of OA in canines. By the end of six weeks, GLM-treated canines showed significant improvement in arthritic score, joint pain, and swelling, without significant improvement in crepitus and range of joint movement. Rialland et al., (2013) evaluated the effect of a GLM-supplemented diet on pain behavior and functioning in dogs. Findings revealed increased concentrations of plasma omega-3 fatty acids (EPA and DHA), improvement of peak vertical force using ground force plate, and reduced OA signs. In all clinical studies, GLM has been found to be safe [11, 13, 39, 82].

Iron transport tocopheryl polyethylene glycol succinate (ITPGS) is a novel formulation consisting of two molecules: the iron transport protein ovotransferrin (IT) and α-tocopheryl polyethylene glycol succinate (TPGS). ITPGS is both a water and fat soluble formulation. ITPGS exerts several biological and pharmacological actions (antioxidative, anti-inflammatory, and immunomodulatory) through multiple mechanisms. ITPGS in Flex Choice™, by serving as a bioenhancer, may have improved the absorption and bioavailability of ingredients that have antioxidant, anti-inflammatory, immunomodulatory, analgesic and anti-OA properties [99]. ITPGS can exhibit additional properties, such as a surfactant, apoptogenic, anticancer, neuroprotectant, and P-glycoprotein (P-gp) inhibiting activity, thereby reversing multidrug resistance (MDR) activity.

Radiographs were taken on Day 0 and Day 150 and then compared to observe whether or not Flex Choice™ could reduce osteophyte formation in thoracic and pelvic limbs and hip joints. Lateral radiographs of elbows, shoulders, and stifles as well as ventral dorsal radiographs of hips, were also obtained on Day 0 and Day 150. Radiograph quality was dependent on the particular x-ray machine's settings and on movement of the dog. Steps were taken to be consistent, but technique and views varied slightly from Day 0 to Day 150. There was no remarkable difference in radiographic evidence on Day 150 and Day 0.

No significant differences were observed in values of serum and blood parameters throughout the study duration. In addition, there were no complaints from owners. These observations suggested that Flex Choice™ was safe and well tolerated by moderately OA dogs.

Conclusions

The present investigation evaluated anti-OA properties of a novel nutraceutical, Flex Choice™ soft chews in moderately OA dogs. Findings of overall pain, pain during limb manipulation, and pain after physical exertion suggested that administration of Flex Choice™ soft chews twice daily significantly reduced pain and inflammation associated with canine OA. Our observations also suggested that long-term use of Flex Choice™ was safe and well tolerated by moderately OA dogs.

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