EVALUATION OF A NUTRACEUTICAL FLEX CHOICE FOR ANTI-ARTHRITIC EFFICACY AND SAFETY IN MODERATELY OSTEOARTHRITIC DOGS

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EVALUATION OF A NUTRACEUTICAL FLEX CHOICE FOR ANTI-ARTHRTIC EFFICACY AND SAFETY IN MODERATELY OSTEOARHTIFIC DOGS

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By Rachael Elizabeth Webber

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EVALUATION OF A NUTRACEUTICAL FLEX CHOICE FOR ANTI-ARTHRITIC EFFICACY AND SAFETY IN MODERATELY OSTEOARTHRITIC DOGS

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Abstract

Arthritis is a very common chronic debilitating disease in people and dogs alike. It does not discriminate based on species, and one in five dogs suffer from osteoarthritis. The purpose of this study was to evaluate the safety and anti-arthritic efficacy of a nutraceutical, Flex Choice, in moderately arthritic canines. Five moderately arthritic canines that weighed between 40-65 pounds were selected for this study. Moderate arthritis was considered a ranking of 4-6 on a scale of 1-10 with 1 being no/minimal pain and 10 being severe and constant pain. All five canines were orally administered one Flex Choice soft chew twice a day for 150 days. There was no control group due to limited population. Day 0 baseline results were used as the control. On days 0, 30, 60, 90, 120, and 150 of the study, the canines were evaluated for overall joint pain, pain during limb manipulation, and pain after physical exertion. Radiographs were also performed on days 0 and 150 to observe if osteophyte formation may have been reduced. Serum chemistry analysis was performed each month as well to see if Flex Choice had any adverse effects on kidney, liver, or heart function. Body weight, heart rate, respiration rate, and temperature were also evaluated throughout the entire study.

Treated canines had a significant reduction in overall pain levels, pain during limb manipulation, and pain after physical exertion at some point during the study with P < 0.05. Serum chemistry indicated no significant side effects to vital organs throughout the entire study. Heart rate, body weight, respiration rate, and temperature were not significantly affected by the administration of Flex Choice chews. This study proves that Flex Choice is a safe and effective way to reduce pain and inflammation associated with canine osteoarthritis and may provide alternatives to NSAIDs.
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Chapter I: Introduction

Arthritis is a chronic, painful disease that affects many humans and targets joints such as the wrist, hip and knee. Just like humans, dogs and other animals can be affected by this disease. There is no cure for arthritis, but veterinarians can perform surgeries and prescribe pharmaceuticals or nutraceuticals to help improve the joint’s flexibility and to help reduce pain (Arthritis in Dogs, n.d.).

Osteoarthritis is the most common type or arthritis in dogs. Osteoarthritis, which is also commonly known as degenerative joint disease, is a debilitating disease that affects one in five dogs (Arthritis in Dogs, n.d.). Osteoarthritis can be either one of two types of arthritis: primary or secondary. Primary osteoarthritis is a condition in which cartilage in the joint degenerates as the dog ages. Secondary osteoarthritis occurs secondary to joint disease, instability of the joint, or abnormal pressure on cartilage in the joint (Texas A&M Veterinary Medicine & Biomedical Sciences, 2016).

Although osteoarthritis is not breed, age, or sex specific, there may be predisposing factors. Senior dogs tend to be more prone to developing arthritis as they age (Innes, 1995). Dogs who are overweight are more prone to arthritis due to increased impact on joints. Other factors, including genetics and injuries, may make some dogs more prone to arthritis than others (American College of Veterinary Surgeons, n.d.).

Symptoms of arthritis in dogs include activity impairment and pain. Dogs who are suffering from osteoarthritis may not exercise as much as they once did, or they may
have a harder time getting up or lying down. They may also experience depression and sleep more than they used to. In order to properly diagnose osteoarthritis, a veterinarian will perform a physical exam to assess joint mobility and pain and radiographs are used to determine the extent of damage to joints caused by arthritis (American College of Veterinary Surgeons, n.d.).

Since there is no cure for this disease, veterinarians do their best to make the dogs more comfortable. Weight management and exercise are common practices in managing arthritis in dogs. Exercise helps loosen stiff joints and helps dogs shed the extra pounds that are adding more stress to joints. Non-steroidal anti-inflammatory (NSAIDs) are a commonly prescribed medication to arthritic dogs. A disadvantage to NSAIDs is they eliminate pain associated with the joint damage caused by arthritis, but do not reverse damage and can produce side effects if given long term. Nutraceuticals are becoming more popular because they not only help with pain associated with arthritis, but they consist of ingredients found in cartilage, therefore promoting healthy joints and preventing further damage (Arthritis in Dogs, n.d.).

**Statement of the Problem**

According to literature, one in five dogs suffer from osteoarthritis and that use of certain pain management drugs, such as NSAIDs, may do more harm than good (Arthritis in Dogs, n.d.). A new nutraceutical, Flex Choice, was tested for efficacy and safety in this project.

**Purpose**

The purpose of this study was to determine anti-arthritic efficacy and safety of Flex Choice in moderately osteoarthritic dogs. A comparison of pain assessment,
radiography, physical examination, and blood tests was used by the researcher to test efficacy and safety of the nutraceutical. This multi-method study was composed of three major components. Assessment of pain was the first component. This was measured using a standardized scale and a physical exam was performed to help determine level of pain. Additionally, blood tests including chemistry panels, complete blood counts (CBC), and erythrocyte sedimentation rate (ESR) were used to assess the effects Flex Choice had on the body. Finally, radiographs were taken to compare the effects of Flex Choice on selected joints.

**Hypothesis**

H0: (Null) There will not be a clinically significant difference in canines treated with Flex Choice.

H1: (Research hypothesis) Canines treated with Flex Choice will have a clinically significant reduction in pain.

**Objective**

The research study was guided by the following objectives:

1. Determine anti-arthritic efficacy of the nutraceutical, Flex Choice, in moderately arthritic canines through subjective data, such as overall pain, pain after limb manipulation and pain after physical exertion.

2. Determine safety and tolerability of the nutraceutical, Flex Choice, in moderately arthritic canines through evaluation of objective data, such as heart rate, respiration rate, temperature, and serum chemistry values.
Definition of Terms

1. Osteoarthritis- the most common form of arthritis. It is caused by the wear and tear of a joint and gradually worsens over time (Center for Disease Control and Prevention, 2018).

2. Joint- Any point in which two or more bones are in contact (Pasquini et al., 2007).

3. Moderate Arthritis- the level of arthritis in which overall pain is described as a 4-6 on a scale of 0-10. Cartilage is compressed/damaged, and the bone becomes thickened. Exercise is painful for moderately arthritic dogs (Gupta et al., 2011).

4. Nutraceutical- a constituent of food that is used for human and animal health benefits for treating and preventing disease

5. Flex Choice- a nutraceutical that is being tested in this study. It is composed of krill oil, hyaluronic acid, astaxanthin, *Boswellia serrata* extract, green lipped mussel, and iron transport tocopheryl polyethylene glycol succinate (ITPGS).

6. NSAID (non-steroidal anti-inflammatory drug)- a class of drug that is commonly given to arthritic animals to reduce inflammation and pain in joints (Innes, 1995).

7. Pain- an unpleasant sensation that is conveyed to the brain through sensory neurons. The physical discomfort may lead to evasive action and cause injury (Gupta et al., 2011).

8. Cartilage- a fibrous connective tissue that is found at the end of bones in the joints that reduces friction and absorbs shock (Pasquini et al., 2007).

Limitations

This research was restricted by the following limitations:

1. The study was limited to dogs between 35 and 65 pounds.
2. The study was limited to 5 dogs that were considered to be moderately arthritic (overall pain of 4-6 on a scale of 0-10).
3. The study was limited to the use of Flex Choice only.
4. The study did not evaluate the effects of Flex Choice on dogs of different ages, sexes, or breeds.
5. The study did not attempt to prove that Flex Choice cures arthritis.
6. The study was limited to 150 days.

**Assumptions**

The following assumptions concerning this study were made:

1. The dogs’ owners would follow directions and give Flex Choice as instructed.
2. All dogs were not given any pain medication or treatments for arthritis for two weeks prior to the start of the study.
3. All dogs were free of any serious health conditions such as hepatic, renal, or heart disease or failure.
4. All owners would be honest in their assessment and accurately record the dogs’ daily pain level.
5. Differences of age, breed, sex, diet, and lifestyle would have no effect on the efficacy of Flex Choice.

**Statement of Significance**

A study to determine the efficacy and safety of new nutraceuticals such as Flex Choice, is important for a couple of reasons. First, if a nutraceutical is proven effective, then many dogs may receive relief from this debilitating disease of osteoarthritis. Second, if a nutraceutical is proven safe, there will be more, safer alternatives to invasive or
noninvasive treatments for osteoarthritis. Flex Choice is not intended to cure arthritis, but if proven effective, it will give relief to dogs who suffer from this disease and give owners a peace of mind that they are helping their dogs stay comfortable.

**Chapter Summary**

Arthritis does not discriminate, but through an understanding of canine osteoarthritis and treatments available, it is confirmed that more alternative measures are needed. In this particular study, it is predicted that Flex Choice will reduce pain and discomfort associated with canine arthritis. There are limitations on weight, duration, sample size and assumptions relying on owner compliance are present, but the researcher can draw significant conclusions based on subjective and objective parameters.
Chapter II: Review of Literature

Introduction

The purpose of this chapter is to establish the background of osteoarthritis in canines and the need for more management options. Literature and research available for osteoarthritis in dogs is vast and many researchers are looking for new ways to treat and manage the disease and pain in affected canines. Topics covered in this literature review were osteoarthritis in canines, joint function and anatomy, and treatment options.

Osteoarthritis in Canines

Osteoarthritis is a degenerative joint disease that affects dogs and humans, along with all mammals. Osteoarthritis is highly prevalent in adult dogs with more than 20% affected. There is no known predisposing factor to osteoarthritis, however, genetics, age, breed, and systemic changes such as hormones and disease have been shown to cause increased susceptibility. This disease is a very painful, inflammatory degenerative joint disease that affects synovial joints including the knee, elbow, hock and stifle. Although this disease is slow to progress, it will eventually lead to loss of mobility (Comblain, et al, 2015).

Although osteoarthritis may be defined in many ways, the American Academy of Orthopedic Surgeons defines osteoarthritis as:

“Osteoarthritic diseases are the result of mechanical and biologic events that destabilize normal coupling of degradation and
Some initiating factors of osteoarthritis include aberrant repair, degeneration of articular cartilage, formation of new bone at the articular margins, sclerosis of subchondral bone and low-grade synovial inflammations (Innes, 1995). These initiating factors, along with the American Academy of Orthopedic Surgeons’ definition, explain that osteoarthritis is caused by the breakdown of cartilage and formation of new bone in its place. This causes bone on bone action in joints and leads to pain.

Currently, there are three types of osteoarthritis: primary, secondary, and erosive osteoarthritis (Innes, 1995). Primary osteoarthritis, also known as idiopathic, has unidentified initiating factors. This type also symmetrically affects joints and can affect the knees, elbows, hocks, stifles, or any combination as long as joints on both sides (left and right limb) are affected. Primary osteoarthritis is not very common in dogs but has been documented in young adult dogs of the Chow Chow, Dalmatian, Samoyed, Labrador Retriever, and Spaniel breeds. Secondary osteoarthritis is the most common type and usually has an identifiable cause (Innes, 1995). Some causes of secondary osteoarthritis include trauma, development issues, inflammatory joint disease, or metabolic disease. Erosive, or atrophic, osteoarthritis is the third type of osteoarthritis. This type is not well documented in dogs, but it may occasionally be detected by erosive changes upon radiography (Innes, 1995).

Diagnosing osteoarthritis can be difficult since many owners do not seek veterinary care until major joint degradation has already occurred or it is brought up in conversation at annual vet visits. A history of the patient and examination of the clinical
Signs is necessary to diagnose osteoarthritis. Signs of osteoarthritis include chronic lameness, stiffness, decreased range of motion, and a thickened joint upon palpation, as well as the presence of crepitation (Merck, 2016). Joint thickening is caused by capsular fibrosis and osteophyte production. Other than evaluation of clinical signs, radiographs may be taken to visualize joints and see the extent of osteoarthritis. Arthrocentesis may also be performed to evaluate synovial fluid color and viscosity (Innes, 1995).

Since osteoarthritis is caused by pathological changes in articular cartilage, there is no known cure. Pain management measures are the only option in affected animals (Innes et al., 2010). There are several forms of pain management that range from invasive to non-invasive.

**Joint Function and Anatomy**

Joints are an important part of an animal’s body. Joints work together with bones, tendons, and ligaments to allow an animal to move. Synovial joints are the joints most commonly affected by osteoarthritis. They are diarthrodial, movable joints that are characterized by mobility, joint cavity, articular cartilage, synovial membrane, and a fibrous capsule (Pasquini et al., 2007).

Joint capsules are a two layered structure that surround joints and are composed of an outer fibrous layer and an inner synovial membrane. Fibrous layers attach to periosteum on or near margins of the articular cartilage and synovial membranes are highly vascular, nerve rich membranes that produce synovial fluid for joints. Synovial fluid has the consistency of a raw egg white and lubricates joints. It helps supply nutrients to joints and removes waste products. Articular cartilage covers ends of bones and helps to reduce friction and concussion. Ligaments are bands of fibrous connective tissue that
connect bone to bone. They help keep everything together but still allow for movement (Pasquini et al., 2007). Tendons are connective tissue that connect muscle to bone.

Synovial joints are called freely movable joints because they can move in different ways depending on what classification they are. Simple joints have two articular surfaces enclosed in a joint capsule while a compound joint has more than two. There are also plane joints that glide, ball-and-socket joints that have a head fitting into a pit, a hinge joint that can either increase or decrease the angles between bones, pivot joints that allow rotation, and ellipsoidal joints that are convex/concave (Pasquini et al., 2007). These are all types of synovial joints that allow the body to move and can be affected by osteoarthritis.

**Treatment Options for Osteoarthritis**

Canine osteoarthritis is a very painful disease that requires treatment to keep animals comfortable. Without some form of pain management, quality of life will be diminished. Fortunately, there are many ways to manage the pain associated with osteoarthritis, although precautions must be taken as some are not without adverse side effects. Some options for treatment include physical therapy, weight management, surgery, medications, and nutraceuticals.

**Physical Therapy and Weight Management**

Since osteoarthritis is a joint disease, mobility may be decreased as the disease progresses. It is important to keep dogs with osteoarthritis physically active to keep joints moving and at an adequate weight to prevent unnecessary force on joints. It is also important to monitor these dogs regularly to ensure they are maintaining movement and not gaining weight. (McKee, 2013). Use of a body condition score to monitor weight is
strongly advised when this method of arthritis management is selected. This method may also be used in conjunction with medication or a nutraceutical.

Obesity is described as accumulation of excess body fat and increased body weight causes excess pressure on joints and cartilage, causing more pain and degradation. Adipose tissue, or fat, is considered metabolically active and proinflammatory which leads to more inflammation in joints (Budsberg & Bartges, 2006). It is important to keep the animal active. Short walks with adequate rest in between are all that is needed.

**Surgery**

Surgery is usually the last resort for dogs with osteoarthritis unless it is secondary to an underlying cause. There are only three instances when surgery would be warranted: a) to treat underlying causes, b) as an attempt to treat osteoarthritis, or c) to remove or replace an osteoarthritic joint (Innes, 1995). There are surgeries that may be performed on any joint that can be affected by osteoarthritis. Common surgeries are a cheilectomy (removal of osteophytes), articular forage (drilling across metaphyseal scars), excision arthroplasty, joint replacement, and arthrodesis (fusion of joints) (Innes, 1995; Mckee, 2013).

**Medications and Nutraceuticals**

There are a few medications available that can be used for managing pain caused by osteoarthritis. There are two classes of drugs that are commonly administered: non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Nutraceuticals are also another option rising in popularity.

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of drug that are considered a mainstay in pain management as they are an analgesic as well as an anti-
inflammatory. These drugs relieve clinical signs associated with osteoarthritis without a myriad of potential metabolic and immunosuppressive adverse effects that are commonly associated with corticosteroids (Merck, 2016). NSAIDs act primarily to reduce biosynthesis of prostaglandins by inhibiting cyclooxygenase (COX). There are two different isoforms of COX that have been discovered (COX-1 and COX-2) and this discovery has led to a greater understanding of the mode of action and adverse effects of NSAIDs. COX-1 is found in most body tissues and is responsible for mediating a variety of normal physiological effects. This includes hemostasis, GI mucosal protection and protection of the kidney from hypotensive insult. COX-2 is activated in damaged and inflamed tissues and also catalyzes the formation of prostaglandin, which is associated with inflammatory response. One downside to use of NSAIDs is that they cannot differentiate between the two isoforms of COX, inhibiting positive COX-1 enzymes. This contributes to common side effects often seen with use of NSAIDs (Merck, 2016).

Commonly used NSAIDs include Carprofen, Metacam, Previcox, and others. All NSAIDs have the potential to induce adverse effects, including vomiting, gastritis and ulceration. GI blood loss may occur and result in iron-deficient anemia. Nephropathies and hepatopathies have also been recognized with long term used of NSAIDs (Merck, 2016). NSAIDs may be used short or long term. However, use must be monitored with regular blood work to ensure that organ function is not compromised.

Corticosteroids are another class of drug used to manage pain associated with osteoarthritis. Glucocorticoids are the most common corticosteroids used. Glucocorticoids suppress inflammatory responses by interacting with specific intracellular receptor proteins in target tissues. Results of the end process include blocked
synthesis of prostaglandins, inhibited production of COX enzymes, cytokines, and enzymes such as collagenases and aggrecanases. Adverse effects of corticosteroid use include polyuria, polydipsia, suppressed immune system, hepatopathy, and reduced collagen synthesis (Merck, 2016). Corticosteroids were once commonly used to treat osteoarthritis, but NSAIDs have now become a popular treatment.

Nutraceuticals are natural supplements that have been shown to reduce osteoarthritis effects in dogs. They can be used individually or in combination to form a supplement in which effects of each compound are combined to reach a desired effect. Nutraceuticals are becoming a popular alternative to other medications such as NSAIDs, as they have little to no side effects and are able to lessen pain and discomfort that is associated with canine arthritis. There are many different nutraceuticals on the market and more are being added regularly due to the increase in popularity. One of the more recently introduced nutraceuticals is Flex Choice.

**Flex Choice**

Flex Choice is categorized as a nutraceutical and was chosen for this study based on its analgesic and anti-inflammatory properties. Flex Choice is a joint supplement that is composed of krill oil, hyaluronic acid, astaxanthin, *Boswellia serrata* extract, green lipped mussel, and iron transport tocopheryl polyethylene glycol succinate (ITPGS).

Krill oil is a source of Omega-3 fatty acids and has been found as a source of phospholipids and antioxidants. Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs) and are beneficial for treatment of canine osteoarthritis. Fish oils, including krill oil, are excellent sources of two out of three types of omega-3 fatty acids involved in mammalian systems: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).
Omega-3 fatty acids act by reducing IL-1β, PGE₂, ADAMTS-4, COX-2, IL-1α, 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 have a way to protect activation of autophagy in chondrocytes by modulating mammalian targets of rapamycin (mTOR) signaling, therefore making them an effective way of treating osteoarthritis (Gupta et al, 2019).

Hyaluronic acid (HA) is an anionic, nonsulfated glycosaminoglycan (GAG) that is produced by chondrocytes and synovial fibroblasts. HA is an important compound in articular cartilage as it coats each chondrocyte. HA size decreases with age but increases in volume. It has been shown that treatment with HA of higher molecular weight is more effective. There are two common methods of administering HA: intra-articularly (IA) and orally. IA treatment with HA has been used for decades as a therapy for osteoarthritis in horses and canines. In this method, HA is injected directly into affected joints. Oral administration of high molecular weight HA has been proven to also reach joints, making it a justified route of supplementation. There is a third method of administration, intravenous, but it has not gained popularity and has only been tested in horses. HA is effective at treating osteoarthritis as it may have a role in regulating synthesis of proteoglycans during maturation of articular cartilage. It may also play a role in repair processes of articular joints. 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 is associated with pain from osteoarthritis. In osteoarthritis, treatment is usually the only option for management, but studies have shown that HA prevents degradation of cartilage and may even promote
regeneration. There are some adverse side effects associated with HA, but they are mainly associated with IA administration route. These side effects include muscle pain, cramping, pain in the injected joint, and swelling of arms and legs. This may make movement difficult. Overall, HA is a slow acting anti-osteoarthritic agent that can be used as an anti-inflammatory disease-modifying agent in osteoarthritis (Gupta, 2016; Gupta et al, 2019a, b).

Astaxanthin (AXT) is a xanthophyll carotenoid that is present in many microalgae and yeasts. AXT possesses strong antioxidant activity because it neutralizes singlets of oxygen, scavenges free radicals, inhibits lipid peroxidation, enhances immune system function, and regulates gene expression. AXT also contributes to glucose metabolism and decreases lipid accumulation in the liver (Gupta, 2016). Although AXT does not have any direct links to managing or treating osteoarthritis, it does have many health benefits that contribute to its use as a supplement. AXT can be used for neuroprotection by reducing oxidative stress, suppressing expression of inflammatory cytokines, protecting gastric mucosal linings by stimulating the anti-oxidative mechanism, and by boosting the immune system. There have not been any side effects reported from use of AXT as a dietary supplement (Gupta, 2016).

*Boswellia serrata*, commonly called Indian Frankincense, is a plant that is native to India and its extract has many health benefits. Boswellic acids (alpha- and beta-boswellic acids) are the main component of *B. serrata* extract. These are organic acids that consist of a pentacyclic triterpene, a carboxyl group, and at least one other functional group. Acetyl-keto-beta-boswellic acid (AKBA) contributes most to health benefits and is present in concentrations of 2-3% of the extract. Boswellic acids have been effective in
treatments for ulcerative colitis, chemically induced hepatic damage, bronchial asthma, and other diseases such as osteoarthritis. Acetyl-boswellic acids have exhibited anti-inflammatory properties by inhibiting leukotriene synthesis. In some studies, 30% AKBA provided improvement in joint mobility and comfort in as little as one week (Gupta, 2016).

Green lipped mussel (GLM), *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 treatment of osteoarthritis as they exert anti-inflammatory activities and lubricate joints. ETA also plays a role as it acts as a dual inhibitor of arachidonic acid oxygenation by COX and lipoxygenase pathways. Use of GLM has a similar mechanism of action as NSAIDs without adverse side effects. GLM is a gastroprotective agent and does not affect platelet aggregation. This suggests that ETA may be selective in only blocking COX-2 while sparing COX-1. There have been no reported adverse side effects from administration of GLM (Gupta et al, 2019a).

Iron transport tocopheryl polyethylene glycol succinate (ITPGS) exerts several biological and pharmacological actions (antioxidative, anti-inflammatory, and immunomodulatory) through multiple mechanisms. ITPGS in Flex Choice, by serving as bioenhancer, might have improved the absorption and bioavailability of ingredients that have anti-osteoarthritic properties (Srivastava et al., 2019).

**Theoretical Framework**

At the base of this theoretical framework is knowledge that there is no known cure for osteoarthritis. Management practices are the only way to keep affected animals comfortable and these practices include medications, nutraceuticals, weight management,
and surgery. With this knowledge, several researchers have set out to try and find new medications, procedures, and nutraceuticals that will provide animals utmost comfort and relief from pain.

Nutraceuticals are becoming more and more popular for use in veterinary medicine to help alleviate signs and symptoms of common problems, including osteoarthritis. New nutraceuticals are being discovered and tested by researchers on a regular basis. Flex Choice is a new nutraceutical that was studied as a part of this thesis study in hopes of finding a new product to help with signs and symptoms associated with osteoarthritis.

By providing more than one option to manage osteoarthritis, owners will have a chance to choose which product or procedure that will fit best with their budget and lifestyle. Medications are an option and relieve symptoms while nutraceuticals have been shown to provide joints with ingredients needed to prevent further damage. Surgery is an option, although expensive, if medications or nutraceuticals are not effective or an underlying issue is causing osteoarthritis.

Summary

This review of literature described many methods that provide pain management for dogs affected by osteoarthritis. There is no cure for this debilitating disease, but there are steps that can be taken to minimize pain. Owners can choose to put their dogs on medication to alleviate pain, try administering a nutraceutical to help with signs and symptoms, use weight management and physical therapy to help prevent further damage, or surgery. Out of these options, nutraceuticals are popular as there are new ones put on the market all the time. According to literature, there are already many nutraceuticals
available today, but researchers are trying and testing new compounds and combining compounds in hopes of finding one that is even better and has less long-term effects than the ones before. This will give owners options to try different formulas to see what works best for their dog. The purpose of this study was to test the efficacy and safety of a new nutraceutical, Flex Choice, in osteoarthritic dogs.
Chapter III: Methodology

Introduction

The purpose of this study was to observe and analyze effects of Flex Choice (provided by Vets Plus, Inc. of Menomonie, WI) on moderately osteoarthritic dogs over a 150-day period. The study was designed to examine whether or not Flex Choice would alleviate some of the pain associated with arthritis. This study was also conducted to determine if any adverse effects occur in liver, kidney, or heart function due to administration of Flex Choice. Data was collected through subjective observations, including limb manipulation, physical exertion and overall pain levels and vital signs and blood serum chemistries. Objectives of this study were as follows:

1. Determine if Flex Choice decreases pain and inflammation caused by arthritis based on subjective data, such as overall pain, pain during limb manipulation, and pain after physical exertion.

2. Determine if Flex Choice may increase flexibility and range of motion in affected joints.

3. Determine safety of Flex Choice by observing overall health, such as heart rate, respiration rate, temperature, and liver, kidney, and heart function.

4. Evaluate before and after radiographs to determine if affected joints exhibited improvements.
Population and Sample Selection

This study was composed of five moderately osteoarthritic dogs that were selected based on limitations stated in Chapter I, such as weight and level of arthritis. Sources used to obtain dogs included flyers posted around Murray, KY, Humane Society of Calloway County, posts published on social media websites such as Facebook, and flyers sent to faculty and students of Murray State University. Limitations on weight reduced possible candidates as most interest came from owners of 70+ pound dogs. Population owners consisted of students and faculty of Murray State University. Owners volunteered their dogs by signing a consent form consenting the use of their dog, administering Flex Choice as directed, and meeting with the researcher every 30 days. This form is located in Appendix B. This selection was purposive and dependent on owners’ willingness to provide dogs. All five canines were used for duration of the study.

Context of the Study

Study location remained consistent throughout the entire 150 days. Owners brought their dogs to Carmen Pavilion at Murray State University each month. Carmen Pavilion provided a neutral location for evaluation and had all amenities necessary for proper evaluations. Carmen Pavilion Laboratories provided ample room to perform physical examinations and pain evaluations as well as centrifuge blood samples. Long hallways located inside provided ample room for physical exertion, and radiograph equipment was available in a nearby suite of rooms. Weight in pounds was obtained through use of an electronic scale located just outside the radiology suite. Research was performed during normal business hours without interruption to scheduled classes.
Research Design

Time series design is best suited to represent data within this study. All 5 dogs were included in the treated group and baseline values collected on Day 0 were used as controls. Dogs received Flex Choice soft chews orally twice daily. Subjective and objective data were collected on each dog starting with baseline values on Day 0 and ending on Day 150 with sample collections every 30 days in between. Due to some scheduling conflicts, some collections were not obtained exactly 30 days apart. Table 1 below illustrates this study’s design.

Table 1: Simple Time Series Design

<table>
<thead>
<tr>
<th>Date</th>
<th>12/4 Day 0</th>
<th>30 Days</th>
<th>1/7 Day 30</th>
<th>30 Days</th>
<th>2/7 Day 60</th>
<th>30 Days</th>
<th>3/7 Day 90</th>
<th>30 Days</th>
<th>4/2 Day 120</th>
<th>30 Days</th>
<th>5/6 Day 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
<td>Obs Tx</td>
</tr>
</tbody>
</table>

*Obs (Observation)*  
*Tx (Treated)*

Data Collection Methods

All data were collected from each of five canines every 30 days for the entire study duration, a total of 150 days. Dogs in this study were assessed using six different methods of subjective and objective measurements: pain assessment, physical examination, blood chemistry, complete blood counts, erythrocyte sedimentation rate, and radiography. Owners also received a questionnaire to fill out monthly to assess their dogs pain levels at home. This form is located in Appendix C. Physical exams and pain assessments were conducted by the researcher on Day 0 and every 30 days during study duration. See Appendix D for researcher physical exam and pain assessment forms.
**Subjective Data**

There were three main scales used to observe pain in arthritic canines:

1. Overall pain was observed on a scale of 1-10 with 1 equaling slight pain and 10 equaling severe and constant pain. Evaluations for overall pain included gait quality, rising from a sitting or lying position, and lowering into a sitting or lying position. Owner evaluations were also taken into consideration.

2. Pain during limb manipulation was observed on a scale of 0-4 with a 2-2.5 indicating moderate arthritis. Pain was assessed based on vocalization, body posture, resistance, flexibility, and range of motion.

3. Pain after physical exertion was observed on a scale of 0-4 with 2-2.5 equaling moderate arthritis. Pain was assessed 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.

**Objective Data**

Objective data were recorded during physical examination on Day 0 and every 30 days until Day 150. Heart rate and respiration rate were observed using a stethoscope. Results were recorded in beats per minute. Body temperature was recorded using a digital rectal thermometer set to degrees Fahrenheit. Body weight was measured using an electronic scale and recorded in pounds. Radiographs were taken on Days 0 and 150 to determine if administration of Flex Choice may reverse some arthritic changes in joints.

**Blood Collection**

Blood samples were collected from canine research subjects using jugular or cephalic veins with a 20-gauge needle and a 5cc syringe. Samples were then placed into a
3.5 ml serum separator tube and a 2 ml EDTA tube. Serum separator tubes were allowed to clot for 20 minutes and then were centrifuged for ten minutes. Serum was then removed from the clot and placed in a 2 ml red top tube using a pipette. Samples were then refrigerated until transport to Murray State University Breathitt Veterinary Center Diagnostic Laboratory. Ms. Jean Miller of the clinical pathology department at Breathitt analyzed serum for kidney, liver, heart and muscle function biomarkers using a Beckman AU 480 serum analyzer and whole blood was used for an erythrocyte sedimentation rate (ESR) and complete blood count (CBC) using a Sysmex XT-2000iV. ESR was used to test for inflammatory biomarkers.

**Data Analysis**

All data were subjected to Microsoft Excel data analysis. Data were analyzed using simple paired T-tests. Values from the T-tests with \( P < 0.05 \) were considered statistically significant when compared to Day 0 values. Graphs were also created using Microsoft Excel.

**Reliability and Validity**

As with any research study, precautions must be taken in order to ensure credibility and trustworthiness of collected data and methods of analysis. In this study, steps were taken to ensure validity. For collection of subjective and objective data, exams and assessments were performed consistently in manner and order each month. This improved consistency when data was recorded in numerical form. Radiographs were also performed using identical techniques to ensure comparisons were accurate when evaluating joint changes.
Chapter IV: Results

Overview

All five canines that participated in this study were used in the treatment group due to low population size selected for this study. Each owner was given 150 days’ worth of Flex Choice soft chews. Owners were instructed to orally administer one soft chew in the morning and one soft chew in the evening for 150 days. The researcher evaluated canine subjects on Days 0, 30, 60, 90, 120, and 150 at Carmen Pavilion at Murray State University. Each canine was evaluated for overall pain by observing pain from rising from a sitting or lying position, lowering into a sitting or lying position, overall gait, and owner’s observation of overall pain. Each canine was also evaluated for pain during limb manipulation by observing vocalization, flexibility, resistance, and range of motion. Pain after physical exertion was also evaluated after two minutes of jogging. Radiographs were performed on days 0 and 150 and then compared to observe any internal changes in joints. Blood samples were collected on Day 0 and every 30 days for the duration of the study, and serum samples were analyzed for changes in liver, kidney, and heart and muscle function.

All data were recorded and statistically analyzed and mean ± standard error of means were calculated for each parameter on Day 0 and every 30 days thereafter. All values are shown in Tables 2 through 9. Some values could not be calculated, such as respiration rates due to panting.
Oral administration of Flex Choice soft chews led to a significant reduction in pain in at least one of the final months of treatment and evaluation. Overall pain was significantly reduced starting on Day 30 (P < 0.05) and pain during limb manipulation was significantly reduced on Days 30 and 120 (P < 0.05) and pain after physical exertion was significantly reduced on Days 120 and 150 (P < 0.05). Body weight, temperature, respiration, and all but one blood serum chemistry analysis showed no statistical significance. Creatine kinase showed a significant reduction on Days 30 and 150 (P < 0.05) and heart rate showed a significant reduction starting on Day 60 (P < 0.05).

**Overall Pain**

Overall pain was evaluated in five canines over a period of 150 days. Baseline values were collected on Day 0 and canines were reevaluated every 30 days for study duration. Levels of overall pain were observed based on a scale of 1 to 10 with 1 indicating slight pain and 10 indicating severe and constant pain. Canines used in this study had a baseline value of overall pain ranging from 4 to 6. Determination of pain levels were observed based on quality of gait, ability to rise from a sitting or lying position, ability to lower into a sitting or lying position, range of motion, vocalization, and body posture. Input from owners on monthly questionnaires were also taken into account as owners saw their dog on a daily basis.

Table 2 shows results from Day 0 through Day 150. Baseline values on Day 0 showed a mean value of 4.6 ± 0.40, indicating pain from moderate arthritis. Overall pain was significantly reduced (P < 0.05) starting on Day 30 to 3.6 ± 0.40 and continued to have a significant reduction (P < 0.05) for Days 60, 90, 120, and 150 (3.6 ± 0.60, 3.4 ± 0.51, 3.2 ± 0.49, and 2.4 ± 0.40 respectively) from Day 0.
Table 2: Effects of Flex Choice on Overall Pain Level in Arthritic Canines

<table>
<thead>
<tr>
<th>Day</th>
<th>Dog</th>
<th>Level</th>
<th>Mean ± SEM</th>
<th>Day</th>
<th>Dog</th>
<th>Level</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Bailey Reecey</td>
<td>5</td>
<td>4.6 ± 0.40</td>
<td>90</td>
<td>Bailey Reecey</td>
<td>3</td>
<td>3.4 ± 0.51 *</td>
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<tr>
<td></td>
<td>Brutus</td>
<td>4</td>
<td></td>
<td></td>
<td>Brutus</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>6</td>
<td></td>
<td></td>
<td>Goblin</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>4</td>
<td></td>
<td></td>
<td>Lucchese</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Bailey</td>
<td>3</td>
<td>3.6 ± 0.40 *</td>
<td>120</td>
<td>Bailey</td>
<td>3</td>
<td>3.2 ± 0.49 *</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
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<td></td>
<td></td>
<td>Reecey</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
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<td>Brutus</td>
<td>5</td>
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<tr>
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<td></td>
<td>Lucchese</td>
<td>3</td>
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</tr>
<tr>
<td>60</td>
<td>Bailey</td>
<td>3</td>
<td>3.6 ± 0.60 *</td>
<td>150</td>
<td>Bailey</td>
<td>2</td>
<td>2.4 ± 0.40 *</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
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<td></td>
<td>Reecey</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
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<td></td>
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<td>Brutus</td>
<td>4</td>
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<tr>
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<td>Goblin</td>
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<td></td>
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<td></td>
<td></td>
<td>Lucchese</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically different from the value of Day 0 (P < 0.05)
Pain During Limb Manipulation

Pain during limb manipulation was evaluated in five canines over a period of 150 days. Baseline values were collected on Day 0 and canines were reevaluated every 30 days for study duration. Levels of pain were observed on a scale of 0 to 4 with 0 indicating no pain and 4 indicating severe and constant pain. Moderate arthritic canines would exhibit a 2 to 2.5 on this scale. Each limb was assessed in standing recumbency while simultaneously observing vocalization, body posture, stiffness/resistance, integrity, and crepitus in the joints.

Table 3 shows results of pain during limb manipulation from Day 0 to Day 150. Baseline values on Day 0 show a mean value of 2.0 ± 0.00. Pain during limb manipulation showed a significant reduction (P < 0.05) in pain to 1.6 ± 0.10 on Day 30 and again on Day 120 to 1.3 ± 0.12 compared to Day 0.
Table 3: Effects of Flex Choice on Pain from Limb Manipulation in Arthritic Canines

<table>
<thead>
<tr>
<th>Day</th>
<th>Dog</th>
<th>Level</th>
<th>Mean ± SEM</th>
<th>Day</th>
<th>Dog</th>
<th>Level</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Bailey</td>
<td>2</td>
<td>2.0 ± 0.00</td>
<td>0</td>
<td>Lucchese</td>
<td>2</td>
<td>1.8 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Bailey</td>
<td>1.5</td>
<td>1.6 ± 0.10 *</td>
<td>120</td>
<td>Bailey</td>
<td>1.5</td>
<td>1.3 ± 0.12 *</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Bailey</td>
<td>2</td>
<td>1.5 ± 0.22</td>
<td>150</td>
<td>Bailey</td>
<td>2</td>
<td>1.4 ± 0.24</td>
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<tr>
<td></td>
<td>Reecey</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically different from the value of Day 0 (P < 0.05)
Pain After Physical Exertion

Pain after physical exertion was observed in five canines starting on Day 0 until Day 150. Baseline values were collected on Day 0 and canines were reevaluated every 30 days for the study duration. Pain was observed on a scale of 0 to 4 with 0 indicating no pain and 4 indicating severe and constant pain. Moderately arthritic canines will exhibit a 2 to 2.5 on this scale. All five dogs were assessed for pain after limb manipulation based on vocalization, body posture, flexibility, resistance, and range of motion after 2 minutes of jogging. Canines were also observed for evidence of lameness and exercise intolerance.

Table 4 shows results of pain after physical exertion from Day 0 to Day 150. Baseline values on Day 0 exhibited a mean value of 2.0 ± 0.00. Evaluation of pain after physical exertion resulted in statistically significant reduction (P < 0.05) in pain to 1.2 ± 0.12 on Day 120 and again on Day 150 to 1.2 ± 0.25 compared to values on Day 0.
Table 4: Effects of Flex Choice on Pain after Physical Exertion in Arthritic Canines

<table>
<thead>
<tr>
<th>Day</th>
<th>Dog</th>
<th>Level</th>
<th>Mean ± SEM</th>
<th>Day</th>
<th>Dog</th>
<th>Level</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Bailey</td>
<td>2</td>
<td>2.0 ± 0.00</td>
<td>90</td>
<td>Bailey</td>
<td>2</td>
<td>1.7 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
<td>2</td>
<td></td>
<td></td>
<td>Reecey</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>2</td>
<td></td>
<td></td>
<td>Brutus</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>2</td>
<td></td>
<td></td>
<td>Goblin</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>2</td>
<td></td>
<td></td>
<td>Lucchese</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Bailey</td>
<td>2</td>
<td>2.1 ± 0.10</td>
<td>120</td>
<td>Bailey</td>
<td>1</td>
<td>1.2 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
<td>2</td>
<td></td>
<td></td>
<td>Reecey</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>2.5</td>
<td></td>
<td></td>
<td>Brutus</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>Goblin</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Lucchese</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Bailey</td>
<td>2</td>
<td>1.8 ± 0.20</td>
<td>150</td>
<td>Bailey</td>
<td>1.5</td>
<td>1.2 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
<td>1.5</td>
<td></td>
<td></td>
<td>Reecey</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>2.5</td>
<td></td>
<td></td>
<td>Brutus</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>1.5</td>
<td></td>
<td></td>
<td>Goblin</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>1.5</td>
<td></td>
<td></td>
<td>Lucchese</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically different from the value of Day 0 (P < 0.05)
Effects of Flex Choice on Body Weight, Heart Rate, Respiration Rate, Temperature, and Biological Parameters in Serum.

Body weight, heart rate, respiration rate, temperature and biological parameters in serum were evaluated in all five canines starting with baseline values on Day 0 and reevaluated every 30 days thereafter for study duration.

Table 5 presents results of body weight in pounds for all five canines participating in this study. Body weight was recorded using an electronic scale located near the radiology suite in Carmen Pavilion. There were some fluctuations in weight, including some that went outside limitations of this study. Body weights were recorded in lbs (mean ± SEM). Mean value 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.

Table 6 presents results of heart rate in beats per minutes of five canine participants. Heart rate was observed using a stethoscope early in data collection process and values were recorded. The researcher attempted to keep canines as calm as possible to get an accurate recording. Some fluctuations were present due to canine excitement and nervousness. Mean value on Day 0 was 132.8 ± 11.55 with a significant difference (P < 0.05) observed starting on Day 60 (88.4 ± 7.33) and continued to have a significant reduction on Days 90, 120, and 150 (109.6 ± 15.88, 107.2 ± 10.84, and 112.8 ± 13.05 respectively) compared to Day 0. This statistical significance could be due to many factors including level of excitement, level of pain, and nervousness. On Day 0, canines were very nervous and excited as they were in a new place with a new person performing
examinations. As each evaluation was complete, canines may have become more familiar with surroundings and comfortable with the researcher, therefore reducing heart rate.

Table 7 presents results of respiration rate in terms of breaths per minute of all five canines. Respiration rate was measured using a stethoscope early in data collection process and values were recorded. The researcher attempted to get an accurate measurement but panting interfered with valid measurement and values were unable to be obtained. Therefore, statistical analysis, as well as evaluation of the mean ± SEM, were not available.

Table 8 presents the results of temperature in terms of degrees Fahrenheit of all five canines. Temperature was measured by using a digital rectal thermometer and values were recorded. Mean value on Day 0 was 101.9 ± 0.27 and Day 150 was 101.4 ± 0.24. No significant differences (P > 0.05) were observed in all canines throughout study duration.

Table 9 presents data from serum chemistry parameters of all five canine participants. Blood chemistry parameters included BUN, creatinine, total bilirubin, direct bilirubin, ALT, AST, and creatine kinase. Blood samples were collected through venipuncture of either jugular or cephalic vein and serum was separated and transported to Breathitt Veterinary Center for analysis. There were some slight variations present possibly due to sample hemolysis and time between collection and analysis. Results of serum chemistries for BUN, creatinine, total bilirubin, direct bilirubin, ALT, and AST were not statistically significant (P > 0.05) from values of Day 0 throughout study duration. Creatine kinase was significantly reduced (P < 0.05) on Day 30 (106.0 ± 19.15) and Day 150 (112.8 ± 15.81) when compared to Day 0 (165.6 ± 18.17). This reduction
could be due to delayed sample analysis or individual canine variation as CK has a very short half-life in the blood. Even with a statistical reduction, the values for CK remained within reference interval.

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.
Table 5: Effects of Flex Choice on Body Weight (lb.) of Arthritic Canines

<table>
<thead>
<tr>
<th>Day</th>
<th>Dog</th>
<th>Weight</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Bailey</td>
<td>63.4</td>
<td>52.4 ±5.71</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
<td>56.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>64.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>41.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>36.4</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Bailey</td>
<td>63.4</td>
<td>52.8 ± 5.89</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
<td>57.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>65.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>42.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>35.6</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Bailey</td>
<td>65.6</td>
<td>53.0 ± 6.72</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
<td>58.8</td>
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</tr>
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<td></td>
<td>Brutus</td>
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</tr>
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<td>Lucchese</td>
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<tr>
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<td>Bailey</td>
<td>65.6</td>
<td>53.1 ± 6.71</td>
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<td>Reecey</td>
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<tr>
<td></td>
<td>Brutus</td>
<td>68.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>41.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>34.1</td>
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</tr>
<tr>
<td>120</td>
<td>Bailey</td>
<td>62.1</td>
<td>52.9 ± 6.42</td>
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<td>Reecey</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>67.0</td>
<td></td>
</tr>
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<td></td>
<td>Goblin</td>
<td>41.2</td>
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</tr>
<tr>
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<td>Lucchese</td>
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<tr>
<td>150</td>
<td>Bailey</td>
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<td>52.6 ± 6.57</td>
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<td>Brutus</td>
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<td></td>
</tr>
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<td>Goblin</td>
<td>39.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
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No statistically significant differences from the value of Day 0 (P > 0.05)
Table 6: Heart Rate/Min. of Arthritic Canines Treated with Flex Choice

<table>
<thead>
<tr>
<th>Day</th>
<th>Dog</th>
<th>BPM</th>
<th>Mean ± SEM</th>
<th>Day</th>
<th>Dog</th>
<th>BPM</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Bailey</td>
<td>168</td>
<td>132.8 ±11.55</td>
<td>90</td>
<td>Bailey</td>
<td>140</td>
<td>109.6 ±15.88*</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
<td>108</td>
<td></td>
<td></td>
<td>Reecey</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>152</td>
<td></td>
<td></td>
<td>Brutus</td>
<td>152</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>116</td>
<td></td>
<td></td>
<td>Goblin</td>
<td>104</td>
<td></td>
</tr>
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<td></td>
<td>Lucchese</td>
<td>120</td>
<td></td>
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<td>Lucchese</td>
<td>72</td>
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<td>30</td>
<td>Bailey</td>
<td>120</td>
<td>120.8 ±12.55</td>
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<td>Bailey</td>
<td>144</td>
<td>107.2 ±10.84*</td>
</tr>
<tr>
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<td>Reecey</td>
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<td></td>
<td>Reecey</td>
<td>80</td>
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<td></td>
<td>Brutus</td>
<td>116</td>
<td></td>
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<tr>
<td></td>
<td>Goblin</td>
<td>116</td>
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<td>Goblin</td>
<td>96</td>
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<td></td>
<td>Lucchese</td>
<td>96</td>
<td></td>
<td></td>
<td>Lucchese</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Bailey</td>
<td>102</td>
<td>88.4 ± 7.33 *</td>
<td>150</td>
<td>Bailey</td>
<td>140</td>
<td>112.8 ±13.05 *</td>
</tr>
<tr>
<td></td>
<td>Reecey</td>
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<td></td>
<td>Reecey</td>
<td>92</td>
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<td></td>
<td>Brutus</td>
<td>84</td>
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<td></td>
<td>Brutus</td>
<td>148</td>
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<td></td>
<td>Goblin</td>
<td>108</td>
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<td>Goblin</td>
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<td></td>
<td>Lucchese</td>
<td>68</td>
<td></td>
<td></td>
<td>Lucchese</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically different from the value of Day 0 (P < 0.05)
Table 7: Respiration Rate/Min. of Arthritic Canines Treated with Flex Choice

<table>
<thead>
<tr>
<th>Day</th>
<th>Dog</th>
<th>BPM</th>
<th>Mean ± SEM</th>
<th>Day</th>
<th>Dog</th>
<th>BPM</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Bailey</td>
<td>Pant 24</td>
<td>N/A</td>
<td>90</td>
<td>Bailey</td>
<td>Pant 24</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Reecey Blown</td>
<td>Pant</td>
<td></td>
<td></td>
<td></td>
<td>Pant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>Pant</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>Pant</td>
<td></td>
<td></td>
<td></td>
<td>Pant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>Pant</td>
<td></td>
<td></td>
<td></td>
<td>Pant</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Bailey</td>
<td>Pant 28</td>
<td>N/A</td>
<td>120</td>
<td>Bailey</td>
<td>Pant 20</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Reecey Blown</td>
<td>Pant 32</td>
<td></td>
<td></td>
<td></td>
<td>Pant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>Pant</td>
<td></td>
<td></td>
<td></td>
<td>Pant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>Pant</td>
<td></td>
<td></td>
<td></td>
<td>Pant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>Pant</td>
<td></td>
<td></td>
<td></td>
<td>Pant</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Bailey</td>
<td>Pant 28</td>
<td>N/A</td>
<td>150</td>
<td>Bailey</td>
<td>Pant 40</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Reecey Blown</td>
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<td></td>
<td>Pant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>Pant</td>
<td></td>
<td></td>
<td></td>
<td>Pant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lucchese</td>
<td>Pant</td>
<td></td>
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</tbody>
</table>
Table 8: Temperature (°F) of Arthritic Canines Treated with Flex Choice

<table>
<thead>
<tr>
<th>Day</th>
<th>Dog</th>
<th>Temp (°F)</th>
<th>Mean ± SEM</th>
<th>Day</th>
<th>Dog</th>
<th>Temp (°F)</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Bailey</td>
<td>102.9</td>
<td>101.9 ± 0.27</td>
<td>90</td>
<td>Bailey</td>
<td>102.9</td>
<td>101.8 ± 0.36</td>
</tr>
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<td>101.6</td>
<td></td>
<td></td>
<td>Reecey</td>
<td>101.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>102.2</td>
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<td>Brutus</td>
<td>101.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>101.4</td>
<td></td>
<td></td>
<td>Goblin</td>
<td>102.5</td>
<td></td>
</tr>
<tr>
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<td>Lucchese</td>
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<td>Lucchese</td>
<td>101.2</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Bailey</td>
<td>104.2</td>
<td>101.9 ± 0.62</td>
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<td>Reecey</td>
<td>101.5</td>
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</tr>
<tr>
<td></td>
<td>Brutus</td>
<td>101.6</td>
<td></td>
<td></td>
<td>Brutus</td>
<td>101.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goblin</td>
<td>101.4</td>
<td></td>
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</tr>
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<td>Lucchese</td>
<td>101.6</td>
<td></td>
<td></td>
<td>Lucchese</td>
<td>101.6</td>
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</tr>
<tr>
<td>60</td>
<td>Bailey</td>
<td>102.6</td>
<td>101.5 ± 0.45</td>
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</tr>
<tr>
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<td>Goblin</td>
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No statistically significant differences from the value of Day 0 (P > 0.05)
Table 9: Biochemical Parameters of Canines Treated with Flex Choice

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

*Statistically different from the value(s) of Day 0 (P < 0.05)
Radiographs

Radiographs were taken on Day 0 and Day 150 on all five canines. Care was taken to attempt to keep technique and positioning the same on each day to improve comparison. There were no appreciable differences between radiographs from Day 0 to Day 150 observed on any canine. 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. 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 canines. 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. See Appendix E for radiographs.
Chapter V: Discussion and Conclusion

Discussion

Osteoarthritis is a chronic debilitating disease that does not discriminate. It effects both human and animals, with dogs suffering more often than other species due to excessive exercise, injury, obesity, genetic disposition, or poor nutrition. Approximately 20 to 25% of the roughly 90 million dogs in the United States have been diagnosed with arthritis. This leads to researchers testing different remedies of easing pain, improving joint flexibility, and improving quality of life in those canines affected with this disease. Lifestyle changes such as weight loss management may help some canines, but others require medication to reduce the pain associated with arthritis. Non-steroidal anti-inflammatories (NSAIDs) are the most commonly prescribed medications for management of arthritis. These medications, however, are not without risk. Canines may exhibit decreased appetite, vomiting, gastrointestinal irritation or ulceration, or hepatopathies with long term use (Merck, 2016). Due to these side effects, researchers and veterinarians are looking for new methods of pain management.

Nutraceuticals, such as Flex Choice, have become a popular choice among owners and veterinarians as they provide excellent pain management with little to no side effects and do not require a prescription. Canine participants in this study were given one Flex Choice soft chew twice daily for 150 days. Baseline values for overall pain, pain during limb manipulation, pain after physical exertion, vital signs, weight, and blood
chemistry parameters were obtained on Day 0 and reevaluated every 30 days for study duration. Evaluation of overall pain was based on a scale of 1 to 10 and canines were observed for quality of gait, vocalization, ability to sit, lay down, or stand, and body posture. Evaluation of pain during limb manipulation was based on a scale of 0 to 4 observing vocalization, body posture, flexibility and range of motion, resistance, and crepitus of joints. Evaluation of pain after physical exertion was based on a scale of 0 to 4, observing canines for vocalization, lameness, reluctance to move, and overall attitude after two minutes of jogging. Body weight, heart rate, respiration rate, temperature, and serum chemistry analysis were evaluated each time to ensure the safety of the canine throughout the entire duration of the study. Radiographs were taken on Day 0 and Day 150 and then compared to observe if Flex Choice could reduce osteocyte formation in thoracic and pelvic limbs and hip joints.

Evaluations provided by canine owners were taken into consideration, and the researcher statistically analyzed data to determine if there was a significant reduction in pain. Administration of Flex Choice twice daily showed significant reduction in pain from arthritis over a 150-day period. Several owners commented about how they felt their dog was feeling better as they noticed a change in physical activity level during the study. One dog in particular was noted to be able to stand on slick floors again near Day 120 of the study. Difference in canines were visually noticeable upon study’s conclusion.

Figures in the following section provide graphical evidence of significant differences in pain throughout the study.

Figure 1 presents overall pain level of canines treated with Flex Choice. There was a significant reduction in pain by Day 30. Pain levels continued to reduce by Day
150. All canines benefited from treatment and pain levels were reduced from moderate pain to mild pain, with some showing more of a difference than others.

Figure 2 presents pain during limb manipulation of canines treated with Flex Choice. There was a significant reduction in pain observed on Day 30 and Day 120, but then it increased slightly by Day 150.

Figure 3 presents pain after physical exertion in treated canines. There was a significant reduction in pain by Day 120 and again on Day 150. Owners noticed a difference in their dog’s activity level around Day 90 and no comments about decreased activity were made at study’s conclusion on Day 150.

Lateral radiographs of elbows, shoulders, and stifles as well as ventral dorsal radiographs of hips were obtained on Day 0 and Day 150 so comparison could be made. Radiograph quality was dependent on x-ray machine settings and movement of canines. Steps were taken to attempt consistent quality, but technique and views varied slightly from Day 0 to Day 150. There were no appreciable differences between Day 0 and Day 150 radiographs.

Physical examinations and blood collections were performed each month during study duration to ensure safety of Flex Choice. Serum samples were transported to Breathitt Veterinary Center for analysis. No significant differences overall were observed in canine serum chemistry values except for CK. CK was significantly reduced on Day 30 and Day 150. This could be due to time from sample collection to analysis. One canine had elevated BUN values as a baseline at Day 0 of study, but level returned within reference intervals by Day 150. ALT level was elevated at Day 150 for this same canine. Body weight, heart rate, respiration rate, and temperature were also evaluated for
significant differences. Body weight did fluctuate slightly throughout study duration, but no significant differences were observed. Temperature remained steady throughout study duration. Heart rate seemed to lower as the study progressed, with a significant reduction starting on Day 60 and continued to be reduced for Days 90, 120, and 150. This could be due to canines becoming familiar with surroundings where research took place or reduced anxiety due to anticipation of treats. Respiration rate fluctuated throughout the study, but all canines except one were panting during each evaluation. There were no complaints from owners about loss of appetite, vomiting, diarrhea, or overall negative effects.

Figure 1

*Statistically different based on value of Day 0 (P < 0.05)*
**Figure 2**

![Figure 2](image)

*Statistically different based on value of Day 0 (P < 0.05)*

**Figure 3**

![Figure 3](image)

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

Results presented for overall pain, pain during limb manipulation, and pain after physical exertion concluded that administration of Flex Choice soft chews twice daily significantly reduced pain and inflammation associated with canine arthritis. Although there was no control group to compare results to, comparisons were made using baseline values from Day 0. Through physical examination, pain level analysis, and serum chemistry analysis, Flex Choice has been proven as an effective and safer alternative to conventional pharmaceuticals commonly used for canine arthritis.
Appendix A: Pictures of Canine Participants

Bailey

Reecy

Brutus

Goblin

Lucchese
Appendix B: Owner Consent Form

Owner’s Names: ____________________________________________________________

Address: ____________________________________________________________________

Phone: ____________________________ Alternate Phone: _________________________

Canine’s Name: _____________________________________________________________

Breed: _________________________________ Age: _____________________________

As the owner or authorized agent of the above animal, I give consent for the use of this animal in the research project of Rachael Webber. The study is outlined as follows: To observe the effects of this joint supplement on moderately arthritic canines that are not currently receiving treatment. I understand that participation is voluntary and will continue for a period of 5 months (150 days). During the 150 days, I am responsible for giving the canine oral soft chew daily throughout the entire time period. Other responsibilities will include completion of monthly questionnaires and monthly meetings with Rachael Webber, who will perform physical observations and collect blood samples. These meetings will occur on Day 0, Day 30, Day 60, Day 90, Day 120, and Day 150 of the study. I also consent to withdraw all other arthritis medications the canine may be receiving throughout the duration of the study. Other medications, such as heartworm and other parasite control and prevention, can still be administered.

Owner Signature: ____________________________________________________________

Date: ________________________________________________________________________

Researcher Signature: _________________________________________________________

Date: _________________________________________________________________________
Appendix C: Owner Questionnaire

Animal Name: ____________________________________
Owner Name: ____________________________________

1) On a scale of 1-10 (10 being most painful), rank your pet's current pain: ____

Please use the following scale to answer question 2-9:
- 0=no pain
- 1=mild pain
- 2=moderate pain
- 3=severe pain
- 4=severe and constant pain

2) Does your dog have a difficult time raising his/her limbs? ______

3) Does your dog experience stiffness in the limbs after extended periods of standing or sitting? ______

4) Does your dog express or whimper in pain when limbs are manipulated? ______

5) Is your dog tender when touched in the hip/rear area? ____

6) Does your dog experience difficulty going up or downs stairs? ______

7) Is your dog more stiff/painful during times of cold weather? _____

8) Does your dog experience lameness after physical exertion (i.e., walks, running, playing)? _____

9) Does your dog have difficulty jumping on to furniture, cars, or other objects? ____

Please Answer Yes or No for Questions 10-13:

10) Does your dog whimper, growl, snap, or any other negative outrages when touched? _____

12) Has your dog been diagnosed with being overweight by a veterinarian? _____

13) Has your dog been diagnosed by a licensed veterinarian with arthritis? _____
Appendix D: Researchers Forms

Patient Name: ________________
Owner Name: ________________
Date of Observation: ____________
Weight: ________________
Heart Rate: _____________
Respiration Rate: ________
Temperature: ____________

Right Front Leg:

Left Front Leg:

Right Rear Leg:

Left Rear Leg:

Vocalization:

Condition after 3 minutes of physical exertion:

Extra Information:
Evaluation Date: ____________

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<tr>
<th>Overall perceived pain level:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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</thead>
<tbody>
<tr>
<td>1=No pain evident during exam</td>
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<td>5=Moderate pain evident</td>
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<tr>
<td>10=Severe and constant pain evident</td>
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<tr>
<th>Pain while rising from a sitting position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<th>Pain while rising from a lying position</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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<th>6</th>
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<th>8</th>
<th>9</th>
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<tr>
<th>Pain while lowering body into sitting or lying positions</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tr>
<th>Pain during limb manipulation</th>
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<th>1</th>
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<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>0=No pain evident</td>
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<tr>
<td>1=Mild pain evident</td>
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<tr>
<td>2=Moderate pain evident</td>
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<tr>
<td>3=Severe pain evident</td>
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<td>4=Severe and constant pain evident</td>
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<tr>
<th>Apparent lameness after 3-minute physical exertion</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
</table>

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<th>Apparent pain after 3-minute physical exertion</th>
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<th>4</th>
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</table>
Appendix E: Radiographs

Radiographs of Bailey

Day 0: Right Lateral view of Thoracic Limbs

Day 150: Right Lateral view of Thoracic Limbs

Day 0: Right Lateral view of Stifles

Day 150: Right Lateral view of Stifles
Day 0: VD view of Hips

Day 150: VD view of Hips
Radiographs of Brutus

Day 0: Right Lateral view of Elbows

Day 150: Right Lateral view of Elbows

Day 0: Left Lateral view of Stifles

Day 150: Left Lateral view of Stifles
Day 0: VD view of Hips

Day 150: VD view of Hips
Radiographs of Goblin

Day 0: Left Lateral view of Thoracic Limbs

Day 150: Left Lateral view of Thoracic Limbs
Day 0: VD view of Hips

Day 150: VD view of Hips
Radiographs of Lucchese

Day 0: Left Lateral view of Elbows

Day 150: Left Lateral view of Elbows
Day 0: VD view of Hips

Day 150: VD view of Hips
Radiographs of Reecey

Day 0: Left Lateral view of Thoracic Limbs

Day 150: Left Lateral View of Thoracic Limbs

Day 0: Right Lateral view of Stifles

Day 150: Right Lateral view of Stifles
Day 0: VD view of Hips

Day 150: VD view of Hips
Appendix F: IACUC Approval

August 13, 2018

Dr. Ramesh Gupta  
Breathitt Veterinary Center  
Murray State University  
101 MSU Drive  
Hopkinsville, KY 42240

Dear Dr. Gupta:

It is with pleasure I inform you that the Murray State University Institutional Animal Care and Use Committee (IACUC) has approved your research protocol for the project titled, “Evaluation of a Nutraceutical VPI-G5 for Anti-arthritic Efficacy and Safety in Moderately Osteoarthritic Dogs.”

The teaching protocol timeline is approved through April 30, 2019. Please use the Animal Use Report (attached) to keep up-to-date information about the animals. At the termination of the protocol, you will need to complete the Conclusion Report (attached) and list final information concerning the animals.

The IACUC sincerely wishes you the best in your teaching pursuits. If you have any questions, please contact me at 270-809-3534.

Sincerely,

Kristi Stockdale  
IACUC Coordinator

cc:  
IACUC File
References


