CHONDROPROTECTIVE EFFICACY OF UNDENATURED COLLAGEN TYPE II ON CANINE
OSTEOARTHRITIS SECONDARY TO MEDIAL PATELLAR LUXATION

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จุฬาลงกรณ์มหาวิทยาลัย
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ประสิทธิภาพในการปกป้องผิวกระดูกอ่อนของคอลลาเจนที่คงตัวชนิดที่สองในสุนัขที่เป็นโรคข้อเสื่อม
ที่เกิดจากโรคสะบ้าเคลื่อนด้านใน

น.ส.สุโสฬส คุณสุวรรณชัย

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

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สุโสพส คุณสุวรรณชัย : ประสิทธิภาพในการปกป้องผิวกระดูกอ่อนของคอลลาเจนที่คง
ตัวชนิดที่สองในสุนัขที่เป็นโรคข้อเสื่อมที่เกิดจากโรคสะบ้าเคลื่อนด้านใน. (
CHONDROPROTECTIVE EFFICACY OF UNDENATURED COLLAGEN TYPE II ON
CANINE OSTEOARTHRITIS SECONDARY TO MEDIAL PATELLAR LUXATION) อ.
ที่ปรึกษาหลัก : ผศ. น.สพ. ดร.กัมปนาท สุนทรวิภาต

โรคข้อเสื่อมมีลักษณะของโรคคือมีการเสื่อมสลายของกระดูกและมีการเปลี่ยนแปลง
ภายในข้อซึ่งจะเป็นการอักเสบแบบช้า ในสุนัขพันธุ์เล็กโรคข้อเสื่อมมักเกิดจากโรคสะบ้าเคลื่อนด้าน
ใน การรักษาโรคสะบ้าเคลื่อนด้านใน ควรทำการผ่าตัดแก้ไขในกรณีที่เกิดปัญหาโครงสร้างของ
กระดูกที่ผิดปกติ และการบรรเทาความเจ็บปวดในสุนัขที่เป็นโรคนี้ส่วนใหญ่จะให้ยาลดอักเสบ แต่
การให้ยาลดอักเสบนั้น ไม่สามารถให้ติดต่อกันเป็นระยะเวลานานได้ ปัจจุบันจึงนิยมใช้ ผลิตภัณฑ์
อาหารเสริมที่มีคุณสมบัติในการลดการอักเสบ หรือลดการทำลายกระดูกอ่อนภายในข้อได้ และ
สามารถให้ติดต่อกันเป็นระยะเวลานานได้โดยที่ไม่ได้ส่งผลข้างเคียงใด โดยงานวิจัยนี้ เป็นการศึกษา
ประสิทธิภาพในการปกป้องผิวกระดูกอ่อนของคอลลาเจนที่คงตัวชนิดที่สองในสุนัขที่เป็นโรคข้อ
เสื่อมที่เกิดจากโรคสะบ้าเคลื่อนด้านใน ที่มีสุนัขพันธุ์เล็กที่เป็นสะบ้าเคลื่อนด้านใน 9 ตัว ได้รับ
คอลลาเจนที่คงตัวชนิดที่สอง เป็นเวลา 120 วัน โดยจะประเมินผลโดยการให้คะแนนภายในข้อ
จากอัลตร้าซาวน์ เอ็กซเรย์ ผลเลือด การให้คะแนนความเจ็บปวดขา และการให้คะแนนความ
เจ็บปวดของสัตว์โดยให้เจ้าของทำแบบสอบถาม โดยจะเปรียบเทียบผลระหว่างก่อนและหลังทำ
การทดลอง ซึ่งการวิเคราะห์ข้อมูลทั้งหมดจะใช้ วิธีการวิเคราะห์ความแปรปรวนทางเดียว
ส่วนข้อมูลผลเลือดจะใช้วิธีการทดสอบความแตกต่างของค่ากลางของสองประชากรไม่อิสระ ผล
การทดลองพบว่าเมื่อเปรียบเทียบก่อนและหลังการทดลองสุนัขมีคะแนนจากการทำอัลตร้าซาวน์
และคะแนนความเจ็บปวดของสัตว์โดยให้เจ้าของทำแบบสอบถาม ที่ดีขึ้นแบบมีนัยสำคัญ (p value
<0.05) คะแนนจากการทำอัลตร้าซาวน์ที่ดีขึ้นอย่างมีนัยสำคัญนั้น จะมีเพียงน้ำในข้อเท่านั้น ส่วน
การให้คะแนนภายในข้อจากเอ็กซเรย์ ผลเลือด การให้คะแนนความเจ็บปวดขานั้นไม่พบว่ามีความ
แตกต่างทางนัยสำคัญ โดยสรุปการให้คอลลาเจนที่คงตัวชนิดที่สอง อาจเป็นอีกหนึ่งในทางเลือก
ของอาหารเสริมที่ปกป้องผิวกระดูกอ่อน ที่สามารถใช้ร่วมกันในการจัดการโรคสะบ้าเคลื่อนด้านใน

สาขาวิชา   ศัลยศาสตร์ทางสัตวแพทย์   ลายมือชื่อนิสิต ....

ปีการศึกษา   2562   ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

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KEYWORD: chondroprotective effect, dog, medial patellar luxation, osteoarthritis, undenatured collagen type II

Susolos   Kunsuwanachai   :   CHONDROPROTECTIVE   EFFICACY   OF UNDENATURED COLLAGEN TYPE II ON CANINE OSTEOARTHRITIS SECONDARY TO   MEDIAL   PATELLAR   LUXATION.   Advisor: Asst.   Prof.   KUMPANART SOONTORNVIPART, D.V.M., Ph.D., D.T.B.V.S.

Osteoarthritis (OA) is found secondary from medial patellar luxation (MPL) which is one of the most common diseases in small-breed dogs. Managements of the disease are surgical correction or a medical treatment. In the patients who are unable to do the surgical correction, non-steroidal anti-inflammatory drugs (NSAIDs) remains the drug of choice for OA therapy. Nowadays nutraceuticals have become more popular since there are no side effects once given for a long period of time. The purpose of this study was to evaluate the clinical chondroprotective effects of undenatured collagen type-II (UCII) on OA secondary to canine medial patellar luxation. Nine small-breed dogs received UCII for 120 days. Ultrasonographic, radiographic examinations, lameness score, blood collection and CBPI were evaluated for comparison the results between before and after received UCII. One-Way Anova and paired T-test was used to analyze the statistical significance of data. The results revealed a statistically significant difference between before and after received UCII in CBPI (p-value <0.05). Lameness score, radiographic examinations and blood collection were no significantly different. CBPI and the ultrasonographic scores has significantly improve even if only joint effusion from all of ultrasonographic examination was significant difference. In conclusion, UCII may be the choice of OA nutraceutical chondroprotective products which could be a co-treatment with the MPL treatment as UCII can improve the clinical signs.

Field of Study:	Veterinary Surgery	Student's Signature	....
Academic Year:	2019	Advisor's Signature	....

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CHAPTER I

INTRODUCTION

Important and rationale

Osteoarthritis (OA) is a degenerative joint disease which characterized by degenerative articular cartilage and formation of new bone in the affected joint surfaces which also cause the changes in the synovial membrane. Osteoarthritis is a slow progressive inflammatory disease causing a lameness, decreased weight-bearing capacity and occasional joint effusion (Vaughan-Scott and Taylor, 1997; Alam et al., 2011a; Alam et al., 2011b).

The etiology of osteoarthritis in a particular joint still be unclear. However, the susceptibility to osteoarthritis is involved with some factors such as age, genetics and obesity. Secondary osteoarthritis of stifle joint is a common result from medial patellar luxation (MPL) (Martinez, 1997; Alam et al., 2011a). In Thailand, Medial patellar luxation is one of the most common orthopedic disorders in small breed dogs. The prevalence of medial patellar luxation (MPL) and lateral patellar luxation (LPL) in small-breed dogs is 87% and 13%, respectively. Pomeranians are presently the highest-ranking breed for patellar luxation in the USA, with 42.4% of dogs affected (Salg et al., 2006; Soontornvipart et al., 2013). Patellar luxation influenced 1.3% of the dogs in the study of 210,824 dogs in England (O'Neill et al., 2016).

The degeneration of articular cartilage is an important point to detect OA in early stage. The most common diagnostic technique is radiography which cannot reveal the changes of articular cartilage or synovial membrane. Others diagnostic technique such as arthroscopy, force plate gait analysis, biomarkers detection, computerized tomography (CT) scan or magnetic resonance imaging (MRI), would give more details than radiography, nonetheless these techniques are more invasive and expensive. Recently in human studies, ultrasonography technique is recommended for detection an early stage of OA by detecting the abnormalities of articular cartilage surface. Moreover, this technique is desirable as a quick and easy screening method

for diagnosis of human knee osteoarthritis with its non-invasiveness and cost-effectiveness (Okano et al., 2016). Therefore, the ultrasound will be a technique used for diagnosis and follow up in this study.

Management of osteoarthritis is most often conservative methods with multimodal to control pain and clinical signs for a better quality of life, and to slower progressive of OA by given Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) combined with nutraceuticals and weight and exercise management. NSAIDs remained the drug of choice for OA therapy (Brosseau et al., 2003). Nevertheless, NSAIDs cannot be given continuously for a long period of time due to its adverse effects, such as gastrointestinal ulceration and renal papillary necrosis (Vaughan-Scott and Taylor, 1997).

Nutraceuticals have become more popular since there are no side effects once given for a long period of time. At present, there are many current nutraceutical compounds that could be used as integrators in everyday, such as in olive oil, fish oil, GAGs (Glucosamine Sulfate, Chondroitin Sulfate, and Hyaluronic Acid), Methionine, and botanical extracts (Castrogiovanni et al., 2016). One of the nutraceuticals that can be used to slower the progress of OA is undenatured collagen type II. Undenatured collagen type II is a glycoprotein from chicken sternum (Bagchi et al., 2002), for decreasing inflammation resulted in reducing pain for osteoarthritis patient (Deparle et al., 2005; Bagi et al., 2017). Bagi et al. (2017) have studied an orally undenatured collagen type II given to OA induced rats. The outcome of that study showed less destruction of cartilage than the rats without undenatured collagen type II treatment. Even though, there are several earlier researches studies on the effectiveness of using undenatured collagen type II in both human and animals (Deparle et al., 2005; D'Altilio et al., 2007; Gupta et al., 2012), none has been done regarding chondroprotective effect of undenatured collagen type II on OA dogs by using ultrasonography. Therefore, this study aims to investigate the chondroprotective effects of undenatured collagen type II on dog with OA secondary to medial patellar luxation.

CHAPTER II

LITERATURE REVIEW

2.1 Osteoarthritis and medial patellar luxation

Osteoarthritis is the most common cause of arthritis in the dog which affects up to 20 percent of the adult canine population. In dogs, both primary and secondary forms of osteoarthritis have been reported although the secondary form is far more common (Alam et al., 2011b; Pettitt and German, 2015). The characterized clinical signs are lameness, stiffness, exercise intolerance. The clinical signs can be credited to the primary inciting cause, pain associated with arthritis or a combination of both (Pettitt and German, 2015).

Secondary osteoarthritis is a common result of medial patellar luxation (MPL) that is one of the most orthopedic disorders in dogs. It has b een reported that the medial patellar luxation (MPL) rises the stress on the cranial cruciate ligament (CCL), predisposing the structure to degeneration and rupture (Alam et al., 2011b). The clinical signs of patellar luxation are found in mature dog while the disease is developed and progressed since it was young (Di Dona et al., 2018). In small breed dogs, medial patellar luxation can be found more than lateral patellar luxation with 87% and 13%, respectively (Wangdee et al., 2017).

Di Dona et al. (2018) report that patellar luxation is a developmental disorder. Although the etiology of patellar luxation is not concluded yet, malalignment of quadriceps muscle group in patellar luxation dogs may implies the cause of the disease. The abnormalities of alignment of quadriceps muscle groups, patella, trochlear groove, patellar ligament and tibial tuberosity have a direct effect in the abnormal extensor mechanisms of the stifle joint and can lead to the change of the distal femur anatomy, proximal tibial and consequent patellar instability during the growth stage. There abnormal development lead to femoropatellar instability. The absence of the physiological pressure from the patellar on the articular cartilage of

the trochlear groove during growth, may prevent the development of an sufficiently deep and wide groove (trochlear hypoplasia) Repetitive medial luxation of the patella can induce lesions on the articular surface of both the medial femoral condyle and the patella (Daems et al., 2009).

Articular cartilage mainly composes of proteoglycans and collagen type II that is designed to decrease friction and to absorb shock. In normal dynamic tissue, chondrocytes continually synthesize products that repair aged or damaged cartilage. OA is the condition that catabolic process exceeds anabolic process and that regeneration of cartilage becomes ineffective which caused cartilage degeneration progresses by the loss of proteoglycans and hyaluronic acid. Synovial cells and chondrocytes response according to a number of cytokines, including prostaglandin E_2, interleukin-1 (IL1), interleukin-6 (IL6), and tumor necrosis factor (TNF) in cartilage catabolism. These cytokines are released into the joint space. The catabolic enzymes cause direct damage to the cartilage and may influence proteoglycan synthesis by chondrocytes. These result in decreased load-bearing capacity and localized areas of softening in the cartilage. Moreover, flaking and fissuring of cartilage occurs as the result of the exposure of underlying bone (Vaughan-Scott and Taylor, 1997).

Clinical signs of medial patellar dogs associated to the grade of skeletal malformations. Mild-to-moderate weightbearing lameness are occurred intermittently or continuously with occasional lifting of the limb. When patella luxated, the dog will attempt to stretch the leg backward to permit the patella to seat back into the groove. Which is a typical sign of the patellar luxation. Dogs with grade I patellar luxation are usually asymptomatic; although, they can present sometimes a "skipping" type lameness which is typical of dogs with grade II patellar luxation. Occasionally, a mild internal rotation of the tibia and abduction of the hock can be detected when examination. Grade II luxation can progress to a grade III due to the progressive cartilage erosion of the trochlear ridge and lead to more severe clinical signs, as a result of chronic degenerative joint changes. Grade III and IV patellar luxations are commonly considered by determined lameness and abnormal posture.

Orthopedic examination is done by gait evaluation, joint flexion–extension movements and range of motion assessment, and tendency of the patella to luxation

and grading. Physical examination is performed during the patient standing optimal evaluated the symmetry between limbs and stifle joint effusion. At walk and trot, determining the degree of lameness is detected apparent skeletal deformities. Patellar luxation in dogs has been classified as follows Fauron and Perry (2016).

Grade I – the patella can be luxated manually but returns to the normal position in the trochlear groove when released. There is no crepitus noted during stifle range of motion and bone deformity is absent. Clinical signs are typically not present.

Grade II – spontaneous luxation occurs during a normal range of motion, but the patella normally resides within the trochlear groove. Clinical signs may be present, the most typical being a non-painful, "skipping" type of lameness, where the dog will intermittently limb and hold the leg up for a few steps before returning to normal. Dogs might also stretch the leg backwards in an effort to reduce the patella. This condition may progress to a grade III or IV, as erosion on the patellar and trochlear surfaces occur and/or cranial cruciate ligament disease develops.

Grade III – the patella is permanently luxated but can be reduced manually. More severe deformities of the tibia and femur may be present. A shallow trochlear groove may be palpable.

Grade IV – this is a severe condition, with permanent, non-reducible luxation of the patella.

Medial patellar luxation has two treatments; conservative management and surgical treatments. Surgical treatments are suggested for dogs with an intermittent or permanent lameness as an outcome of patellar luxation or in young dogs in an effort to relieve the negative effects of the condition on growing bone. Joint pathology rises with age and luxation grade, and surgical correction should be presented at the initial chance to limit additional development of skeletal abnormalities or DJD.

Conservative management may be indicated in dogs with grade I patellar luxation if the episode of lameness is mild and infrequent, and the degree of osteoarthritis is mild. Non-surgical management typically includes administration of nonsteroidal anti-inflammatory drugs (NSAIDs) in association, or not, with other

analgesic drugs to reduce pain. Physical rehabilitation exercises are useful to enhance quadriceps mechanism. Weight control is essential to reduce undue stress on the stifle joint. Also, massage therapy and hydrotherapy may help to promote wellness and comfort (Di Dona et al., 2018).

In OA treatment, pharmacological management is the common therapy, while complementary therapy such as physical rehabilitation, weight reduction and exercise control help to promote better quality for OA patients. Prevention of OA progression could be served by pharmacologic management to slow a severe stage of OA, especially as prevention of cartilage damage. Although, NSAIDs is the most common medication for OA treatment, but long-term usage will cause severe side effects to patients. Therefore, the use of nutraceutical has been increased in order to inhibit the inflammatory pathways which cause a reduction of cartilage degeneration (D'Altilio et al., 2007; Bagi et al., 2017).

2.2. Osteoarthritis and nutraceuticals

The term "nutraceutical" was derived from "nutrition" and "pharmaceutical" in 1989 and was defined as "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease" (Vandeweerd et al., 2012). Nutraceuticals may be used to increase life expectancy, delay the aging process, improve health, prevent chronic diseases, or support the structure or function of the body (Nasri et al., 2014). The goal of OA treatment is reducing pain and inflammation. NSAIDs, the common pharmacologic drug, can eliminate pain but chronic use of NSAIDs is associated to many side effects. Therefore, the use of nutraceuticals has been introduced in order to alleviate pain, control inflammation, preserve joint ability in OA patient with desirable therapeutic outcome with few side effects (Kalra, 2003; Deparle et al., 2005; Nasri et al., 2014).

At present, undenatured collagen type II has been used to control pain, reduce inflammation, promote health joint, and improve joint motility and flexibility in arthritis cases (Deparle et al., 2005; Bagi et al., 2017). Undenatured collagen type II is from chicken sternum which was prepared using low temperature to preserve its undenatured form and ensure biological activity. When undenatured collagen type II

is digested resulting in chains of soluble collagen molecules of varying length containing biologically active epitopes which eventually interact with Peyer's Patch and trigger the complex series of immunological events. Undenatured collagen type II functions through a process called oral tolerization i.e. the ability of mucosal immune system to actively inhibit systemic immune response to fed antigens has been used as a therapy for some chronic inflammatory and autoimmune conditions(C Crowley et al., 2009). A small amount of undenatured type II collagen (10 mg) taken orally has been shown to turn off the immune response targeted (T-cell) at type II collagen in joint cartilage and there are no adverse side effects associated with the intake of this nutraceutical (Bagchi et al., 2002; Deparle et al., 2005). In contrast, while denatured collagen may provide a nutritional source of substrate for joint cartilage synthesis, research demonstrates that it does not induce immunological hypo-responsiveness and has not demonstrated an effect on reducing pain and inflammation. Deparle et al. (2005) reported in arthritic dogs that undenatured collagen type II is significantly more effective than denatured against arthritis. In addition, recent pilot study, reported for the first time that daily administration of glycosylated undenatured type II collagen (40 mg of UC-II providing 10 mg/day) for 90 days significantly improved the signs and symptoms of arthritis in dogs (D'Altilio et al., 2007).

2.3 Ultrasonography in osteoarthritic patients

Diagnostic methods of osteoarthritis are commonly determined by orthopedic and radiographic examination findings. Though, radiography can reveal joint effusion, it is difficult to be detected as only small amount of joint effusion occurred. Moreover, there are many changes in soft tissue structures occurred prior to a detection of bony changes in radiographic findings (Chappard et al., 2006; Ramirez-Flores et al., 2017). For the last few decades, methods for diagnosing and monitoring osteoarthritic disease in dogs was developed which including arthroscopy, magnetic resonance imaging (MRI), scintigraphy, serum OA biomarkers and ultrasonography.

Many authors discovered ultrasonography is a desirable additional method to orthopedic and radiological examination for diagnosing stifle diseases (Kramer et al., 1999; Reed et al., 2005). Ultrasonography can be used for examining a joint disease as

a diagnostic tool due to its beneficial non- invasiveness and capability of soft tissue visualization (Reed et al., 2005; Ramirez-Flores et al., 2017). The use of ultrasonography for detection of subtle soft tissue structure changes in early OA joint has been proposed (Arnault et al., 2009; Nishitani et al., 2014). This early detection will help practitioners to manage the disease before it becomes more complicated stage. In humans, it is used as a screening diagnosis of stifle disease, especially for assessing early changes of cartilage in OA patients (Okano et al., 2016). Thereby the combination of radiography and ultrasonography for OA diagnosis has been recommended (Kramer et al., 1999; Goranov et al., 2013).

CHAPTER III

MATERIALS AND METHODS

3.1 Animals

Small breed dogs (eg. Pommerinian, Chihuahua, Yorkshire terrier, etc) with lameness problem were diagnosed and those with medial patellar luxation (severity grade I-III) was selected into the study group. The severity classification of patellar luxation was based on Fauron and Perry (2016). Briefly, grade I - patella can be manually luxated but returns to normal position when released. Grade II – patella luxates with stifle flexion or on manual manipulation and remains luxated until stifle extension or manual replacement occurs. Grade III – patella luxated continually and can be manually replaced but will re-luxate spontaneously when manual pressure is removed. Grade IV – patella luxated continually and cannot be manually replaced (table1). The animals included in this study must be more than 2 years of age and had no other diseases (e.g. neurologic disease, systemic disease, others orthopedic diseases).

The animals were evaluated for body condition score. Only animal with body condition score 3-6/9 was selected to the study. The evaluation of body condition score scale was based on Nine-Interger BSC scale system (Laflamme, 1997) (table2) All dogs must not be treated for MPL and not taken NSAIDs or any pain killers at the time they were in the study. The animals must be fed only commercial standard food. The owners of the dogs must inform the consent of the permission for the study (figure1)

The study was approved by Chulalongkorn University Animal Care and Use Committee (CU-ACUC) Bangkok, Thailand. The approved number is 1931008.

Table 1 Classification of patellar luxation based on Fauron and Perry (2016)

Grade	Description
I	The patella can be luxated manually but returns to the normal position in the trochlear groove when released. There is no crepitus noted during stifle range of motion and bone deformity is absent. Clinical signs are typically not present.
II	Spontaneous luxation occurs during a normal range of motion, but the patella normally resides within the trochlear groove. Clinical signs may be present, the most typical being a non-painful, "skipping" type of lameness, where the dog will intermittently limp and hold the leg up for a few steps before returning to normal. Dogs might also stretch the leg backwards in an effort to reduce the patella. This condition may progress to a grade III or IV, as erosion on the patellar and trochlear surfaces occur and/or cranial cruciate ligament disease develops.
III	The patella is permanently luxated but can be reduced manually. More severe deformities of the tibia and femur may be present. A shallow trochlear groove may be palpable.
IV	This is a severe condition, with permanent, non-reducible luxation of the patella.

Table 2 Classification of the body condition score based on Nine-Interger BSC scale system (Laflamme, 1997)

Score	Description
1	Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No observable body fat. Obvious loss of muscle mass.
2	Easily visible of ribs, lumbar vertebrae and pelvic bones. Some evidence of other bony prominences. Minimal loss of muscle mass.
3	Easily palpation of ribs. Evidence of top of lumber vertebrae and prominence of pelvic bone. Obvious waist and abdominal tuck.
4	Easily palpation of ribs with minimal fat covering. Easily noted of waist from the top view. Abdominal tuck evident.
5	Ribs palpable without excess fat covering. Waist observed behind ribs from the top view. Abdominal tuck up when viewed from side.
6	Ribs palpable with slight excess fat covering. Waist discernible from the top view without prominence. Appearance of abdominal tuck.
7	Difficult palpation of ribs under excess fat covering. Noticeable fat deposits over lumbar area and base of tail. Absent or barely visible of waist. Abdominal distention may be presented.
8	No palpation of ribs without pressure. Heavy fat deposits over lumbar area and base of tail. Absent of waist and abdominal tuck. Obvious abdominal distention may be presented.
9	Massive fat deposits over thorax, spine and base of tail. Absent of waist and abdominal tuck. Fat deposits on neck and limbs. Obvious abdominal distention.

ใบยินยอมเข้าร่วมการวิจัย (Consent Form)

โครงการวิจัยเรื่อง: ประสิทธิภาพในการปกป้องผิวกระดูกอ่อนของคอลลาเจนที่คงตัวชนิดที่สองในสุนัขที่เป็นโรคข้อเสื่อมที่เกิดจากโรคสะบ้าเคลื่อนด้านใน

วันที่ให้คำยินยอม   วันที่........................เดือน...พ.ศ...................

1. ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจาก สัตวแพทย์หญิงสุโสพส คุณสุวรรณชัย (ผู้วิจัย) ถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย และมีความเข้าใจดีแล้ว

2. ผู้วิจัยรับรองว่าจะตอบคำถามต่าง ๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

3. ข้าพเจ้ามีสิทธิ์ที่จะบอกเลิกการเข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้ และเข้าร่วมโครงการวิจัยนี้โดยสมัครใจ

4. ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ จะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลของตัวข้าพเจ้าต่อหน่วยงานต่าง ๆ ที่เกี่ยวข้องต้องได้รับอนุญาตจากข้าพเจ้าแล้วจะกระทำได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

5. ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม...ผู้ยินยอม

(...)

ลงนาม...พยาน

(...)

ลงนาม...ผู้ทำวิจัย

(...)

Figure 1 The consent of the permission for the study

3.2 Study designs

The 21 stifle joints from 13 dogs were treated with undenatured collagen type II (SC II) at recommended for 10 mg per animal once a day for 16 weeks. The animals were evaluated both stifle for clinical outcomes before treatment at week 0 (D0) and post-treatment program at week4 (W4), week8 (W8), week12 (W12) and week16 (W16). The clinical evaluations included ultrasonographic findings, radiographic findings, lameness scores, blood profiles and owner questionnaires. In each visit, the patients were assessed for monitoring all the clinical evaluations except the blood profiles which were examined on D0 and W16 for any adverse effects and health status. The study was complete at the end of W16.

3.2.1 Lameness score

The gait of the animals was evaluated weekly by walking and trotting from Lameness score criteria that are shown in table 3.

Table 3 Lameness score criteria (Impellizeri et al., 2000)

Score	Signs
0	Normal gait when walking and trotting
1	Slight algetic gait when walking and normal gait when trotting
2	Obvious algetic gait when walking and normal gait when trotting
3	Difficulty walking and algetic gait when trotting
4	Non-weight bearing when walking and algetic gait when trotting
5	Non-weight bearing when walking and trotting

3.2.2 Radiographic examination

A conventional radiography of both stifle joints was taken in two views: mediolateral and caudocranial views to evaluate osteoarthritis score. This osteoarthritis score was based on OA grading system by Wessely et al. (2017). Fifteen locations of each stifles were evaluated for OA grading system, in which 11 locations were in the mediolateral view and 4 locations in the caudocranial view in the radiography (Figure 2 and 3). Each location was graded on a numeric scale from 1 to 4 as mild to severe

lesion shown on the radiographs (Table 4). The overall OA scores were calculated in range between 15 and 60.

To obtain the standard mediolateral view, each dog was positioned in lateral recumbency with flexion the stifle joint at an angle of 90 degree and the femoral condyles was superimposed meanwhile for the standard caudocranial view, each dog was positioned in sternal recumbency with the affected limb extended along the long axis of the femur and parallel to the long axis of the tibia.
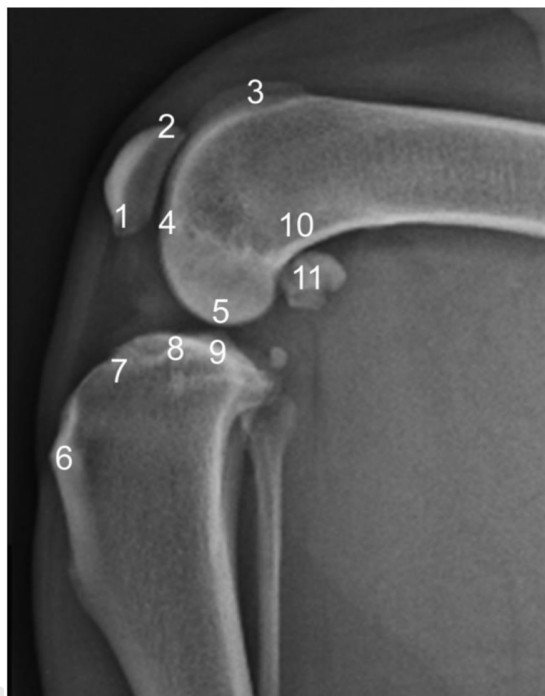

Figure 2 Assessment points in the mediolateral view. 1, patellar apex; 2, patellar base; 3, proximal trochlear ridge; 4, distal trochlear ridge; 5, femoral condyle; 6, tibial tuberosity; 7, cranial aspect tibial plateau; 8, caudal aspect tibial plateau; 9, central aspect tibial plateau; 10, popliteal surface femur; 11, sesamoid bones (Wessely et al., 2017).

Figure 3 Assessment points in the caudocranial view. 12, lateral tibial and femoral condyle; 13, medial tibial and femoral condyle; 14, intercondylar notch; 15, patella (Wessely et al., 2017).

Table 4 Osteoarthritic grading system and corresponding radiographic changes (Wessely et al., 2017)

Grade	Severity	Radiographic changes
1	No	Radiographic normal/no evidence of sclerosis or osteophyte
2	Mild	Mild osteophyte and/or mild sclerosis (mild arthrosis)
3	Moderate	Moderate osteophytes and moderate sclerosis (moderate arthrosis)
4	Severe	Marked osteophytes and severe sclerosis (severe arthrosis)

3.2.3 Blood profile

The blood test was assessed at D0 before the study and W16 at the end of study to evaluate the health status and adverse effects. Blood

sample were collected from either cephalic or saphenous veins to evaluate hematological profile (Red blood cell count, Hemoglobin, Hematocrits, Platelets count, white blood differentiate cell count and serum biochemistry which included blood urea nitrogen (BUN), creatinine, alanine transferase (ALT), alkaline phosphatase (ALP) and total protein (TP). The blood sample will be separated into two equal portions. The hematological profile was evaluated using ethylenediamine tetra acetic acid (EDTA) contained blood sample while serum biochemistry was evaluated by using heparin contained blood sample.

3.2.4 Ultrasonographic examination

The dogs were shaved at the one-third of the distal femur to the one-third of the proximal tibia to exam stifle from proximal to distal area in the supine position. Ultrasonography was used to evaluate stifle joint alterations with real-time 7.5-MHz linear (Kramer et al., 1999; Ramirez-Flores et al., 2017). Stifle joint was examined following to acoustic approach by Kramer et al. (1999). Some standard views were listed appropriately according to the objective of this study in Table 5.

Table 5 Ultrasonographic Standard Views for Evaluating the Stifle joint modified from Kramer et al. (1999)

Standard regions / views	Implementation
Suprapatellar region	
Parallel view on the femoral trochlea	Knee flexed at an angle of 45 degrees Parallel sagittal image
(B) Suprapatellar views on the femoral trochlea	Knee flexed as far as possible Transverse image
Infrapatellar region	
(C) Infrapatellar view	Knee flexed an angle of 90 degrees Sagittal image

Moller et al. (2008); Sathienbumrungkit (2018) explained that the normal stifle joint, in the sagittal view, suprapatellar recess was above the femoral condyle in the suprapatellar region. The synovial fluid was found in anechoic content with homogeneous echogenicity in the infrapatellar region. Hyaline cartilage was the anechoic band with a smooth and sharp hyperechoic margin along femoral condyles of the suprapatellar and infrapatellar region. The bone surface was the hyperechoic line with homogeneous and smooth below the articular cartilage.
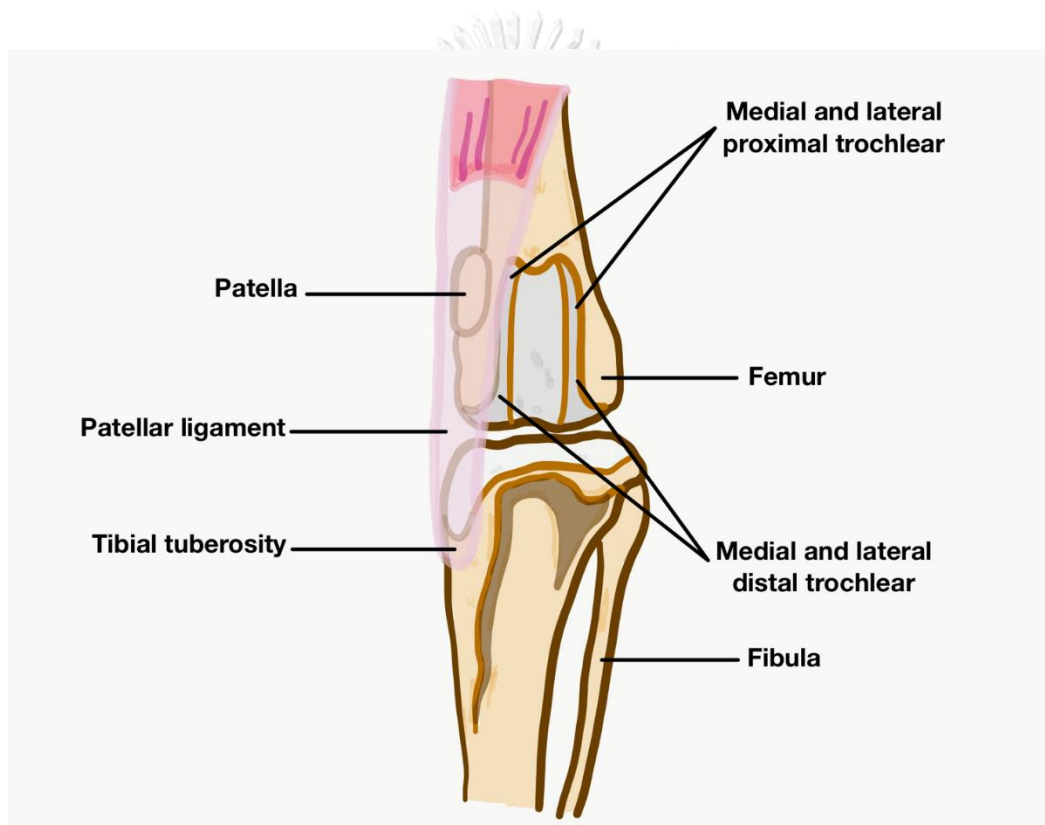

Figure 4 Anatomy of left femoral condyle

To evaluate a suprapatellar region with parallel view on the femoral trochlea, the stifle was flexed from its axis at an angle of 45 degrees and the ultrasound probe was placed on the femoral condyle in a sagittal view (Figure 5A). The joint effusion, the proximal femoral condyles and the articular cartilage covering them became apparent (Figure 6). The joint effusion, the articular cartilage and bone surface of medial and lateral proximal femoral condyle was determined and recorded.

Then when performing a transverse examination under maximal flexion of the stifle, the ultrasound probe was placed up on the patella in a transverse view (Figure 5B), a surface and depth of the articular cartilage at the proximal femoral trochlea was determined and recorded (Kramer et al., 1999) (Figure 8).
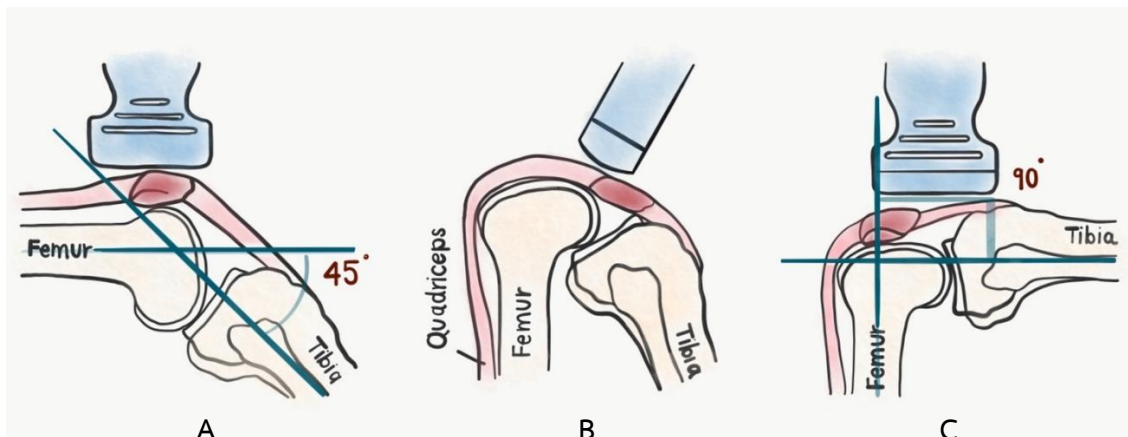

Figure 5 Drawing of stifle flexion from its axis at an angle of 45 degree (A), maximum stifle flexion (B) and stifle flexion from its axis at an angle of 90 degree with ultrasound probe (C) (Kramer et al., 1999)
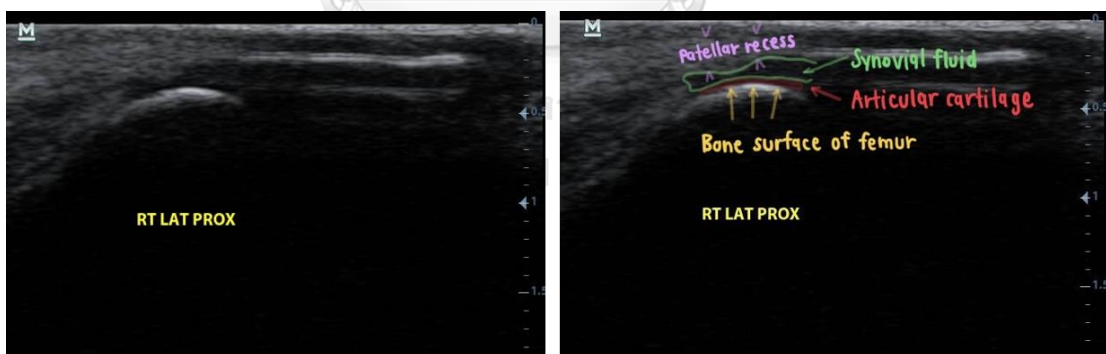

Figure 6 Suprapatellar view with sagittal image of stifle joint (A: in figure5) shows synovial fluid, femoral condyles and its cartilage

For infrapatellar view, the knee was flexed from its axis at an angle of 90 degree and the ultrasound probe was placed on the femoral condyle in a sagittal view (Figure 5C), the sagittal plane image showed the patella, the patellar ligament, the infrapatellar fat body, femoral cartilage surface as well as joint effusion (Figure 7).

This view was used for evaluation a score of articular cartilage and bone surface of both ridges of the distal femoral condyle. Moreover, a joint effusion was considered in cooperate with the suprapatellar view.
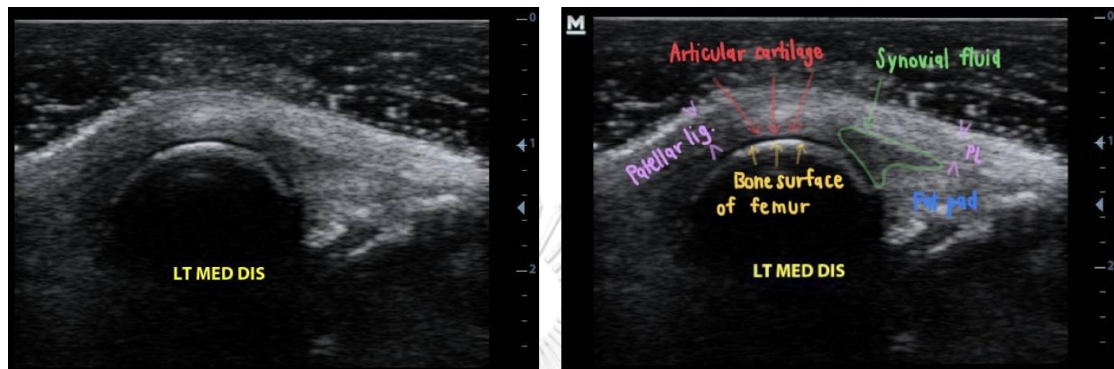

Figure 7 Infrapatellar view with sagittal image of stifle joint (C: in figure5) shows joint effusion, articular cartilage of the femoral condyle

Figure 8 Transverse view of stifle joint (B: in figure5) shows articular cartilage of the femoral condyle

The ultrasound evaluation was analyzed base on ultrasound scoring system of Goranov et al. (2013). Synovial fluid, articular cartilage and bone surface of the femoral condyles score was evaluated from 4 parts of the femoral condyles; the proximal and distal of both medial and distal of both medial and lateral area (table 6, 7 and 8).

Individual scores range was between 0 and 28 points. Furthermore, the articular cartilage thickness of the femoral condyles was recorded in millimeters.

Table 6 Ultrasound Scoring System of OA Stifle Joint modified from Goranov et al. (2013) (Figure 9)

| Synovial fluid score	Description
0	Normal appearance of joint fluid; anechoic content
1	Mild increasing opacity of joint fluid
2	Obvious increasing opacity of joint fluid with slightly heterogenous echogenicity
3	Slightly hyperechoic appearance of joint fluid with heterogenous echogenicity
4	Obvious hyperechoic appearance of joint fluid; echogenicity similar to bone
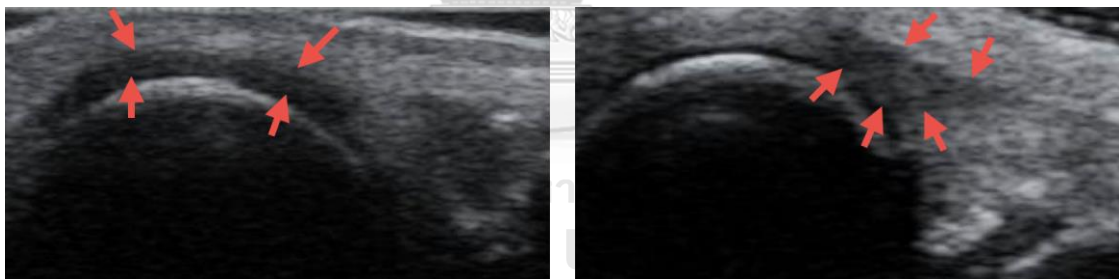
Figure 9 Example for the synovial fluid score 2 in the left image and score 3 in the right side

Table 7 Ultrasound Scoring System of OA Stifle Joint : Articular cartilage score modified from Goranov et al. (2013) (Figure 10)

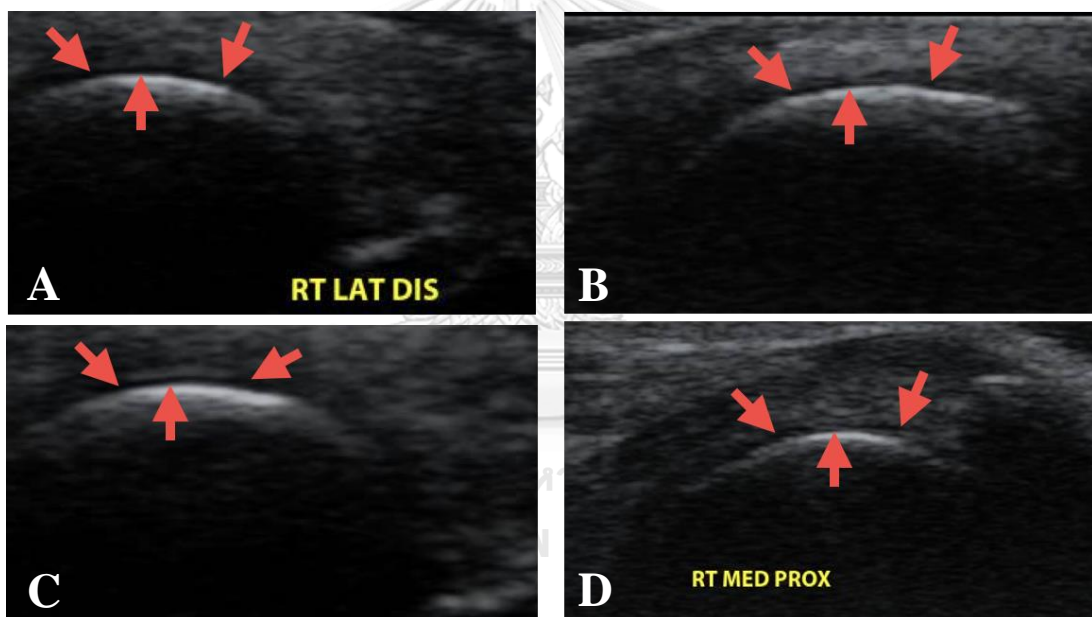| Articular cartilage score	Description
0	Normal appearance; anechoic band with smooth margin
1	Hypoechoic appearance of the hyaline cartilage with irregular margin
2	Hyperechoic appearance of the hyaline cartilage with irregular margin
3	Heterogeneous appearance of the hyaline cartilage with irregular margin

Figure 10 Example for the articular cartilage score 0, 1, 2 and 3 in the image A, B, C and D respectively

Table 8 Ultrasound Scoring System of OA Stifle Joint : Bone surface score modified from Goranov et al. (2013) (Figure 11)

| Bone surface score	Description
0	Normal appearance; absent irregular and/or rounded interruptions of the hyperechoic boundary of bones
1	Mild irregular and/or rounded interruptions of the hyperechoic boundary of bones
2	Moderate irregular and/or rounded interruptions of the hyperechoic boundary of bones
3	Severe irregular and/or rounded interruptions of the hyperechoic boundary of bones
4	Very severe irregular and/or rounded interruptions of the hyperechoic boundary of bones
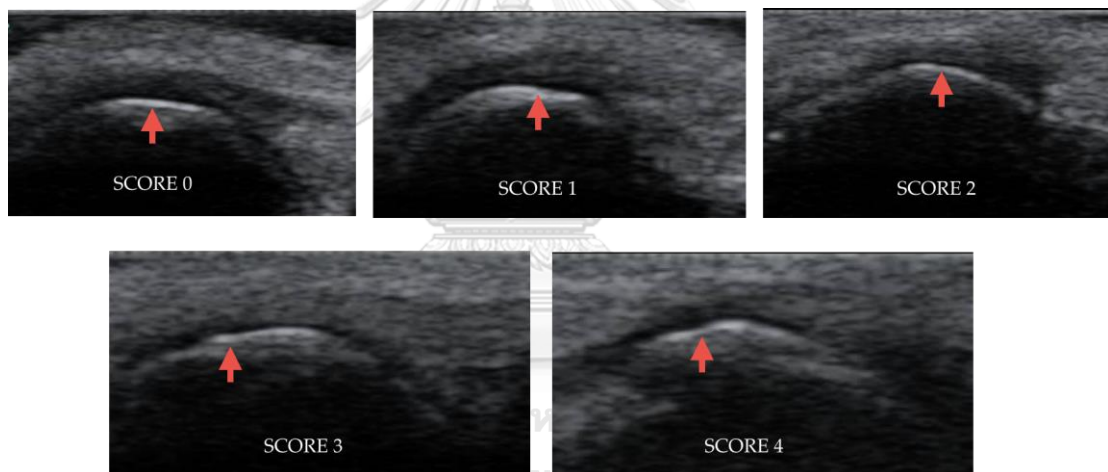
Figure 11 Example for the bone surface score 0, 1, 2, 3 and 4

3.2.5 Owner questionnaires

The owner were assessed by using Canine Brief Pain Inventory (Canine BPI) questionnaire by University of Pennsylvania to detect the severity of osteoarthritis pain (Figure 12) (Cleeland, 2006). The questionnaire had divided to 2 parts; pain evaluation and the quality of life in every week. Pain score start from 0 (not painful) to 10 (the most painful). In another part was the quality of life score that identified by 1- poor, 2- fair, 3- good, 4- very good and 5- excellent.

แบบสอบถามเกี่ยวกับระดับความเจ็บปวดในโครงการ "ประสิทธิภาพในการปกป้องผิวกระดูกอ่อนของคอลลาเจนที่คงตัวชนิด
ที่สองในสุนัขที่เป็นโรคข้อเสื่อมที่เกิดจากโรคสะบ้าเคลื่อนด้านใน"

ชื่อสุนัข .... ครั้งที่ ....วันที่..

-	เกี่ยวกับระดับความเจ็บปวด : ให้คะแนนระหว่าง 0 (น้อยที่สุด) ถึง 10 (มากที่สุด)									
1. ระดับความเจ็บปวดที่แย่ที่สุดในช่วง 7 วันที่ผ่านมา										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
2. ระดับความเจ็บปวดที่น้อยที่สุดในช่วง 7 วันที่ผ่านมา										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
3. ระดับความเจ็บปวดเฉลี่ยในช่วง 7 วันที่ผ่านมา										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
4. ระดับความเจ็บปวดในปัจจุบัน										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
- เกี่ยวกับหน้าที่การทำงานของขาในช่วง 7 วันที่ผ่านมา โดยสังเกตว่าความเจ็บปวดส่งผลต่อการใช้ขาของสุนัขมาก น้อยเพียงใด : ให้คะแนนระหว่าง 0 (ความเจ็บปวดมีผลน้อยที่สุด) ถึง 10 (ความเจ็บปวดมีผลมากที่สุด)										
5. กิจกรรมทั่วไป										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
6. ความสนุกในชีวิต										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
7. ความสามารถในการลุกยืนหลังจากนอน										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
8. ความสามารถในการเดิน										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
9. ความสามารถในการวิ่ง										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
10. ความสามารถในการเดินขึ้นบันได										
O 0	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
- ความพึงพอใจโดยรวมในด้านคุณภาพชีวิตของสุนัขในช่วง 7 วันที่ผ่านมา										
O น้อยมาก O น้อย O ปานกลาง O ดี O ดีมาก										

Figure 12 Canine Brief Pain Inventory (Canine BPI) by Dr. Dorothy Cimino Brown modified from Cleeland (2006)

3.3. Statistical analysis

The ultrasound scoring system, articular cartilage thickness of the femoral condyles, osteoarthritis scoring system, lameness scores, pain scores and quality of life scores from owner's questionnaires were calculated using One-way anova. The blood profile was calculated using paired T-test. All statistical analysis was performed using Prism program version7. *P-value* < 0.05 was considered as statistically significant.

CHAPTER IV

RESULTS

In this study, 13 dogs (21 stifle joints) were examined for 16 weeks. However, one dog had sudden dead and others systemic diseases was found in three dogs during the study. Therefore, only 13 stifles were observed through 16 weeks period of study. From total sample observed, 61.5% was Pomeranian, 23% was Chihuahua and 15.5% was Yorkshire terrier. Among 9 dogs, there was six male dogs (45.15%) and three females (53.85%).

Mean ± SD age and body weight of this study group was 7 ± 4.5 and 3.35 ± 1.043 respectively, and the detail of the studied patients are shown in table 9. The severity of medial patellar luxation divided into MPL grade1 (7.7%), MPL grade 2 (30.77%) and MPL grade 3 (61.53%). This data is shown in table 10

Table 9 Mean ± SD of age and body weight and the number of gender of dogs in this study

Demographic data		Treatment group (N = 9)
Age (year)		7 ± 4.5
Body weight (kg)		3.35 ± 1.043
Gender (N)	Male (N)	5
	Female (N)	4

Table 10 number of dogs categorized from severity of MPL

Severities of Medial patellar luxation (MPL)	Treatment group
MPL I	7.7% (1/13)
MPL II	30.77% (4/13)
MPL III	61/53% (8/13)

4.1 Blood profiles

        In this study the blood sample had been collected from left forelimb for the complete blood count (CBC) and serum chemistry before starting the experiment (day 0; D0) and after the experiment (week 16; W16), to assess a health status of the patients pre and post treatment including the effect of the collagen type II to the serum chemistry.

        The blood test results (Mean± SD) shown in table 11. The results revealed a non-significant difference with p-value >0.05 between D0 and W16 exception blood urea nitrogen (BUN) was p-value <0.05.

Table 11 comparison blood profile between pre (D0) and post (W16) treatment within group

Parameters	Normal range	Treatment group D0	W16	p-value
RBC (x10^6/ μl)	5.10-8.50	6.952 ± 1.069	7.294 ± 0.657	0.245
Hemoglobin (g/dl)	11.0-19.0	15.19 ± 2.485	15.84 ± 1.58	0.256
Hematocrit (%)	33.0-56.0	44 ± 6.758	45.46 ± 4.437	0.361
Platelet (x10^3 /μl)	117-490	367 ± 116	318.4 ± 87.9	0.165
WBC (x10^3 /μl)	6.0-17.00	12.82 ± 2.469	12.28 ± 2.185	0.570
Neutrophils (x10^3/μl)	3.62-12.30	9.281 ± 2.122	9.254 ± 2.014	0.734
Eosinophils (x10^3 /μl)	0.04-1.62	0.116 ± 0.134	0.07 ± 0.067	0.562
Basophils (x10^3 /μl)	0..00-0.12	0.024 ± 0.073	0	0.999
Lymphocytes (x10^3/μl)	0.83-4.91	3.063 ± 0.878	2.492 ± 0.84	0.096
Monocytes (x10^3 /μl)	0.14-1.97	0.336 ± 0.053	0.388 ± 0.129	0.340
BUN (mg/dl)	7.0-30.0	26 ± 7.365	21.11 ± 9.662	0.047*
Creatinine (mg/dl)	0.6-2.0	0.954 ± 0.177	0.847 ± 0.404	0.497
ALT(SGPT) (g/dl)	4-91	56.44 ± 23.21	57.67 ± 45.06	0.999
ALP (g/dl)	43-115	71 ± 98.83	71.89 ± 92.45	0.910

All *p*-value are obtained from paired t-test

*p-value <0.05.

4.2 Lameness score

       All of the dogs had been evaluated 6 times within 16 weeks. The results of the lameness score had no significant difference (p-value =0.337; p-value> 0.05) in each exam week. But some dogs with both left and right medial patellar luxation revealed an obvious improvement of both legs' lameness score. Mean ± SD of lameness score were shown in figure 13 and the improvement was exhibited in table 12.
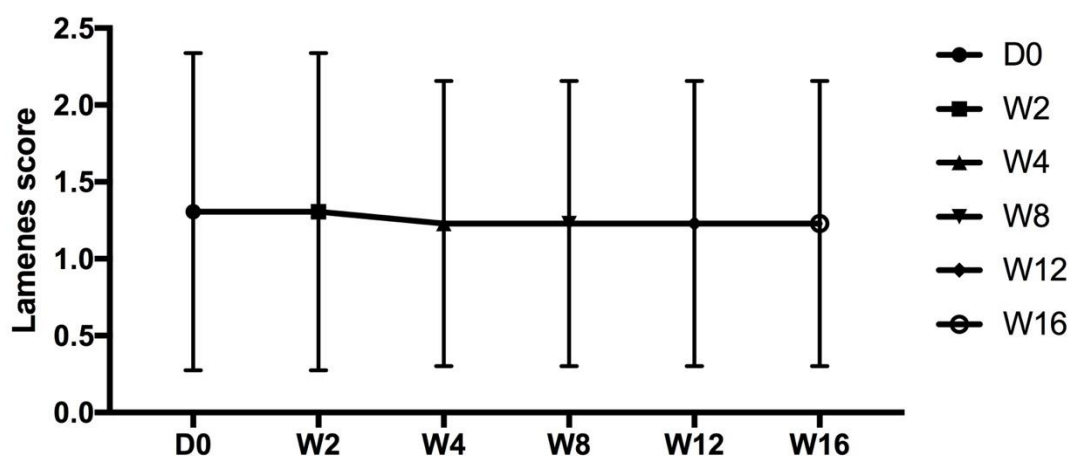

Figure 13 The mean ± SD of lameness score in each monitoring weeks

Table 12 Percentage of the improvement of lameness score at least one degree between adjacent examination: D0-W2, W2-W4, W4-W8, W8-W12 and W12-W16

Week	Improvement of lameness
D0-W2	0% (0/9)
W2-W4	11.11% (1/9)
W4-W8	0% (0/9)
W8-W12	0% (0/9)
W12-W16	0% (0/9)

4.3 Ultrasonographic examinations

In the present study, ultrasonographic score, synovial fluid score, articular cartilage score, bone surface scores and cartilage thickness were analyzed. The data were collected six times within sixteen weeks which (Mean±SD) was shown in table 13 and 14 respectively.

The ultrasonographic score has a significant difference between-treatment (day0) and week 8 to week 16 (p-value<0.05) in contrast to the data between day 0 and week 2 to week4 has no significant difference (p-value>0.05). From the examinations, the most obvious lesion of articular cartilage was found at the medial ridge of the femoral condyle while it was not obvious at the lateral ridge.

30

Table 13 Mean±SD of ultrasonographic, synovial fluid, articular cartilage and bone surface score in every examined week

Ultrasonographic parameters		Treatment group					
		Day 0	Week 2	Week 4	Week 8	Week 12	Week 16
Ultrasonographic score		5.846±1.345^a	5.231±1.092^a	4.923±1.115^a	4.615±1.557^b	4.308±1.109^b	3.692±1.251^b
Synovial fluid score		1.692±0.480^a	1.692±0.480^a	1.462±0.518^b	1.231±0.438^b	1.154±0.375^b	1.154±0.375^b
Articular cartilage score	Proximal aspect of medial femoral condyle	1.692±0.480	1.385±0.506	1.385±0.506	1.308±0.480	1.231±0.438	1.077±0.640
	Distal aspect of medial femoral condyle	1.385±0.506	1.231±0.438	1.077±0.493	1.154±0.688	1.077±0.493	0.923±0.640
Bone surface score	Proximal aspect of medial femoral condyle	0.769±0.599	0.769±0.599	0.769±0.599	0.769±0.599	0.692±0.630	0.384±0.506
	Distal aspect of medial femoral condyle	0.307±0.480	0.153±0.375	0.230±0.438	0.153±0.375	0.153±0.375	0.153±0.375

a, b - values with different superscript letters for each variable in the same row are significantly different (p <0.05)

Table 14 Mean±SD of cartilage thickness in every examined week

Groups	Positions	Day 0	Week 2	Week 4	Week 8	Week 12	Week 16
Treatment group	Medial	0.196±0.059	0.198±0.057	0.200±0.057	0.192±0.049	0.207±0.064	0.196±0.043
	Middle	0.192±0.057	0.188±0.050	0.192±0.049	0.186±0.042	0.2±0.054	0.180±0.043
	Lateral	0.234±0.080	0.226±0.083	0.218±0.064	0.223±0.072	0.230±0.063	0.207±0.049

Values in the same row are not significantly different by One way ANOVA (p value>0.05).

The result of the assessment of the synovial fluids (Mean ± SD) was shown in Table 15 and Figure 14. Significant difference between day0 (pre-treatment) to week 2 to the others. P-value <0.05 was observe.
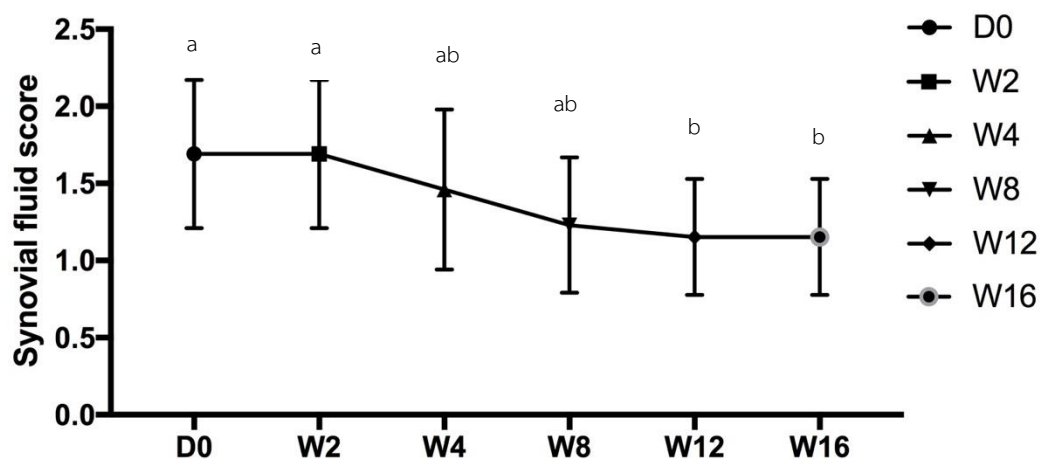
a, b - values with different superscript letters for each variable in the same row are significantly different (p <0.05)

Figure 14 The mean ± SD of synovial fluid score in examined week

Regarding articular cartilage score, slightly improvement was observed between D0 and W16 in seven stifle joints from. However, no significant difference was observed in all examination. Mean ± SD of proximal medial cartilage scores were shown in figure 15. From the treatment found that cartilage thickness had no significant differences (p-value>0.05) in every examination. Mean ± SD of cartilage thickness revealed in table 14.
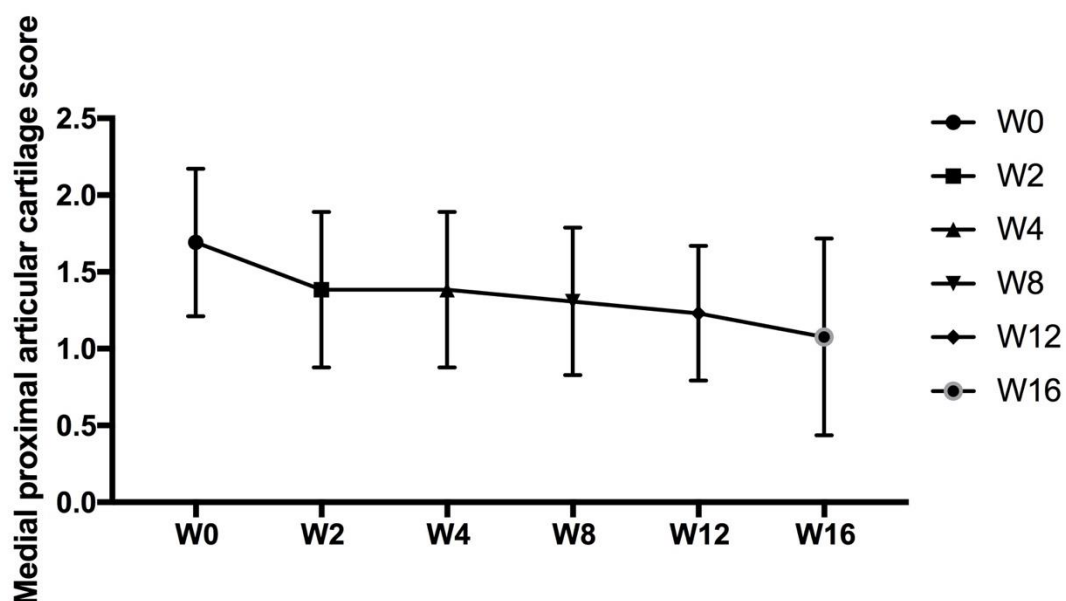
Figure 15 The mean ± SD of proximal medial articular cartilage score in collected weeks

4.4 Radiographic examinations

The stifle joints were assessed to osteoarthritis scores (OA scores) in two standards of the position in radiography. OA scores were evaluated in a range; 0-45. N significant differences (p-value>0.05) was found in any examination. Figure 16 showed the mean ± SD of this data.
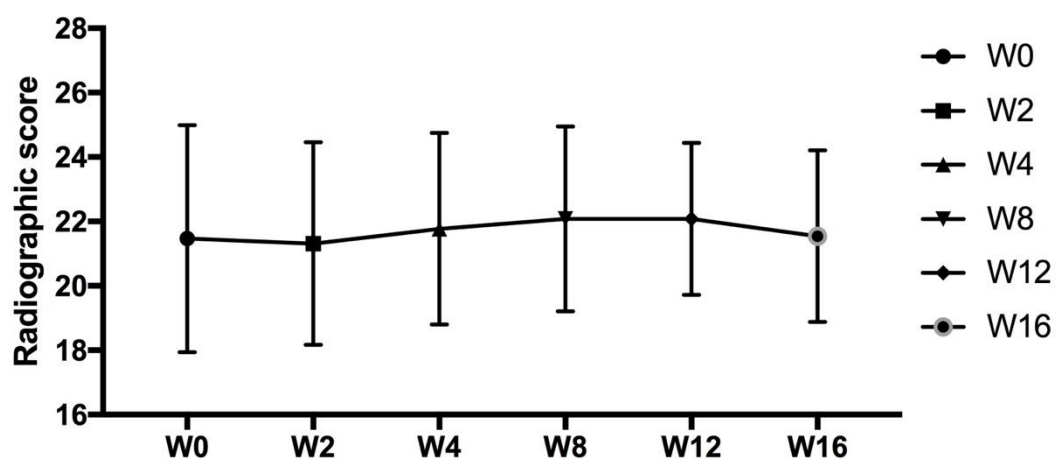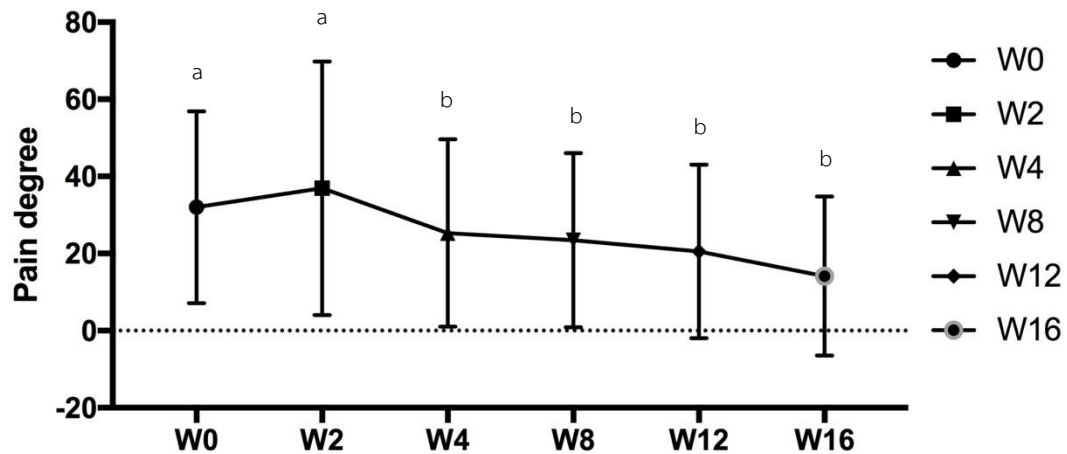

Figure 16 The mean ± SD of radiographic score in each monitoring weeks

4.5 Owner questionnaire

The severity of pain and quality of life was a significantly different (p-value<0.05) between day0 and week 16. The mean ± SD of the data was revealed in Figures 17 and 18 respectively.

a, b - values with different superscript letters for each variable in the same row are significantly different (p <0.05)

Figure 17 The mean ± SD of pain degree in examined weeks
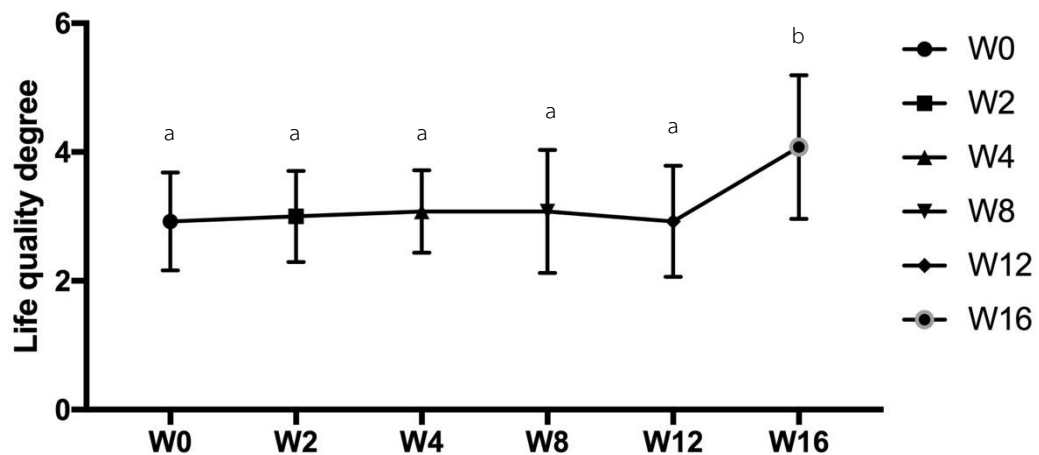
a, b - values with different superscript letters for each variable in the same row are significantly different (p <0.05)

Figure 18 The mean ± SD of life quality degree in each monitoring weeks

CHAPTER V

DISCUSSION

SC II had been used to control pain, reduced inflammation, promoted healthy joints, and improved joint mobility as well as increased flexibility in arthritis cases (Deparle et al., 2005; Bagi et al., 2017). The ability of a mucosal immune system to actively inhibit the systemic immune response to feed antigens had been used as a therapy for some chronic inflammatory and autoimmune conditions (C Crowley et al., 2009).

In this study, It had revealed that age, gender, weight, breed and severity of MPL were not the main factors which affected the results of the study. Sathienbumrungkit (2018), evaluated the efficacy of other marine based nutraceutical product (PCSO-524) by using ultrasonography and provided the data that the factors of age, gender, weight, and severity of MPL of the control and treatment group were not significantly different. Soontornvipart et al. (2013) found that there was a high incidence of patellar location (PL) in Pomeranian dogs from Thailand and that of the 238 Pomeranians indicated, 177 (75%) had PL.

All treated dogs in this study received the nutraceutical (SCII) in exact dose (10 mg/day) and amount of the nutraceutical treatment and the dogs were examined in the following weeks. To assure that the products were given to the dogs, the owners were asked some question and the nutraceutical products were counted at every examination week without prior noticed to owner. There are four excluded dogs in this research because one had died and the others three had got the systemic problems which had to treat by the other drugs. The data of these dogs were not used for analysis.

The blood profile of all dogs between pre and post-treatment were no significantly different. Interestingly, the mean of blood urea nitrogen (BUN) had slightly decreased however it was in the normal range in both pre and post treatment. D'Altilio et al. (2007) also found the same blood profile results as well. It could be concluded

that administration undenatured collagen type II in recommended dose for 10 mg per animal for 120 days did not affected the blood profile.

The result of the lameness score in this study was not significantly different between pre and post-treatment. The lameness score was verified by vet's observation and data were collected by taking video for reviewing the score. In addition, most patients had pre-treatment lameness score at 1-2 out of 5, thus the lameness score of post-treatment was not different within the group. The improvement was found in few of high lameness score dog. The result in this study were different from the previous study by D'Altilio et al. (2007) which found the significant decreases in overall pain, and pain associated with lameness and limb manipulation. The reason of disagreement from the D'Altilio et al. (2007) study might be the assessment method. The study by D'Altilio et al. (2007) had evaluated by gross observing the patients after exertion of some exercise to the patients and observed for limping, holding limb up, rigidly of limbs to assess the pain using DeParle et al. (2005) criteria which grading the pain score from 0-10 (0, no pain ;10, severe and constant pain) while, this present study evaluated by walking and trotting from lameness score criteria of Impellizeri et al. (2000).

The results from CBPI questionnaire found that the evaluation of pain and quality of life had significantly improved between pre and post- treatment. However, Sathienbumrungkit (2018) found that there was a significant difference within placebo group which indicated the placebo effect. Moreover, every groups were treated with conservative management along with medication which may be a factor contributed to the significant improvement of the control groups in Sathienbumrungkit (2018) study. The placebo effect and conservative management should be considered in further study for more accurate result. For the further evaluation, the questionnaires should be done in the dogs that controlled by conservative management or using experimental animals instead of regulation the factors from environmental difference in each patient which may course the inaccurate results.

Osteoarthritis scores (OA scores) from the radiographic examination showed no significant difference between pre and post-treatment. This could be because of OA in medial patellar luxation was the result of a low-grade inflammation and early stage

of OA development in the canine affected stifle (Camber, 2017; Di Dona et al., 2018). Alam et al. (2006) found that the early stages of OA were difficult to diagnose by using radiography. Radiographic changes consist of joint capsular distension, osteoarthritis, soft tissue thickening, narrowing of the joint space and subchondral sclerosis which happened only at the later stages of OA (Goranov et al., 2013).

The results of the ultrasonography scores composed of synovial fluid, medial and lateral proximal and distal articular cartilage and bone surface. Evaluated total scores had differed significantly. When these scores were assessed separately, we found only synovial fluid score had significant difference. The most erosion area of femoral condyle in this study was on the medial proximal condyle ridge which corresponded with the previous report from Jahrupatrakorn (2017). The assessment of medial proximal condyle was shown slight improvement nevertheless it had no significant difference. The articular cartilage thickness from the transverse view of the ultrasound revealed no significant differences in medial, middle and lateral condyle. Using ultrasound to evaluate the joint structures and the synovial fluid echogenicity had to be careful due to the dogs were moveable even under restrained condition and this could affect the probe position during the ultrasound leading to the error of the result. The using of radiographic examinations in order to assess OA scores and ultrasonographic scores, the animals should be under anesthetized to restrain in the correct position as struggling animal could be injured due to the restraining for a proper position.

Bagchi et al. (2002) found that type II collagen was the structural protein which can be found in cartilage and it was responsible for tensile strength and toughness. The undenatured collagen type II is a precursor of collagen producing, however, it cannot be use directly as a joint promoter.

Deparle et al. (2005) mentioned that SCII worked on down regulating the autoimmune response in the case of rheumatoid arthritis. For osteoarthritis patient, SCII could help in reduction inflammation and pain relieving. Furthermore, SCII has the ability to stop the immune system from attacking and damaging its own joint cartilage. Hence, SCII tends to give obviously better outcome which reduced the joint damage in immune mediated osteoarthritis such as rheumatoid.

Study from Prabhoo and Billa (2018) found that small oral doses of glycosylated undenatured collagen type II presents active epitope with corrected three-dimension structures to the gut-associated lymphoid tissue (GALT). This type of collagen increases the immunotolerance. Small doses of SCII prevent the attack by killer T cells. The pathogenesis of osteoarthritis is not related to immune-mediated mechanism. Osteoarthritis is resembled rheumatoid arthritis in the form of cartilage damage and inflammatory response. But this research studies in osteoarthritis dogs that in contrast to the previously referred examination. In addition, the dogs in this study which had osteoarthritis were not show clearly different results between before and after treatment.

For the further study, the author suggested to use other practical instruments that would decrease the effect of other factors and provide higher accurate outcome. For example; Computerized Tomography Scan (CT scan), Magnetic Resonance Imaging (MRI), histopathology, synovial analysis, force plate gait analysis or C-reactive protein test. All of the mentioned appliances may give better and more reliable outcome than radiographic examinations or ultrasonographic scores. The sample size should be increase due to some sample may be excluded from the criteria in clinical trial study.

In conclusion, from the study, UCII can improve the chondroprotective effect in the treatment of the osteoarthritis secondary to medial patellar luxation but not significantly. This may be due to the mechanism that undenatured collagen type II mainly effects on osteoarthritis associated with immune response. For the osteoarthritis, immune system is not involved in the pathogenesis of osteoarthritis. Nevertheless, there are some beneficial aspect of using undenatured collagen type II such as inflammation improvement from the result of joint effusion, CBPI and impression. So SCII has been used to control pain, reduce inflammation, promote health joint, and improve joint motility and flexibility in arthritis cases. (Deparle et al., 2005; Bagi et al., 2017)

REFERENCES

Alam M, Lee H, Kim ms and Kim NS 2011a. Surgical model of osteoarthritis secondary to medial patellar luxation in dogs. Vol 56. In. 123-130.

Alam M, Lee H, Park S, Lee Y, Kim I, Kang H-S and Kim N-S 2006. Changes in the Haematobiochemical Parameters in Experimental Stifle Osteoarthritis in Dogs. Pakistan Journal of Biological Sciences. 9.

Alam MR, Ji JR, Kim MS and Kim NS 2011b. Biomarkers for identifying the early phases of osteoarthritis secondary to medial patellar luxation in dogs. J Vet Sci. 12(3): 273-280.

Arnault F, Cauvin E, Viguier E, Kraft E, Sonet J and Carozzo C 2009. Diagnostic value of ultrasonography to assess stifle lesions in dogs after cranial cruciate ligament rupture: 13 cases. Vet Comp Orthop Traumatol. 22(6): 479-485.

Bagchi D, Misner B, Bagchi M, Kothari SC, Downs BW, Fafard RD and Preuss HG 2002. Effects of orally administered undenatured type II collagen against arthritic inflammatory diseases: a mechanistic exploration. Int J Clin Pharmacol Res. 22(3-4): 101-110.

Bagi CM, Berryman ER, Teo S and Lane NE 2017. Oral administration of undenatured native chicken type II collagen (UC-II) diminished deterioration of articular cartilage in a rat model of osteoarthritis (OA). Osteoarthritis Cartilage. 25(12): 2080-2090.

Brosseau L, MacLeay L, Robinson V, Wells G and Tugwell P 2003. Intensity of exercise for the treatment of osteoarthritis. Cochrane Database Syst Rev. (2): Cd004259.

C Crowley D, Lau F, Sharma P, Evans M, Guthrie N, Bagchi M, Bagchi D, Dey D and P Raychaudhuri S 2009. Safety and efficacy of undenatured type II collagen in the treatment of osteoarthritis of the knee: A clinical trial. Vol 6. In. 312-321.

Camber A-M 2017. Etiology of patellar luxation in small breed dogs.

Castrogiovanni P, Trovato FM, Loreto C, Nsir H, Szychlinska MA and Musumeci G 2016. Nutraceutical Supplements in the Management and Prevention of Osteoarthritis. International journal of molecular sciences. 17(12): 2042.

Chappard C, Peyrin F, Bonnassie A, Lemineur G, Brunet-Imbault B, Lespessailles E and Benhamou CL 2006. Subchondral bone micro-architectural alterations in osteoarthritis: a synchrotron micro-computed tomography study. Osteoarthritis Cartilage. 14(3): 215-223.

Cleeland CS 2006. The Measurement of Pain from Metastatic Bone Disease: Capturing the Patient's Experience. Clinical Cancer Research. 12(20): 6236s.

D'Altilio M, Peal A, Alvey M, Simms C, Curtsinger A, Gupta RC, Canerdy TD, Goad JT, Bagchi M and Bagchi D 2007. Therapeutic Efficacy and Safety of Undenatured Type II Collagen Singly or in Combination with Glucosamine and Chondroitin in Arthritic Dogs. Toxicol Mech Methods. 17(4): 189-196.

Daems R, Janssens LA and Beosier YM 2009. Grossly apparent cartilage erosion of the patellar articular surface in dogs with congenital medial patellar luxation. Vet Comp Orthop Traumatol. 22(3): 222-224.

Deparle LA, Gupta RC, Canerdy TD, Goad JT, D'Altilio M, Bagchi M and Bagchi D 2005. Efficacy and safety of glycosylated undenatured type-II collagen (UC-II) in therapy of arthritic dogs. J Vet Pharmacol Ther. 28(4): 385-390.

Di Dona F, Della Valle G and Fatone G 2018. Patellar luxation in dogs. Vet Med (Auckl). 9: 23-32.

Fauron A and Perry K 2016. Canine patellar luxation part 1: pathophysiology and diagnosis. Veterinary Times. 46: 20-22.

Goranov N, Paskalev M and Kanakov D 2013. Radiological and Ultrasound Imaging Scores in Experimental Sodium Monoiodoacetate Model of Knee Osteoarthritis in Dogs. Vol 391. In. 67-75.

Gupta RC, Canerdy TD, Lindley J, Konemann M, Minniear J, Carroll BA, Hendrick C, Goad JT, Rohde K, Doss R, Bagchi M and Bagchi D 2012. Comparative therapeutic efficacy and safety of type-II collagen (UC-II), glucosamine and chondroitin in arthritic dogs: pain evaluation by ground force plate. J Anim Physiol Anim Nutr (Berl). 96(5): 770-777.

Impellizeri JA, Tetrick MA and Muir P 2000. Effect of weight reduction on clinical signs of lameness in dogs with hip osteoarthritis. J Am Vet Med Assoc. 216(7): 1089-1091.

Jahrupatrakorn S 2017. Intra-articular characteristics of dogs with medial patellar luxation., Chulalongkorn University.

Kalra E 2003. Nutraceutical -Definition and Introduction. Vol 5. In. E25.

Kramer M, Stengel H, Gerwing M, Schimke E and Sheppard C 1999. Sonography of the canine stifle. Vet Radiol Ultrasound. 40(3): 282-293.

Martinez SA 1997. Congenital conditions that lead to osteoarthritis in the dog. Vet Clin North Am Small Anim Pract. 27(4): 735-758.

Moller I, Bong D, Naredo E, Filippucci E, Carrasco I, Moragues C and Iagnocco A 2008. Ultrasound in the study and monitoring of osteoarthritis. Osteoarthritis Cartilage. 16 Suppl 3: S4-7.

Nasri H, Baradaran A, Shirzad H and Rafieian-kopaei M 2014. New Concepts in Nutraceuticals as Alternative for Pharmaceuticals. International journal of preventive medicine. 5: 1487-1499.

Nishitani K, Kobayashi M, Kuroki H, Mori K, Shirai T, Satake T, Nakamura S, Arai R, Nakagawa Y, Nakamura T and Matsuda S 2014. Ultrasound Can Detect Macroscopically Undetectable Changes in Osteoarthritis Reflecting the Superficial Histological and Biochemical Degeneration: Ex Vivo Study of Rabbit and Human Cartilage. PLOS ONE. 9(2): e89484.

O'Neill DG, Meeson RL, Sheridan A, Church DB and Brodbelt DC 2016. The epidemiology of patellar luxation in dogs attending primary-care veterinary practices in England. Canine Genet Epidemiol. 3: 4.

Okano T, Filippucci E, Di Carlo M, Draghessi A, Carotti M, Salaffi F, Wright G and Grassi W 2016. Ultrasonographic evaluation of joint damage in knee osteoarthritis: feature-specific comparisons with conventional radiography. Rheumatology (Oxford). 55(11): 2040-2049.

Pettitt R and German A 2015. Investigation and management of canine osteoarthritis. Vol 37. In. 1-8.

Prabhoo R and Billa G 2018. Undenatured collagen type II for the treatment of osteoarthritis: a review. International Journal of Research in Orthopaedics.

Ramirez-Flores GI, Del Angel-Caraza J, Quijano-Hernandez IA, Hulse DA, Beale BS and Victoria-Mora JM 2017. Correlation between osteoarthritic changes in the stifle

joint in dogs and the results of orthopedic, radiographic, ultrasonographic and arthroscopic examinations. Vet Res Commun. 41(2): 129-137.

Reed AL, Payne JT and Constantinescu GM 2005. ULTRASONOGRAPHIC ANATOMY OF THE NORMAL CANINE STIFLE. Veterinary Radiology & Ultrasound. 36(4): 315-321.

Salg KG, Temwitchitr J, Imholz S, Hazewinkel HA and Leegwater PA 2006. Assessment of collagen genes involved in fragmented medial coronoid process development in Labrador Retrievers as determined by affected sibling-pair analysis. Am J Vet Res. 67(10): 1713-1718.

Sathienbumrungkit R 2018. CHONDROPROTECTIVE EFFICACY OF UNDENATURED COLLAGEN TYPE II ON CANINE OSTEOARTHRITIS SECONDARY TO MEDIAL PATELLAR LUXATION.

Soontornvipart K, Wangdee C, Kalpravidh M, Brahmasa A, Sarikaputi M, Temwichitr J, Lavrijsen IC, Theyse LF, Leegwater PA and Hazewinkel HA 2013. Incidence and genetic aspects of patellar luxation in Pomeranian dogs in Thailand. Vet J. 196(1): 122-125.

Vandeweerd J-M, Coisnon C, Clegg P, Cambier C, Pierson A, Hontoir F, Saegerman C, Gustin P and Buczinski S 2012. Systematic Review of Efficacy of Nutraceuticals to Alleviate Clinical Signs of Osteoarthritis. Journal of Veterinary Internal Medicine. 26(3): 448-456.

Vaughan-Scott T and Taylor JH 1997. The pathophysiology and medical management of canine osteoarthritis. J S Afr Vet Assoc. 68(1): 21-25.

Wangdee C, Leegwater PA, Heuven HC, van Steenbeek FG, Techakumphu M and Hazewinkel HA 2017. Population genetic analysis and genome-wide association study of patellar luxation in a Thai population of Pomeranian dogs. Res Vet Sci. 111: 9-13.

Wessely M, Bruhschwein A and Schnabl-Feichter E 2017. Evaluation of Intra- and Inter-observer Measurement Variability of a Radiographic Stifle Osteoarthritis Scoring System in Dogs. Vet Comp Orthop Traumatol. 30(6): 377-384.

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