COMPARISON OF THE USE OF OMEGA-3 FATTY ACIDS COMPOUND FROM NEW ZEALAND GREEN LIPPED MUSSEL AND FISH OIL IN THE TREATMENT OF CANINE SHOULDER AND COXOFEMORAL OSTEOARTHRITIS

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การเปรียบเทียบการใช้กรดไขมันกลุ่มโอเมกา 3 สกัดจากหอยแมลงภู่นิวซีแลนด์ กับน้ำมันปลา ในการรักษาสุนัขที่เป็นโรคข้อไหล่และข้อสะโพกเสื่อม

นางสาวณัฐวดี มงคล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	COMPARISON OF THE USE OF OMEGA-3 FATTY ACIDS COMPOUND FROM NEW ZEALAND GREEN LIPPED MUSSEL AND FISH OIL IN THE TREATMENT OF CANINE SHOULDER AND COXOFEMORAL OSTEOARTHRITIS
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ณัฐวดี มงคล : การเปรียบเทียบการใช้กรดไขมันกลุ่มโอเมกา 3 สกัดจากหอยแมลงภู่นิวซีแลนด์ กับน้ำมันปลาในการรักษาสุนัขที่เป็นโรคข้อไหล่และข้อสะโพกเสื่อม. (COMPARISON OF THE USE OF OMEGA-3 FATTY ACIDS COMPOUND FROM NEW ZEALAND GREEN LIPPED MUSSEL AND FISH OIL IN THE TREATMENT OF CANINE SHOULDER AND COXOFEMORAL OSTEOARTHRITIS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ. นสพ. ดร. กัมปนาท สุนทรวิภาต 67 หน้า

การศึกษาผลของการใช้กรดไขมันกลุ่มโอเมกา 3 สกัดจากหอยแมลงภู่นิวซีแลนด์กับน้ำมันปลาต่ออาการทางคลินิกและปริมาณตัวบ่งชี้ทางชีวภาพโรคข้อเสื่อม (WF6, HA) ในสุนัขที่เป็นโรคข้อเสื่อมจำนวน 46 ตัว สุนัข 39 ตัวป่วยเป็นโรคข้อสะโพกเสื่อม สุนัข 5 ตัวป่วยเป็นโรคข้อไหล่เสื่อม และสุนัขอีก 2 ตัวป่วยเป็นโรคข้อไหล่ร่วมกับสะโพกเสื่อม ที่เข้ารับการรักษา ณ โรงพยาบาลสัตว์เล็ก จุฬาลงกรณ์มหาวิทยาลัย สุนัขถูกแบ่งเป็น 2 กลุ่มโดยการสุ่มได้รับ PCSO-524 และ น้ำมันปลาเป็นระยะเวลา 8 สัปดาห์ การตรวจประเมินอาการ, ถ่ายภาพรังสี และหาปริมาณตัวบ่งชี้ทางชีวภาพของโรคข้อเสื่อมในซีรัมก่อนรักษาและระหว่างการรักษาในสัปดาห์ที่ 2, 4, 8 ผลการศึกษาพบว่า สุนัขมีอาการเจ็บขาน้อยลง, การลงน้ำหนักมากขึ้น และพิสัยข้อต่อดีขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อได้รับ PCSO-524เป็นระยะเวลา 2 สัปดาห์ซึ่งไม่พบความแตกต่างของอาการเหล่านี้ในกลุ่มที่ได้รับน้ำมันปลา อาการเจ็บขาและลงน้ำหนักของกลุ่ม PCSO-524 ดีขึ้นกว่ากลุ่มน้ำมันปลาในสัปดาห์ที่ 4 และ 8 อย่างมีนัยสำคัญทางสถิติ แม้ว่า ค่าเฉลี่ยคะแนนภาพถ่ายรังสีไม่แตกต่างกันในแต่ละกลุ่ม แต่พบการเปลี่ยนแปลงอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่มข้อสะโพกเสื่อมในสัปดาห์ที่ 8 ความเข้มข้นของคอนดรอยติน ซัลเฟต WF6 อิพิโทป ในซีรัมของสุนัขที่ได้รับ PCSO-524 (262.46±162.24 ng/ml) มีระดับต่ำกว่ากลุ่มที่ได้รับน้ำมันปลา (353.99±132.25 ng/ml) อย่างมีนัยสำคัญทางสถิติ (p<0.05) หลังได้รับการรักษาเป็นระยะเวลา 8 สัปดาห์ แต่ไม่พบความแตกต่างของความเข้มข้นของ HA จากการศึกษานี้สรุปได้ว่า PCSO-524 สามารถชะลอการเสื่อมของข้อไหล่และข้อสะโพกในสุนัขได้

ภาควิชา ศัลยศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา ศัลยศาสตร์ทางสัตวแพทย์ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ .....

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NATWADEE MONGKHON : COMPARISON OF THE USE OF OMEGA-3 FATTY ACIDS COMPOUND FROM NEW ZEALAND GREEN LIPPED MUSSEL AND FISH OIL IN THE TREATMENT OF CANINE SHOULDER AND COXOFEMORAL OSTEOARTHRITIS. ADVISOR : ASSIST. PROF. KUMPANART SOONTORNVIPART, D.V.M., Ph.D., 67 pp.

This study was designed to assess the effects of omega 3 fatty acids compound from New Zealand green lipped mussel (PCSO-524) and omega 3 fatty acids in fish oil on clinical outcomes and on osteoarthritis biomarkers (chondroitin sulfate WF6 epitope and hyaluronan (HA)) in 46 osteoarthritis (OA) dogs; 39 dogs with OA hip joints, 5 dogs with OA shoulder joints and 2 dogs with OA shoulder and hip joints. The animals were presented at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. The dogs were randomly allocated into 2 groups; PCSO-524 and fish oil groups fed with the two preparations for 8 weeks. Lameness score, weight bearing score, radiographic score, range of motion and serum OA biomarkers (WF6, HA) were evaluated before treatment and at 2, 4 and 8 weeks after treatment. Lameness score, weight bearing score and ROM were significantly improved within two weeks after PCSO-524 administration while there were no statistically significant difference in all parameters of the fish oil group. At 4 and 8 weeks, lameness and weight bearing scores of the PCSO-524 group were significantly improved as compared with the fish oil group. Although the mean of radiographic scores was not significant difference among groups, mean of serum WF6 of the PCSO-524 group (262.46±162.24 ng/ml) was significantly ($p<0.05$) lower than that of the fish oil group (353.99±132.25 ng/ml) after eight weeks of administration. In conclusion, PCSO-524 administration could slow progression of osteoarthritis of the shoulder and hip joints in dogs.

Department: Veterinary Surgery Student's Signature.....

Field of Study: Veterinary Surgery Advisor's Signature.....

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Chapter I

Introduction

Importance and rationale

Osteoarthritis (OA) is the most common arthropathy of dogs. OA has been explained as 'an inherently non-inflammatory arthritis' of synovial joints identified by degeneration articular cartilage and by the formation of new bone at the joint surfaces, margins and capsules (Bruce et al., 2006; Nganvongpanit et al., 2008b; Bennett D, 2010). The pathogenesis of OA is still not understood clearly. Many researchers believed that the causes of OA could be the articular cartilage changes such as structure, biochemistry and metabolism, which might be influenced from one or a combination of genetics, overuse, accidents, aging, dietary and inflammatory factors (Bruce et al., 2006; Nganvongpanit et al., 2008b; Bennett, 2010).

The prevalence of canine osteoarthritis has been found to be approximately 20 % among dogs that were more than one year old (Johnston, 1997). In addition, a survey study revealed OA in 52% of hips, 37% of shoulders, 36% of stifles and 5 % of elbows in 100 dogs. These data have shown that the two most important joints were the hips and shoulders. Hip dysplasia (HD) is generally found in dogs and leads to secondary OA. According to Smith et al. (2001), 80% of dogs that had HD were found to also have OA. Canine hip and shoulder osteoarthritis usually also involves limb lameness because of instability, as well as joint pain, joint stiffness, restricted motion and impaired function. The clinical sign of canine hip/shoulder OA is not only limb lameness but also a swollen joint that is a consequence of synovial effusion, joint capsule fibrosis and new periarticular bone formation (Bennett, 2010).

The definite diagnosis of OA cannot be made from the clinical signs and usually the radiographic cannot be indicated at the initial stage of OA. Thus, the novel procedure is to evaluate the biochemical markers of joint disease. Chondroitin sulfate (CS) (Tungyuenyong et al., 2006), keratan sulfate (KS) (Leipold et al., 1989), hyaluranan (HA) (Leipold et al., 1989; Budsberg et al., 2006) or collagen type II (Hollander et al.,

1994) have been shown to be the effective indicators for monitoring treatment, predicting the disease and diagnosing OA.

There is currently no cure for OA because the damage to articular cartilage cannot be truly reversed to restore the normal cartilage (Wang et al., 2004; Bennett, 2010). The two most important problems of OA are pain and a decrease in joint movement that results in discomfort and a reduced quality of life for the dog. Most dogs that have OA require supportive drugs, which are divided into three groups. The first group includes symptom-modifying OA drugs (SYMOA) for pain relief. The second group consists of structural-modifying OA drugs (STMOA), which slow the structural pathological changes. The third group is comprised of symptomatic slow-acting drugs for OA (SYSADOA) (Lequesne et al., 1994; Booth, 2001; Bennett, 2010).

Nutritional supplementation has been studied extensively as a major role in OA management in dogs because it has fewer side effects (Wang et al., 2004; Bennett, 2010). In addition, omega-3 essential fatty acids (omega-3 EFAs) are one of the most popular nutritional supplements. According to several researches on the effects of omega-3 EFAs *in vitro*, omega-3 EFAs supplementation can decrease the inflammation and matrix degradation in articular cartilage (Curtis et al., 2000; Curtis et al., 2002). Initiation and control of the inflammatory process is complicated and governed by an array of biomolecular mechanisms. One important pro-inflammatory mechanism is closely associated with cell-membrane-bound arachidonic acid, which is converted in the body into other compounds that are potent pro-inflammatory substances. This occurs in two major pathways of metabolism: the 5-lipoxygenase pathway, which leads to the formation of leukotrienes; and the cyclo-oxygenase pathway, which leads to the formation of prostaglandins and thromboxanes that induce the degeneration of cartilage (Bruce et al., 2006). A complex lipid compound from the oil of the New Zealand green-lipped mussel (*Perna canaliculus*) contains a unique group of polyunsaturated fatty acids (PUFAs) that includes eicosatetraenoic acid (ETA). It is a source of long-chain omega-3 PUFAs that have been shown to be an effective anti-inflammation compound, which reduced the severity of the symptoms of OA in studies (Tempel et al., 1990;

Sanders, 1993; Curtis et al., 2000). Many researchers have demonstrated that omega-3 PUFAs (especially ETA and EPA) may affect the LOX and COX pathways by reducing the production of leukotrienes and prostaglandins (Whitehouse et al., 1997; Dugas et al., 2000; Murphy et al., 2002). This marine extract has been proved to be a natural, safe and effective inhibitor of the lipoxygenase pathways, one of the principal inflammation pathways in human body (Treschow et al., 2007). There have been few studies in animals. At this time there are many commercial omega-3 compound diets and fish oil in markets that contain omega-3 EFAs such as eicosapentaenoic acid (EPA) and docasahexaenoic acid (DHA).

Although considerable research has been devoted to the improvement of clinical signs, less attention has been paid to OA biomarkers as a way to monitor disease activity and predict disease progression. The aim of this study is to examine the effect of a unique group of omega-3 fatty acids extracted by a patent method from the New Zealand green-lipped mussel in comparison with the effect of omega-3 fatty acids from fish oil for the joint disease patients on the osteoarthritis biomarker level, clinical signs and radiographic findings in the treatment of canine shoulder and coxofemoral osteoartritis.

Objectives of study

To compare the effect of an omega-3 fatty acids compound (PCSO-524) from New Zealand green-lipped mussel with the effect of fish oil on the osteoarthritis biomarker level, clinical signs and radiographic findings in the treatment of canine shoulder and coxofemoral osteoarthritis.

Research frame

This study was designed to use the PCSO-524 and fish oil as nutraceuticals to improve OA in 46 dogs that were presented at the surgery unit of Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. Clinical signs, lameness scores, weight bearing scores, OA scores and ranges of motion (ROM) of the animals were recorded. All dogs were examined clinically and the evidence of OA at

coxofemoral or the shoulder joint was indicated in radiographs (VD-extended coxofemoral projection and lateral projection of the shoulder), then blood collection was taken for CBC, blood chemistry (BUN, creatinine, ALP, ALT) and OA biomarkers (WF6, HA) before treatment. Dogs were divided into two groups by simple randomization. Each group was given a different treatment. The dogs were re-evaluated after two, four and eight weeks of treatment. Parameters such as lameness scores, weight-bearing scores, OA scores and ROM were recorded. The blood for OA biomarkers was centrifuged then the serum was separated and was frozen at -20 ^{o}C until biomarker assays were performed. If the case showed severe liver, gastrointestinal, urogenital, neurological problems, or if the pain score was more than five points on the Glasgow Composite Measure Pain Score-Short Form, the dog was excluded.

Research question

Does this unique group of omega-3 fatty acids from New Zealand green-lipped mussel and fish oil decrease the progression of OA in canine osteoarthritis as determined by decreased level of OA biomarkers (WF6, HA), as well as the improved clinical signs and radiographic findings ?

Keywords (Thai):
สารบ่งชี้ทางชีวภาพ สุนัข น้ำมันปลา โรคข้อเสื่อม พีซีเอสโอ-524

Keywords (English):
Biomarkers, Dogs, Fish oil, Osteoarthritis, PCSO-524

Advantages of study

Use of PCSO-524 (omega-3 PUFAs compound from New Zealand green lipped mussel) is an alternative treatment of canine OA. It can slow progression, return limb to normal function and increase quality of life with minimal complication in OA dogs.

Chapter II
Literature Review

The major chronic disease that leads to joint disability is osteoarthritis (OA), which is a common arthropathy in humans, horses and dogs (Bennett, 2010). The cause of OA is not understood well, but it is generally agreed that the cause of OA involves various factors, such as aging, genetics, diets, trauma, metabolic imbalance and inflammatory pathway responses that lead to the breakdown of articular cartilage (Bruce et al., 2006; Nganvongpanit et al., 2008b; Bennett, 2010). The prevalence of canine osteoarthritis increased from 15% to 67% as dogs aged (Gail et al., 2006). Moreover, another study showed canine OA affected about 20% of dogs that were more than one year old (Johnston, 1997). The pathogenesis of the progression of OA can cause the defects on normal cartilage by altering the balance of joint metabolism in synthesis and degeneration pathways. This leads to a change in the articular cartilage (erosion, osteophyte formation, synovitis) (Bruce et al., 2006; Nganvongpanit et al., 2008b; Bennett, 2010). In small animal practices, OA was recognized a common result of joint diseases. Furthermore, a survey found that the two most effected joints were hip (52%) and shoulder (37%) (Hegemann et al., 2002). These interesting joints are large, so the radiographic diagnosis of OA might be made easily and confidently. The hip and/or shoulder joint have become models for detecting OA.

Joint anatomy

A Joint is the connection or union between bones, which can be divided into three types by motional ability. There are synarthrodal joints (immoveable joint), amphiarthrodal joints (slightly moveable joint) and diarthridal joints (moveable joint) (Riegger-Krugh et al., 2004). The two most prevalent of OA joints were identified at hip and shoulder joints, which are of the diarthridal type. The structure of a diarthrdal joint is composed of bones, ligaments, tendons, a joint capsule and other connective tissues.

Bone ends are covered by cartilage that protects the bone by preventing bone rubbing on bone (Riegger-Krugh et al., 2004).

The chondral bone of articular is hyaline cartilage type (clear, white, smooth characteristics), which consists of chondrocytes and an extracellular matrix (ECM) that provides strong function and elasticity to the joint. In a normal joint, anabolism and catabolism are usually stable. In addition, the absence of blood vessels, lymph vessels and nerve supply are the important properties of the articular cartilage. Since there are no blood vessels, nutritional sources for cartilage are via synovial fluid and subchondral route (Huber, M, et al., 2000).

Pathogenesis and metabolism

The pathogenesis and etiology of OA involve many factors that may be one or combination of age, breed, food, obesity, joint mechanics, injury and biochemical change (Roach and Tilley, 2008). These causes induce alteration of the homeostasis of the joint environment, by which the balance between anabolism and catabolism is broken. The multifactoral influence increases catabolism enzymes such as collagenase, proteinases and aggrecanase, which destroy structures in bone matrix (Nganvongpanit et al., 2008b).

The extracellular matrix molecules in chondral bone are comprised of collagen, hyaluronan and the proteoglycan group (PG). Chondroitin sulfate and keratin sulfate are the structures of this PG group (Nganvongpanit et al., 2008b). The imbalance of anabolism and catabolism leads primarily to more enzyme production (proteinases, matrix metalloprotenases, aggrecanase) that can destroy the ECM (especially collagen and proteogylcan) (Nganvongpanit et al., 2008b). If the activity of catabolism is more progressive than anabolism activity, it destroys not only ECM but also chondrocyte apoptosis.

In a preliminary information of metabolic mechanisms in the pathogenesis of osteoarthritis, this mechanism is based on an inflammatory process that cannot find a balance between the production and destruction of cartilage. This causes the alteration

of fatty acid metabolite, which nourishes osteoarthritis all the time, as well as cartilage degradation (Bruce et al., 2006). The inflammatory pathway arachidonic acid (AA-the primary fatty acid from breakdown of cellular membrane in a condition of acute and/or chronic joint damage) is metabolized by 5-lipoxygenase (5-LOX), which leads to the generation of leukotriene B_4 (LTB_4), which is strongly pro-inflammatory and attracts white blood cells (WBCs) to repair tissue damage. Another enzyme, cyclooxygenase (COX) involves inflammatory process by production of prostaglandinE_2 (PGE_2), prostaglandinF_2 (PGF_2) and thromboxaneB_2 (TXB_2), which are all strongly pro-inflammatory. Increased free radical production, the cytokine synthesis (which induces inflammatory process) and an increase in histamine can cause a frequent breakdown of the articular cartilage if this damage continues past the short term (Bruce et al., 2006).

A major risk factor for OA is aging. Aging is associated often with the accumulation of free radicals, especially nitric oxide, which results in damaged and abnormal articular cartilage (Beckman and Ames, 1998, Thaiklang et al.,2004, Del-carlo and Loeser, 2002). Furthermore, an increasing weakness of the ligament, tendon and joint capsule in adults increases the load on the joint directly. Abnormal loading can occur as a result of trauma or obesity and instable joints induce osteoarthritis (Roach and Tilley, 2008).

Clinical signs

OA presents commonly as one or a combination of clinical signs such as limb pain, decreasing range of motion, joint swelling, joint instability, joint stiffness. There is also a decreased intolerance of exercise and difficulty with tasks such as going up and down stairs. There are also changes in standing and sitting posture (Nganvongpanit et al., 2008b; Ettinger and Feldman, 2010). In the case of chronic osteoarthritis of hip joints, OA dogs always step shortly, stand rigidly and load weight to forelimbs instead of hind limbs, which results in increase in the forelimb muscle mass. As a result of lack of use of the hind limb, the muscles in that area may atrophy (Nganvongpanit, 2008c).

Diagnostic tools

The diagnosis of OA requires a history to be taken, a physical examination, an analysis of synovial fluid and also radiography, which can indicate the late stage of this disease.

Recording the symptoms and history taking of dogs that presents with OA is essential. Many researchers have reported that OA was found mainly in dogs that were old and/or obese. Moreover, a study in 2006 revealed that life-long diet limitation resulted in a lower prevalence and later onset of coxofemeral joint OA in dogs (Smith, 2006).

Physical examination should begin with a general physical examination to rule out systemic problems, then orthopedic examination and neurologic examination should be performed to distinguish the true problem from other possible causes. For patients that have clinical signs of lameness, lameness examination is a beneficial non-invasive diagnostic tool that should be taken by observation of standing, sitting, walking and trot; and palpation of paw, tarsal joint, stifle joint, hip joint, carpal joint, elbow joint and shoulder joint. OA patients exhibit commonly pain and a decrease in range of motion of the OA joint, so radiographs should be taken. The radiographs can also identify other lesion of bone and joint (Schrader et al, 1995).

Radiography is an important tool with which to diagnose osteoarthritis lesions. Mostly, the evidence of OA at coxofemoral or shoulder joints is indicated in radiographs (VD-extended coxofemoral projection and lateral projection of the shoulder). This evidence consists of periarticular osteophyte formation (coxofemoral joint: femoral neck and the cranial part of the acetabulum, shoulder joint: caudal margin of humeral head and glenoid margins), subchondral bone sclerosis, osseous cyst-like lesions, narrowing of the joint space, joint capsule distension and/or periarticular soft-tissue swelling (Sirois et al., 2010; Dennis et al., 2010). Conventional radiographs provide little information during the early stage of OA. Magnetic resonance imaging (MRI) has been applied to diagnose the early stages of OA in humans because of its sensitivity (Blum et al, 1996;

Jazrawi et al, 2011), but MRI in dogs is not done routinely because of the cost and the need for general anesthesia.

Currently, the use of biomarkers to diagnose and monitor the progression of OA, to enable a prognosis to be made and/or to evaluate the effect of OA treatment, might be more useful (Pothacharoen et al., 2006; Pruksakorn et al., 2009).

Some studies tried to apply this method for the assessment of the subclinical changes and/or to diagnose the early stage of OA (Lohmander et al., 1999; Nganvongpanit et al., 2004; Nelson et al., 2006; Pothacharoen et al., 2006; Pruksakorn et al., 2009).

According to previous studies, biomarkers have played a major role in measuring the process of joint tissue destruction. This procedure is used to determine the joint cartilage components correctly, which is possible because the damaged cartilage releases biomolecules into joint fluid and serum (Nelson et al., 2006; Pothacharoen et al., 2006; Pruksakorn et al., 2009).

In animals (horses, dogs) and in humans, many biomarkers can be used to assess the state of joint disease. For example, chondrotin sulfate (3B3, WF6) (Caterson et al., 1990; Hazell et al., 1995; Belcher et al., 1997; Tungyuenyong et al., 2006; Nganvongpanit et al., 2008a), keratan sulfate (KS) (Leipold et al., 1989; Hazell et al., 1995; Belcher et al., 1997; Budsberg et al.,2006), hyaluronan (HA) (Leipold et al., 1989; Budsberg et al.,2006; Nganvongpanit et al., 2008a) or collagen type II (Hollander et al., 1994). As part of the change of the articular cartilage's metabolism, chondrocytes produce biomolecules to restore the imbalance during the early stage of the development of OA. If the catabolism was more than the anabolism, the HA and WF6 biomolecules increased in concentration (Pothacharoen et al., 2006). These biomolecules might be used to determine the state of cartilage metabolism.

The HA (using biotinylated HA-binding protein) relates to an important component of hyaline cartilage. A study on the level of HA, when this study revealed a comparison the biological marker (HA) between non HD and HD dogs, found that the level was significantly different between groups. This result might mean that there has

been catabolic progression in the HD dogs (Nganvongpanit et al., 2008a). The HA serum has shown increased levels, especially from rheumatoid arthritis (RA) that might be inferred by inflammation (Pothacharoen et al., 2006). Many data accepted that the balance of metabolism was mainly disturbed by inducing the articular degradation. In attempt to understand anabolic and catabolic biomarkers, Ong-chai et al. (2002) studied the change in serum CS (WF6) epitope by injection of hydrocortisone into rabbits' joints inducing osteoarthritis. It was interesting to find the result of increasing WF6 epitope, so WF6 epitope might provide information about the catabolism. Many researchers were strongly interested in the use of WF6 epitope as biological marker for the evaluation of joint disease (Tungyuenyong et al., 2006; Nganvongpanit et al., 2008a; Trakulsantirat et al., 2010). Nganvongpanit et al. (2008a) revealed that the average serum level of the WF6 epitope in HD dogs was significantly greater than that in the non-HD dogs: approximately 2,000 ng/ml. Another study in humans examined a comparison of the level of the serum WF6 epitope between 74 healthy people and 33 anterior cruciate ligament rupture patients. The levels of this biomarker in the anterior cruciate ligament rupture patients were significantly greater than those of healthy controls (Pruksakorn et al., 2009). The level of WF6 was found to be useful for the development of a treatment, as well as to monitor the progress of disease. Two different treatment methods (autologous chondrocyte transplantation AC; and subchondral drilling; SD) were studied by evaluation of the serum chondroitin sulfate (WF6) every six weeks (24 weeks). During the 18^{th} and 24^{th} week the CS WF6 epitope decreased slightly in the AC group, similar to the SD group but the levels of this biomarker in the first group were less than the another group during in the same week. Thus, AC treatment was likely to be a superior treatment (Nganvongpanit et al., 2009).

Pain release and OA prevention

According to pathogenesis and clinical outcomes of OA, the purposes of OA management are two, which compose of pain release and OA prevention. Surgical,

medical and neutraceuticals managements are provided to achieve the aims (Wang et al., 2004, Etinger and Feldman, 2010).

Surgery for OA dogs was provided in the case of secondary osteoarthritis, which was induced by conditions such as hip dysplasia, patellar luxation, cranial cruciate ligament rupture and osteochondritis dissecans (OCD).

The correction of these diseases was composed of: 1 pectineus tendon surgery, total hip replacement and pelvic osteotomy for hip dysplasia (Schulz and Dejardin, 2003 ; Liska, WD, 2000; Morgan et al, 2000); 2 femoral trochlear sulcoplasty, desmotomy, imbrications and tibial tuberosity transposition for patellar luxation (Arthurs, G.I. and Langley-Hobbs, S.J., 2006 ; Harasen, G, 2006; Roush, JK, 1993); 3 extracapsular and intracapsular techniques for cranial cruciate ligament rupture; 4 removal of OCD fragment for osteochomdritis dissecans (Fossum, 2007). Although most of surgical technique was applied for pain relief, to restore normal mechanic loading, to reduce the progression of OA and to allow good function of limb, the damaged articular cartilage cannot honestly return to normal cartilage (Fossum, 2007; Etinger and Feldman, 2010; Nganvongpanit, 2008d).

Medical and nutraceutical treatment

There is no the best treatment of choice because the cartilage changes that occur as part of OA cannot be reversed to truly normal cartilage, nevertheless medical management is a good choice to treat OA. The aims of the treatment in canine OA are to diminish the pain and to lessen the structural degeneration. In an attempt to achieve these aims, three groups of drugs are used: symptom-modifying OA drugs (SYMOAD) to reduce pain; structural-modifying OA drugs (STMOAD); and symptomatic slow-acting drugs for OA (SYSADOA) (Ettinger and Feldman, 2010).

In SYMOAD group, non steroidal anti-inflammatory drugs (NSAIDs) have an effect on the inflammatory pathway by inhibiting cyclooxygenase (COX) but use in the long term and/or in high doses induces gastric ulcers and platelet aggregation. This

applies especially to classical NSAIDs. Although specific COX-2 show lesser side effects, this class of drugs is not popular in Thailand (Nganvongpanit et al., 2008b).

Recently, nutritional supplementation (nutraceuticals) has played a major role in OA application because of lesser side effects. Oral glucosamine and/or chondroitin sulfate were studied and research revealed the improvement of clinical signs. Chondroitin sulfate may modulate the articular structure (Uebelhart et al., 1998; Soontornvipart et al., 2006; Uebelhart et al., 2008). In a contrasting study, a placebo-controlled double-blind study in dogs that had OA, owners and veterinarians failed to distinguish between chondroitin sulphate or placebo supplemented in dogs after 12 weeks (Dobenecker et al, 2002).

The current focus of research into the OA pathogenesis has concentrated on documenting the inflammatory process that leads to the cartilage changes.

Thus, the development of nutraceuticals has led to the hope that inhibition of the inflammatory pathway can cause a reduction of articular degradation. For this reason, the anti-inflammatory properties of omega-3 polyunsaturated fatty acids (omega-3 PUFAs) have attracted great interest as a possible OA treatment. There are many types of omega-3 PUFAs, of which eicosapentaenoic acid (EPA), docasahexaenoic acid (DHA) and eicosatetraenoic acid (ETA) are the three known types (Yuan et al., 2006; Treschow el al., 2007; Roush et al., 2010).

An *in vitro* study investigated the effects of omega-3 and omega-6 PUFAs on cartilage metabolism. The degradation and the inflammation in cartilage were decreased by the omega-3 supplementation (Curtis et al., 2002). EPA and DHA can be extracted from fish (a regular source). EPA and DHA are commonly in fish oil supplements and in prescription diets. ETA is found in a little number of food sources. ETA has a carbon chain length of 20 atoms and four double bonds. It is found in the New Zaeland green-lipped mussel (GLM-*Perna canaliculus*), in which it is one of the main PUFAs. The GLM contained not only ETA but also EPA and DHA (Treschow et al., 2007).

An investigator revealed that the average scores of pain were lesser in the EPA group than the ibuprofen group among 26 OA patients at the 24th week (Stammers et al., 1989). Another researcher compared the efficacy of the EPA in cod liver oil with NSAID treatment on joint pain and inflammation. There was no significant advantage to the cod liver oil group compared with the NSAID group in the management of OA (Stammer et al., 1992). Newly, Roush et al., (2010) evaluated the effects of fish oil on weight bearing in OA dogs, test-food group (82%) had significant improvements when compared to control-food group (38%) by using force-plate analysis; moreover, A researcher revealed higher EPA and DHA concentrations can improve the clinical signs (lameness and weight bearing) of OA dogs (Fritsch et al., 2010). On the other hand, there have been more studies into GLM in the treatment of joint disease. A study in The United Kingdom showed that the joint function of 76% of rheumatoid and 70% of OA patients improved significantly within three months (Gibson et al., 1998). The inflammatory process involved in OA has been studied extensively. The two important pathways are the cyclooxygenase (COX) and lipoxygenase (LOX) pathways of arrachidonic (AA) metabolism, the process can produce leukotriene B_4 (LTB_4) like chemoattractant inducing white blood cells (WBCs). Finally, increasing of cytokines, reactive oxygen species and histamine involved cartilage degeneration in OA (Bruce et al., 2006). Identification of GLM components found a predominant PUFA (ETA) which the structure was like AA. As a result of the reduction of the LOX product, the PUFAs in GLM might be a significant anti-inflammatory compound (Treschow et al., 2007). In addition, some studies examined the effect of PUFAs and a decrease of both leukotrienes and prostaglandins was found (Treschow et al., 2007; Bruce et al., 2006). Many researchers have believed that omega-3 PUFAs (especially ETA and EPA) may be able to affect the LOX and COX pathways. A study on the use of GLM in OA dogs found that the clinical signs improved significantly after six weeks (Bierer et al., 2002). Veterinarians and scientists should not confound a stabilized active lipid GLM oil extract with other GLM products, particularly powder extracts and various different union because there is a huge difference in the efficacy between other mussel extracts and powders and the

patent CO_2 extracted oil (Whitehouse et al., 1997). Recently, there has been growing interest in osteoarthritis biomarkers as primary outcome measures. Although considerable research has been devoted to clinical sign improvement rather less attention has been paid to OA biomarkers as a mean by which to monitor disease activity and predict disease progression. Thus, the aim of this study was to examine the effect of PCSO-524 (Antinol – a long–chain omega-3 PUFA compound from the New Zealand green-lipped mussel) and fish oil on the osteoarthritis biomarker level, clinical signs and radiographic findings in the treatment of canine shoulder and coxofemoral osteoarthritis.

Chapter III
Materials and Methods

Animals

Forty-six five-to-10-years-old 15-35 kilogram dogs were participated in this study. They were not restricted on the basis of sex or breed.

Inclusion criteria: all dogs showed signs of coxofemoral or shoulder osteoarthritis, which included limb lameness, joint pain, stiffness and decrease range of motion (ROM) (Millis et al., 2004). Moreover, the evidence of OA at coxofemoral or shoulder joint was indicated in radiographs (VD-extended coxofemoral projection and lateral projection of the shoulder) consisting of periarticular osteophyte formation (coxofemoral joint: femoral neck and cranial part of acetabulum, shoulder joint: caudal margin of humeral head and glenoid margins), subchondral bone sclerosis, narrowing of the joint space, joint capsule distension and/or periarticular soft-tissue swelling (Dennis et al., 2010; Sirois et al., 2010). The dogs were fed standard food (Royal Canin® mature large breed; Table 1) at least 5 days before physical examination and owners were instructed to feed nothing else, including snacks and treats, during the period of the study. The exclusion criteria of our patients during this study were: 1 severe liver, gastrointestinal, urogenital, or neurological problems and/or pregnant; 2 previous OA treatment with other drugs or dietary supplements; and 3 a pain-score evaluation of more than five points on the scale of Glasgow Composite Measure Pain Score-Short Form (Gaynor and Muir, 2009).

Informed owner consents were gained and the trial procedures were approved by the Faculty of Veterinary Sciences, Chulalongkorn University's Ethics Committee, Bangkok, Thailand. (No. 12310001)

Materials and Methods

1. Pretreatment evaluation

There were three steps of pre-treatment. Firstly, dogs were examined physically and orthopedically (including ROM) and the lameness scores were recorded by one veterinarian. Secondly, four milliliter blood samples (per a dog) were taken from each dog to establish baseline hematology, blood chemistry and OA biomarkers (one milliliter for CBC and blood chemistry, three milliliters for OA biomarkers). Finally, radiographs of coxofemoral and/or shoulder joints were interpreted by the same veterinary radiologist (Dennis et al., 2010; Sirois et al., 2010).

2. Treatment procedure

The OA dogs were divided into two treatment groups by random sampling. The first group (PCSO-524 group) were received the omega-3 fatty acids compound from New Zealand green lipped mussel (*Perna canaliculus*) (50mg/10kg body weight once daily) MacLab in Nelson, New Zealand (Sheila, 2000). The second group (fish-oil group) received fish oil (1,000 mg/dog once daily) Mega Lifescience company, Thailand (Table 2). Animals were reassessed at the second week, fourth week and the eighth week for clinical evaluation, blood collection and radiographs. The owner preferences were assessed monthly. Treatment was finished at the end of the second month. The mussel-oil lipid combination used in the trial should not be confused with general freeze-dried mussel powder compounds.

3. Assessment procedure

The severity of clinical lameness was evaluated and recorded before and after treatment, was well as at the second, fourth and eighth weeks of treatment by the use of the clinical scoring system (Table 3) and the measurement of range of motion (ROM) by the same veterinarian (Millis et al. 2004; McCarthy et al., 2007). The veterinarian was blinded to group classification when he scored. Radiographs were taken of the OA joint of the dogs (ventro-dorsal extended coxofemoral projection or lateral projection of

shoulder radiographs) four times (pretreatment, the second week, fourth week and eighth week). These radiographs were interpreted by the same veterinary radiologist who selected the Takahashi scoring system (Table 4) (Takahashi et al., 2004). Three milliliters of blood from the cephalic vein or the saphenous vein were collected to evaluate OA biomarker levels at the second, fourth and eighth week.

Table 1. Composition of the standard diet (Royal Canin® mature large breed)

Ingredient	g/kg diet
Protein	250.0
Fat	170.0
Carbohydrate	371.0
Dietary fibre	77.0
Crude fibre	20.0
EPA+DHA	4.0
Chondroitin+Glucosamine	1.0
Calcium	7.0
Phosphorus	6.0
Zinc	0.225
Polyphenols from grape and green tea	0.15
Vitamin E	0.6
Vitamin C	0.2
Taurine	2.0
Lutein	0.005

* Royal canin® mature large breed: volume according to energy requirement per day

Table 2. Composition of PCSO-524* and fish oil**

Group of treatment	Composition of a capsule	Dose
PCSO-524	*Perna Canaliculus* oil50 mg Vitamin E 0.225 mg Others; Olive oil, gelatin and glycerine	50 mg/capsule 1 capsule per 10 kg daily (8 weeks)
Fish oil	Fish oil 1,000 mg (EPA 180 mg, DHA 120 mg and vitamin E 1.4 mg)	1000 mg/capsule 1 capsule once daily (8 weeks)

* MacLab in Nelson, New Zealand ** Mega Lifescience company, Thailand

Table 3. Clinical scoring system (McCarthy, 2007)

Criteria: lameness

Grade	Clinical evaluation
1	Walk normally
2	Slightly lame when walking
3	Moderately lame when walking
4	Severely lame when walking
5	Reluctant to rise and will not walk more than five paces

Criteria: Weight bearing

Grade	Clinical evaluation
1	Equal on all limbs standing and walking
2	Normal standing; favors affected limb when walking
3	Partial weight-bearing standing and walking
4	Partial weight-bearing standing; non- weight-bearing walking
5	Non- weight-bearing standing and walking

Table 4. Radiographic scoring system (Takahashi et al., 2004)

Grade		Radiographic evaluation
0	Normal	Not affected
1	Mild	Doubtful narrowing of joint space and possible osteophytic lipping
2	Moderate	Definite osteophytes and possible narrowing of joint space
3	Severe	Moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour
4	Very severe	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour

4. **Clinical score**

It was necessary that dogs must be able to walk and trot about six meters three times for evaluation by veterinarian. The canine specific lameness and weight-bearing were assessed, recorded and calculated (McCarthy et al., 2007; Nganvongpanit et al., 2009). The mean of a clinical scoring system was used to determine the effect of each treatment. These evaluations were performed by the same veterinarian, about 15 minutes apart.

5. **Range of motion**

ROM was measured and recorded by a veterinarian who used a goniometer to measure the ROM of joints (Figure 1) at pre-treatment and the second, fourth and eighth week of treatment. A reading of the degree of motion was recorded on the goniometer placed over the fulcrum of the joint. There were two moveable arms to stay straight on landmarks. It was important that dogs did not move their body while moving the joint so that a more accurate angle could be measured. The measurement of flexion and extension of the OA joint were applied. Hip joint angles, the line connecting the lateral femoral epichondyle of femur and greater trochanter and a line connecting the tuber sacrale and ishiadicum were measured (Figure 2, Figure 3) (Millis et al., 2004). Shoulder joint angles, the line connecting the latral humeral epicondyle and the point of insertion of infraspinatus muscle and the spine of the scapular were measured (Figure 4, Figure 5) (Millis, et al, 2004). Double measurement was required in each position of assessment, the mean was calculated and recorded.

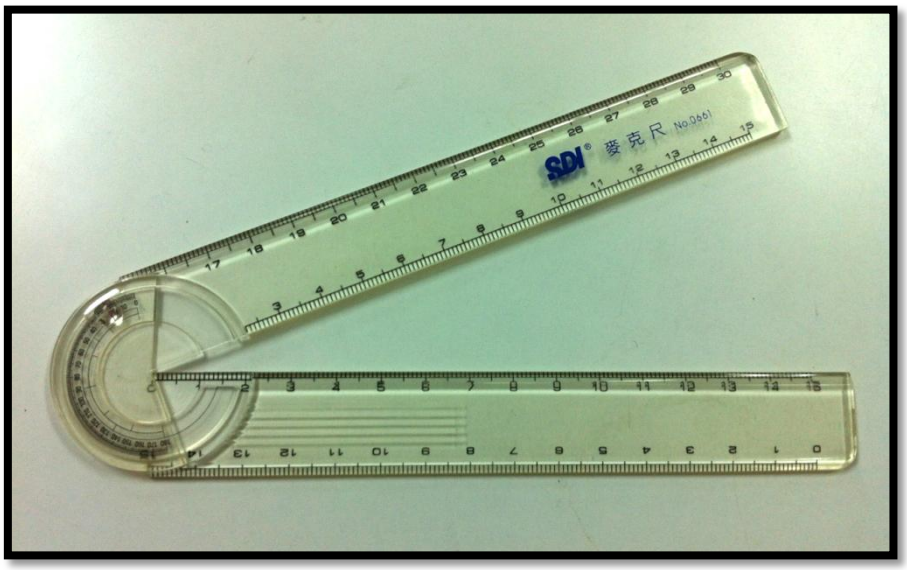
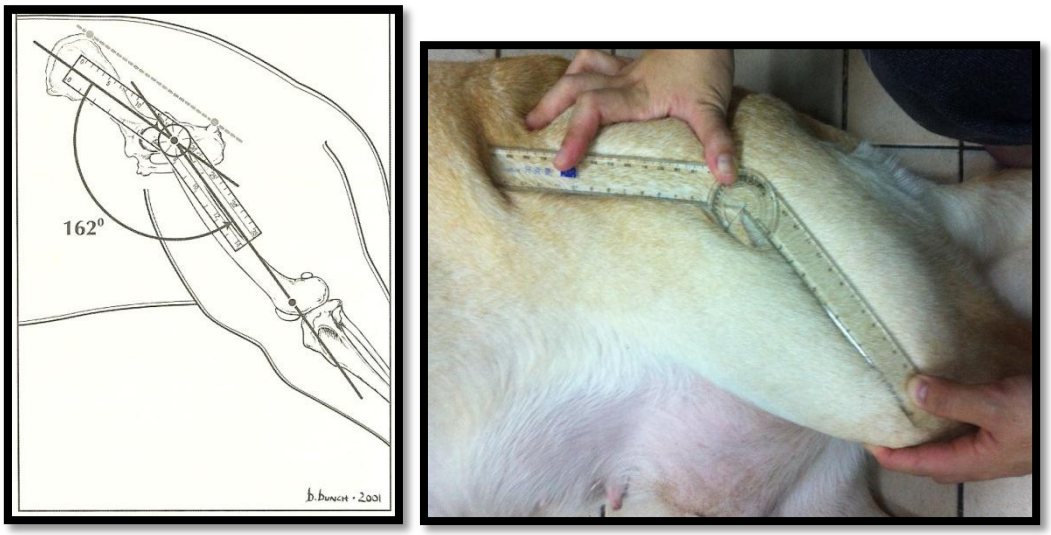
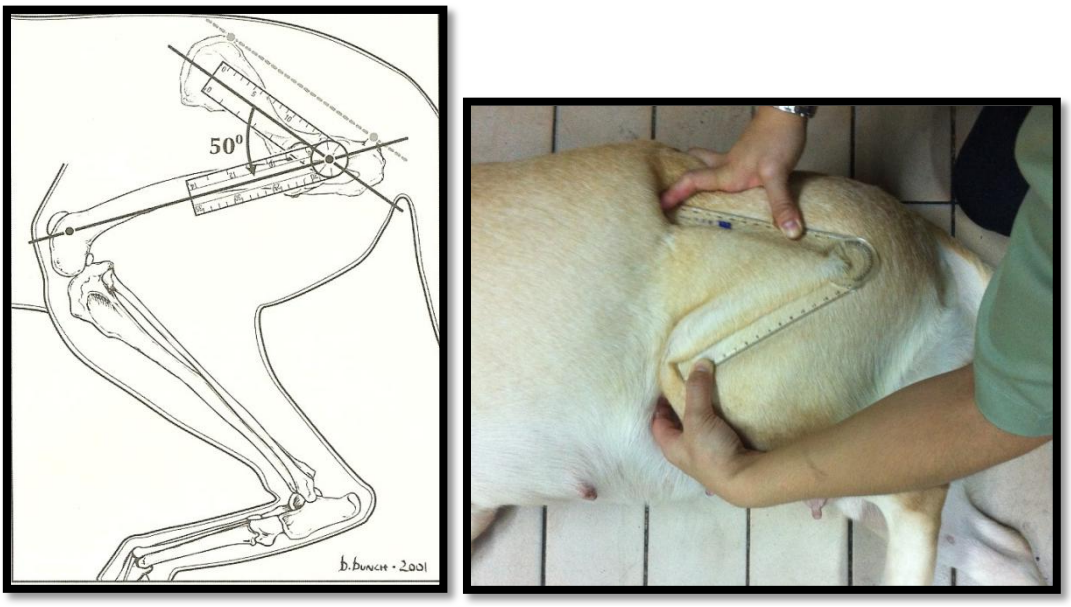


Figure1. Goniometer



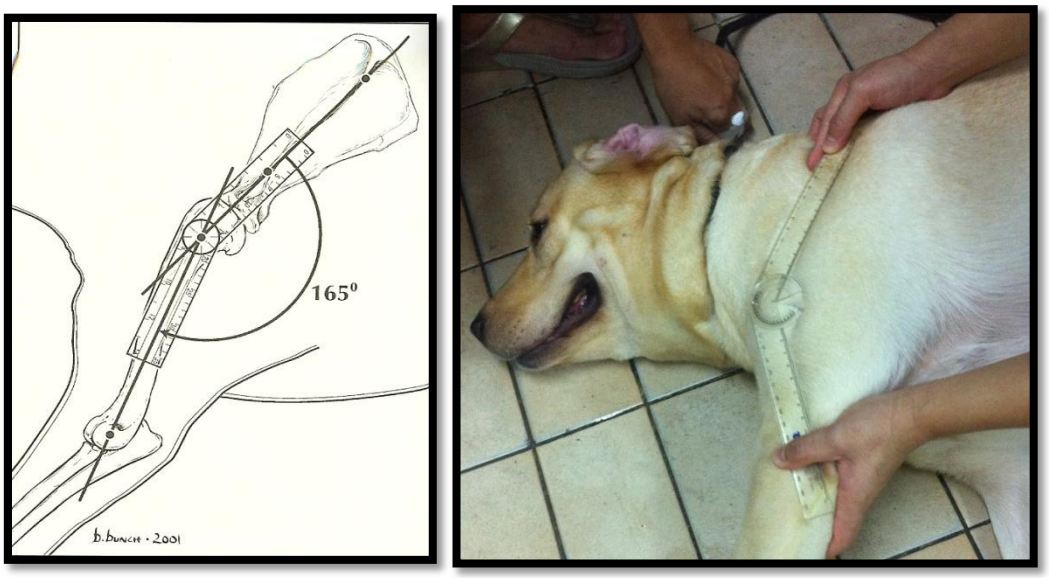
A B

Figure 2. A) Extension: Landmarks of the hip joint composed of lateral epichondyle of the femur, greater trochanter, the tuber sacrale and ischiadicum (Millis et al., 2004) B) Hip joint extension.



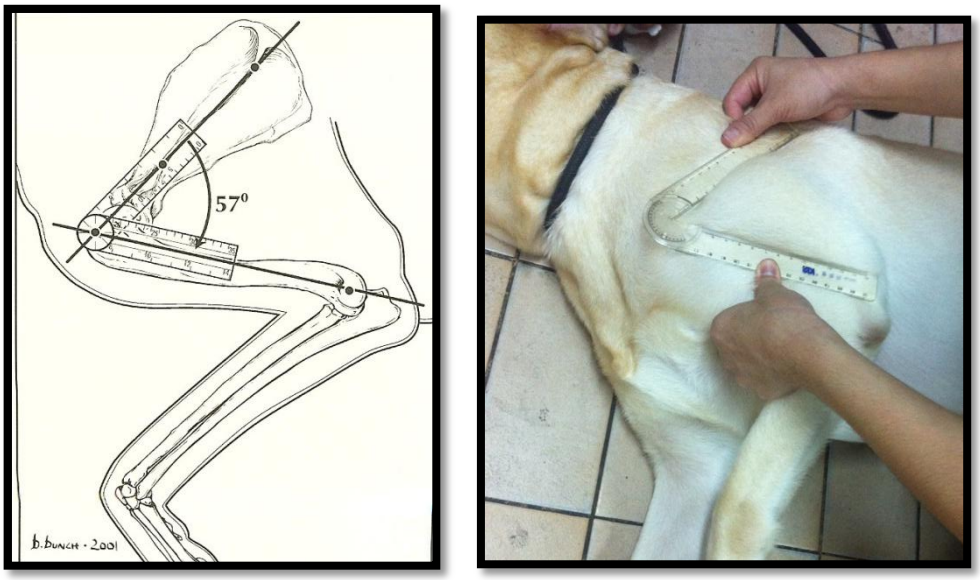
A B

Figure 3. A) Flexion: Landmarks of the hip joint composed of lateral epichondyle of the femur, greater trochanter, the tuber sacrale and ischiadicum (Millis et al., 2004) B) Hip joint flexion



A B

Figure 4. A) Extension: Landmarks of the shoulder joint composed of lateral epicondyle of humerus, the point of insertion of infraspinatus muscle and the scapula's spine (Millis et al., 2004) B) Shoulder joint extension



A B

Figure 5. A) Flexion: Landmarks of the shoulder joint composed of lateral epicondyle of humerus, the point of insertion of infraspinatus muscle and the scapula's spine (Millis et al., 2004) B) Shoulder joint flexion

5. **Radiographs**

The coxofemoral or shoulder joints of each dog were serially required taking ventro-dorsal extended coxofemoral projection (Figure 6) or lateral projection of shoulder (Figure 7) radiographs at pre-treatment and the second, fourth and eight week after treatment commenced. Repeatable radiographs were obtained of limbs in the same positions (Sirois et al., 2010), the same radiographic setting (i.e. kilovolts, milliamperes and milliseconds) and the same technician who used a digital X-ray machine. According to Takahashi's technique, the structural articular changes were assessed on consecutive radiographs (Takahashi et al., 2004). There were four radiographs per a dog. Also, all radiographs were interpreted by the same veterinary radiologist who used the standard in Table 2 (Takahashi et al., 2004; Nganvongpanit et al., 2009). These radiographs were recorded on compact disc (CD).

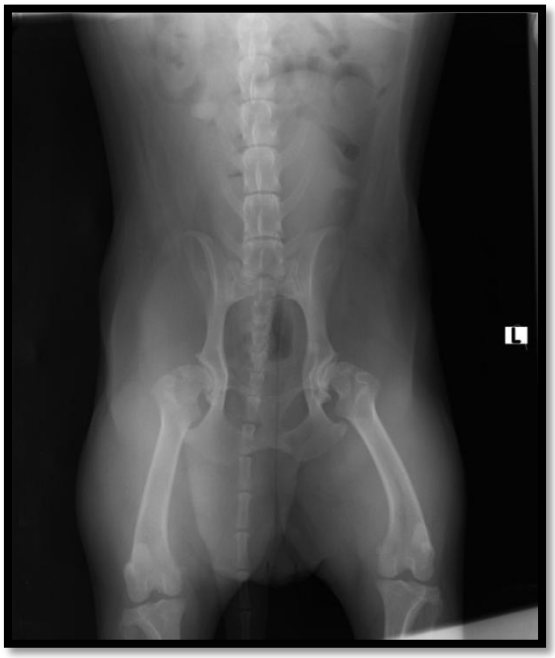


Figure 6. Hip joint: ventro-dorsal extended coxofemoral projection (VD)

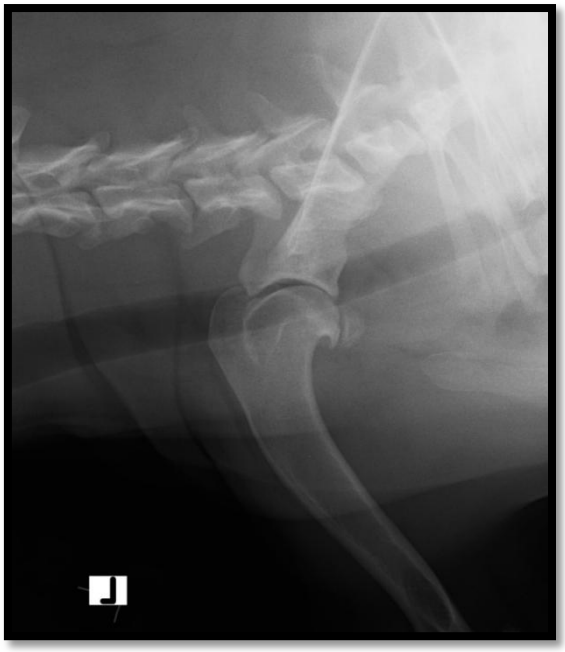


Figure 7. Shoulder joint: lateral projection of shoulder

6. **Blood collection**

Four milliliter blood samples were collected from the cephalic vein or saphenous vein of each dog. The blood collections of all dogs were taken in the morning before the dogs were fed. One milliliter was separated into two parts for complete blood counts

(CBCs) and blood chemistry tests. The CBCs samples were kept in anticoagulant (100IU/ml heparin; APS Finchem, Australia). The blood chemical samples were kept in plain tubes without anticoagulant. These samples were kept at 4°C and sent to the small animal hospital, Faculty of Veterinary Science, Chulalongkorn University within 24 hours. To prepare OA biomarker samples, three milliliters blood was firstly centrifuged at 7,000xg for 15 min to obtain about one milliliter of serum. This serum was frozen at -20 °C until a biomarker assay was performed (Figure 8).

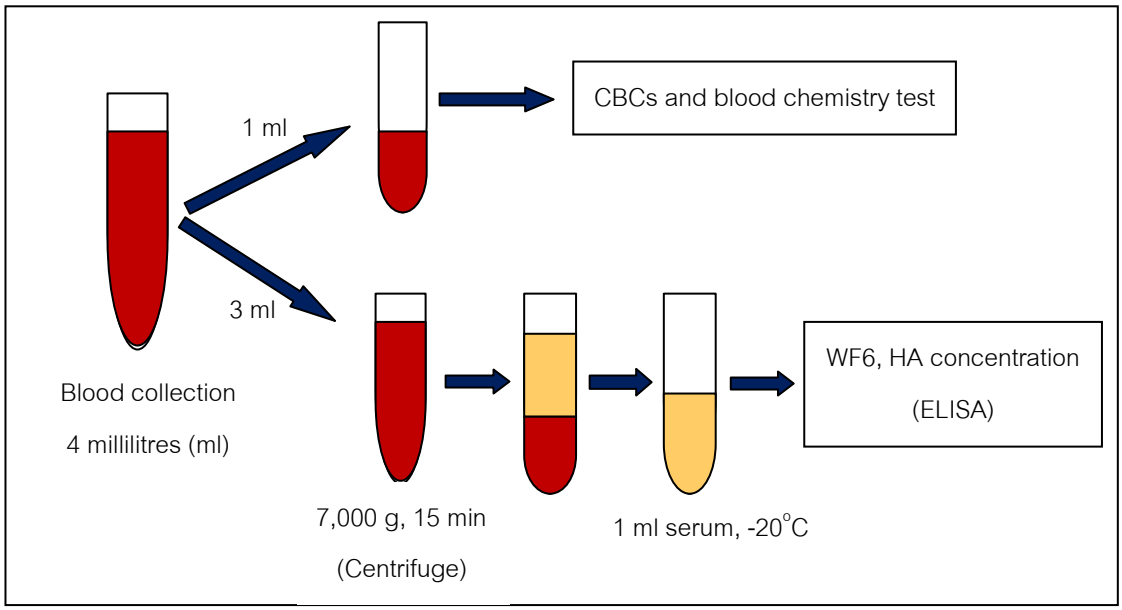


Figure 8. Blood collection for monitoring and OA biomarkers concentration

7. **Hematology and biochemistry**

The biochemical analyses, complete blood counts (CBCs) and blood chemistry tests were conducted at the Small Animal Hospital, Faculty of Veterinary Medicine, Chulalongkorn University, Bangkok Thailand.

One milliliter of the blood sample was divided into 2 parts. The first part was analyzed for the CBC, including the red blood cell count (RBC), white blood cell count (WBC), the hematocrit, the haemoglobin level and the platelet count. Another part was analyzed for blood chemistry profiles, including alanine aminotransferase, aspartate

aminotransferase, blood urea nitrogen and creatinine. These analyses were performed only twice, before and after treatment, to evaluate the health of animals.

Buccal mucosal bleeding time was done before and the forth, eighth week after PCSO-524 and fish oil administration for routine coagulation test as screening. If dogs had prolonged bleeding time (more than 2.6 minutes) (Jergans et al., 1987), the coagulogram (activated parital thromboplastin time, prothrombin time and thrombin time) was performed.

8. **Biomarker assay**

Laboratory of Thailand Excellence Center for Tissue Engineering, Department of Biochemistry, Faculty of Medicine, Chiang Mai Thailand

ELISA-based assay for the hyaluronan (HA)

The competitive inhibition ELISA method was applied. The quantitative ELISA for the HA was detected by biotinylated HA-binding proteins (HABPs) which was the primary antibody. Then the samples were added to microplate wells which were beforehand coated with umbilical cord HA to be a coating antigen, or to be a competitor. Next, peroxidase conjugated anti-biotin antibody was added as a secondary antibody and the incubation of the sample was conducted. To encourage the concentration of the HA, the substrate was added and the absorbance was determined. Finally, the concentration of the HA was calculated and recorded.

ELISA-based assay for the chondroitin sulfate WF6 epitope

The competitive inhibition ELISA method was applied. The quantitative ELISA for the WF6 epitope was detected by monoclonal antibody WF6 which was the primary antibody (Tangkijvanich et al., 2003). Then, the samples were added to microplate wells that had been beforehand with embryonic shark skeletal cartilage aggrecan to be a coating antigen, or to be a competitor. Next, peroxidase conjugated anti-mouse IgM antibody was added as secondary antibody and the incubation of the sample was conducted. To encourage the concentration of the epitope WF6, the substrate was

added and the absorbance was determined. Finally, the concentration of the epitope WF6 was calculated and recorded.

Statistical analysis

The OA biomarkers concentration (HA, WF6) from the serum and ROM were be reported as means ± SD in each week in the same treatment. The paired t-test procedure was used to test for differences between before and after treatment (at the second, fourth and eighth weeks) in the same group. Comparison between groups was analyzed using unpaired t-test. The radiograph and clinical sign scores were calculated as means ± SD that used the non-parametric two samples Mann Whitney procedure. The relative data was analyzed using the SPSS program (SPSS). *P*- values less than 0.05 were considered to be significant.

Chapter IV
Results

Animals

Of the 46 OA dogs 22 were Labrador retrievers, ten were mixed-breed dogs, eight were Golden retrievers, two were German shepherds, two were Cockers spaniels and there was one Alaskan malamute and one Rottweiler. The dogs were divided into two groups (Table 5): PCSO-524 group, means (SD) of animal age and body weight were 7.65 (1.84) years (rage, 5-10 years) and 27.61 (4.57) kg (range, 17-31.4 kg); fish-oil group, means (SD) of animal age and body weight were 6.65 (1.79) year (range, 5-10 years) and 24.37 (5.57) kg (range, 17- 32 kg). There were 82 hip joints and 11 shoulder joints in this study. Twenty-five female dogs and 21 male dogs were participated.

Table5. Signalment of animals

Group	Number (N)	Joint (n)	Age (Year) (Mean(SD))	Body weight (kg) (Mean(SD))	Sex (N)
PCSO-524	23	Hip (42)	7.65 (1.84)	27.48 (4.98)	Female (14)
		Shoulder (7)			Male (9)
Fish oil	23	Hip (40)	6.65 (1.79)	24.37 (5.57)	Female (11)
		Shoulder (4)			Male (12)

Hematology and biochemistry

Complete blood count and serum chemistry were evaluated in all dogs at the pre-treatment (D0) examination and at the end of the treatment (W8). The parameters included red blood cell, hemoglobin, hematocrit, platelet, white blood cell, neutrophils, eosinophils, basophils, lymphocytes, monocytes, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen and creatinine. The mean values for each group, most of the values were not significantly different between the pre-treatment (D0) and at the end of the treatment (W8) ($p > 0.05$) (Table 6). All mean of parameters were in the

normal range at both the pre-treatment (D0) and the end of the treatment (W8) in two groups (Table 6). There was no significantly different in CBC and blood chemistry between PCSO-524 group and fish oil group in both DO and W8 (table 6).

Table 6. (A) Comparisons of complete blood counts and blood chemistry profiles between the pre-treatment (D0) and the post treatment (W8) within group. (B) Comparisons of complete blood counts and blood chemistry profiles between groups.

PCSO-524 group (A)

Parameters	Normal range	D0	W8	*p-value*[†]
R.B.C.x10^3 (cell/µl)	5.2-8.06	6.15±1.32	6.14±1.19	0.4935
Hemoglobin (g/dl)	12.4-19.1	14.41±2.47	14.42±2.24	0.4925
Hematocrit (%)	29.8-57.5	41.66±6.21	41.60±6.19	0.4868
Platelet x10^3 (cell/µl)	160-525	289.47±81.29	293.52±67.65	0.4276
W.B.C. x10^3 (cell/µl)	5.4-15.3	10.00±3.76	10.02±2.55	0.4903
Neutrophils (%)	51-84	71.60±9.92	71.21±8.10	0.4421
Eosinophils (%)	0-9	2.26±2.92	2.52±2.84	0.3802
Basophils (%)	0-1	0.21±0.51	0.17±0.65	0.4016
Lymphocytes (%)	8-38	17.34±7.46	16.61±5.18	0.2427
Monocytes(%)	1-9	6±2.29	5.90±2.87	0.4518
ALT (U/l)	4-91	34.43±10.16	38.95±16.15	0.1312
ALP	3-60	57.21±31.44	65.43±33.09	0.1128
BUN (mg%)	7-26	16.17±7.85	15.29±5.65	0.3319
Creatinine (mg%)	0.6-1.4	1.1±0.38	1.09±0.36	0.4533

[†]The p- values between the pre-treatment (D0) and the end of treatment (W8) in PCSO-524 group. R.B.C: red blood cell, W.B.C: white blood cell, ALT: alanine aminotransferase, ALP: alkaline phosphatase, BUN: blood urea nitrogen. Data were expressed as mean±SD

Fish oil group (A)

Parameters	Normal range	D0	W8	*p-value* †
R.B.C.x10^3 (cell/µl)	5.2-8.06	6.62±0.78	6.88±0.80	0.1442
Hemoglobin (g/dl)	12.4-19.1	15.35±1.68	15.72±1.58	0.2233
Hematocrit (%)	29.8-57.5	44.22±4.80	45.31±5.26	0.2333
Platelet x10^3 (cell/µl)	160-525	297.47±61.64	298±48.28	0.4873
W.B.C. (cell/µl)	5.4-15.3	11.10±2.86	10.23±1.80	0.1127
Neutrophils (%)	51-84	72.95±6.88	71.08±8.17	0.2030
Eosinophils (%)	0-9	2.91±2.69	3.17±2.97	0.3784
Basophils (%)	0-1	0.04±0.20	0.13±0.34	0.1529
Lymphocytes (%)	8-38	16.13±5.96	15.91±7.44	0.4567
Monocytes(%)	1-9	6.00±1.88	6.23±1.93	0.5000
ALT (U/l)	4-91	58.89±27.79	38.30±14.99	0.3134
ALP (IU/L)	3-60	62.86±31.39	60.86±32.03	0.4158
BUN (mg%)	7-26	14.65±3.92	16.95±5.05	0.3390
Creatinine (mg%)	0.6-1.4	0.94±0.22	0.91±0.22	0.3241

† The p- values between the pre-treatment (D0) and the end of treatment (W8) in fish-oil group. R.B.C: red blood cell, W.B.C: white blood cell, ALT: alanine aminotransferase, ALP: alkaline phosphatase, BUN: blood urea nitrogen. Data were expressed as mean±SD

(B) Comparision of complete blood counts and blood chemistry between groups.

Parameters	Normal range	PCSO (Do)	Fish oil (Do)	p-value$^{\tau}$ (D0)	PCSO (W8)	Fish oil (W8)	p-value* (W8)
R.B.C.x10^3 (cell/μl)	5.2-8.06	6.15±1.32	6.62±0.78	0.074	6.14±1.19	6.88±0.80	0.072
Hemoglobin (g/dl)	12.4-19.1	14.41±2.47	15.35±1.68	0.070	14.42±2.24	15.72±1.58	0.092
Hematocrit (%)	29.8-57.5	41.66±6.21	44.22±4.80	0.063	41.60±6.19	45.31±5.26	0.082
Platelet x10^3 (cell/μl)	160-525	289.47±81.29	297.47±61.64	0.354	293.52±67.65	298±48.28	0.398
W.B.C. x10^3 (cell/μl)	5.4-15.3	10.00±3.76	11.10±2.86	0.135	10.02±2.55	10.23±1.80	0.376
Neutrophils (%)	51-84	71.60±9.92	72.95±6.88	0.297	71.21±8.10	71.08±8.17	0.478
Eosinophils (%)	0-9	2.26±2.92	2.91±2.69	0.218	2.52±2.84	3.17±2.97	0.225
Basophils (%)	0-1	0.21±0.51	0.04±0.20	0.073	0.17±0.65	0.13±0.34	0.389
Lymphocytes (%)	8-38	17.34±7.46	16.13±5.96	0.272	16.61±5.18	15.91±7.44	0.481
Monocytes(%)	1-9	6±2.29	6.00±1.88	0.500	5.90±2.87	6.23±1.93	0.357
ALT (U/l)	4-91	34.43±10.16	58.89±27.79	0.129	38.95±16.15	38.30±14.99	0.443
ALP	3-60	57.21±31.44	62.86±31.39	0.272	65.43±33.09	60.86±32.03	0.318
BUN (mg%)	7-26	16.17±7.85	14.65±3.92	0.463	15.29±5.65	16.95±5.05	0.149
Creatinine (mg%)	0.6-1.4	1.1±0.38	0.94±0.22	0.093	1.09±0.36	0.91±0.22	0.052

$^{\tau}$The p- values between groups in the pretreatment (DO). * The p- values between groups in the end of treatment (W8). R.B.C: red blood cell, W.B.C: white blood cell, ALT: alanine aminotransferase, ALP: alkaline phosphatase, BUN: blood urea nitrogen. Data were expressed as mean±SD

Clinical outcomes

Lameness scores

Hip

Eighty-two hip joints were evaluated for lameness scores at pre-treatment (D0) and at the second (W2), the fourth (W4) and the eighth (W8) week post treatment (Figure 9, Table7).

The results revealed that the mean scores for lameness in the PCSO-524 group showed significant improvement ($p<0.05$) at the second week, the fourth week and the eighth week respectively, while in the fish-oil group the lameness scores were not significantly different within treatment period. The means of lameness scores were significantly different between groups during the fourth week and the eighth week ($p<0.05$).

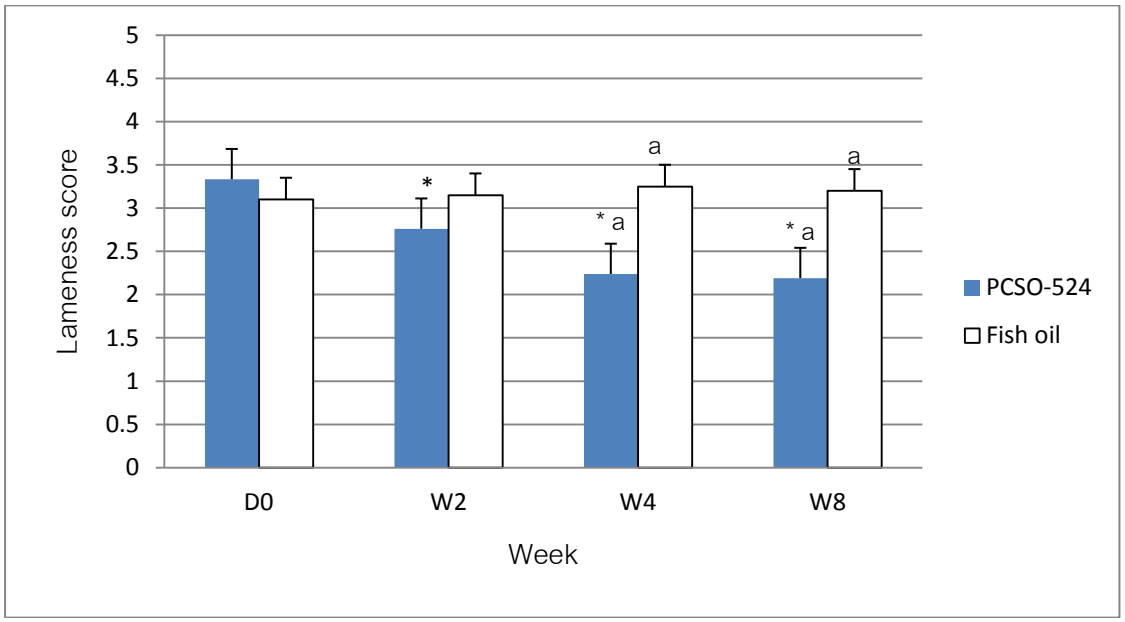


Figure 9. Mean of lameness scores (hip joints). * Values were significantly different compared with the pre-treatment (D0) within the groups ($p<0.05$). [a] Values were significantly different between groups within the week ($p<0.05$).

Table 7. Mean of lameness scores of hip joints

Group (n)	Mean (SD) of lameness scores of hip joints (N=82)			
	D0	W2	W4	W8
PCSO-524 (42)	3.33 (0.73)	2.76* (0.94)	2.23*[a] (0.94)	2.19*[a] (0.81)
Fish oil (40)	3.10 (0.55)	3.15 (0.58)	3.25[a] (0.71)	3.20[a] (0.83)

* Values were significantly different compared with the pre-treatment (D0) within the groups ($p<0.05$). [a] Values were significantly different between groups within the same week ($p<0.05$).

Shoulder

Eleven shoulder joints were evaluated lameness score at the pre-treatment (D0) and the second (W2), the fourth (W4) and the eighth (W8) week after treatment commenced (Figure 10, Table 8). The results revealed that the mean scores for lameness in the PCSO-524 group showed significant improvement ($p<0.05$) at the fourth week and the eighth week compared with the pre-treatment; while in the fish oil group it was not significantly different within treatment period. The means of lameness scores were significantly different between group within the fourth week and the eighth week ($p<0.05$).

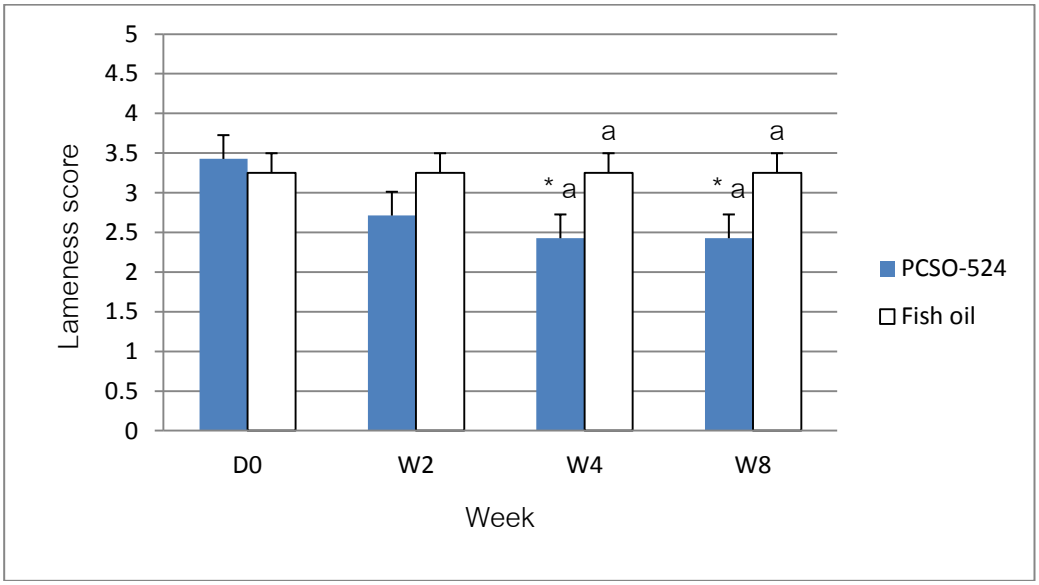


Figure 10. Mean of lameness scores (shoulder joints). * Values were significantly different compared with the pre-treatment (D0) within the groups ($p<0.05$). [a] Values were significantly different between groups within the week ($p<0.05$).

Table 8. Mean of lameness scores of shoulder joints

Group (n)	Mean (SD) of lameness scores of shoulder joints (N=11)			
	D0	W2	W4	W8
PCSO-524 (7)	3.42 (0.53)	2.71 (0.95)	2.42*[a] (0.53)	2.42*[a] (0.53)
Fish oil (4)	3.25 (0.50)	3.25 (0.50)	3.25[a] (0.50)	3.25[a] (0.50)

* Values were significantly different compared with the pre-treatment (D0) within the groups ($p<0.05$). [a] Values were significantly different between groups within the same week ($p<0.05$).

Weight bearing score

Hip

The evaluations of the weight bearing score in 82 hip joints were performed at the pre-treatment (D0) and the second (W2), the fourth (W4) and the eighth (W8) week post treatment (Figure 11, Table 9). The results revealed that the mean scores for weight bearing in the PCSO-524 group showed significant improvement ($p<0.05$) at the second week, the fourth week and the eighth week respectively, while in the fish-oil group it was not significantly different within treatment period. The means of weight-bearing scores were significantly different between groups within the fourth week and the eighth week ($p<0.05$).

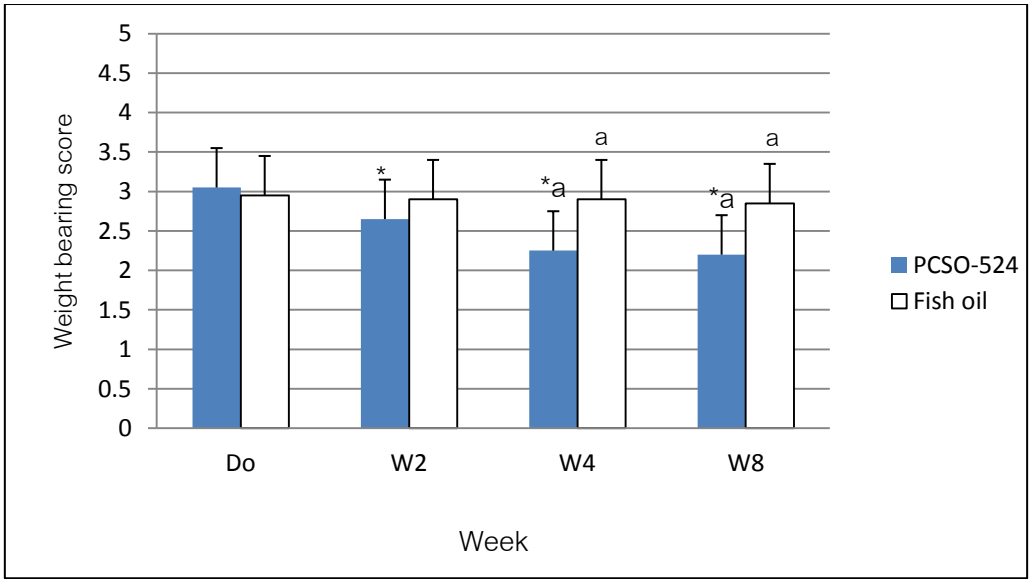


Figure 11. Mean of weight bearing scores (hip joints). * Values were significantly different compared with the pre-treatment (D0) within the groups ($p<0.05$). [a] Values were significantly different between groups within the week ($p<0.05$).

Table 9. Mean of weight bearing scores of hip joints

Group (n)	Mean (SD) of weight bearing scores of hip joints (N=82)			
	D0	W2	W4	W8
PCSO-524 (42)	3.05 (0.60)	2.65* (0.58)	2.25*[a] (0.71)	2.20*[a] (0.69)
Fish oil (40)	2.95 (0.51)	2.90 (0.44)	2.90[a] (0.55)	2.85[a] (0.49)

* Values were significantly different compared with the pre-treatment (D0) within the groups ($p<0.05$). [a] Values were significantly different between groups within the same week ($p<0.05$).

Shoulder

Four shoulder joints were evaluated weight bearing score at the pre-treatment (D0) and the second (W2), the fourth (W4) and the eighth (W8) week post treatment (Figure12, Table 10). The results revealed that the mean scores for weight bearing in the PCSO-524 group showed significant improvement ($p<0.05$) at the second week, the fourth week and the eighth week compared with the pre-treatment; while in the fish-oil group it was not significantly different within treatment period. The means of weight-bearing scores were significantly different between groups in the fourth week and the eighth week ($p<0.05$).

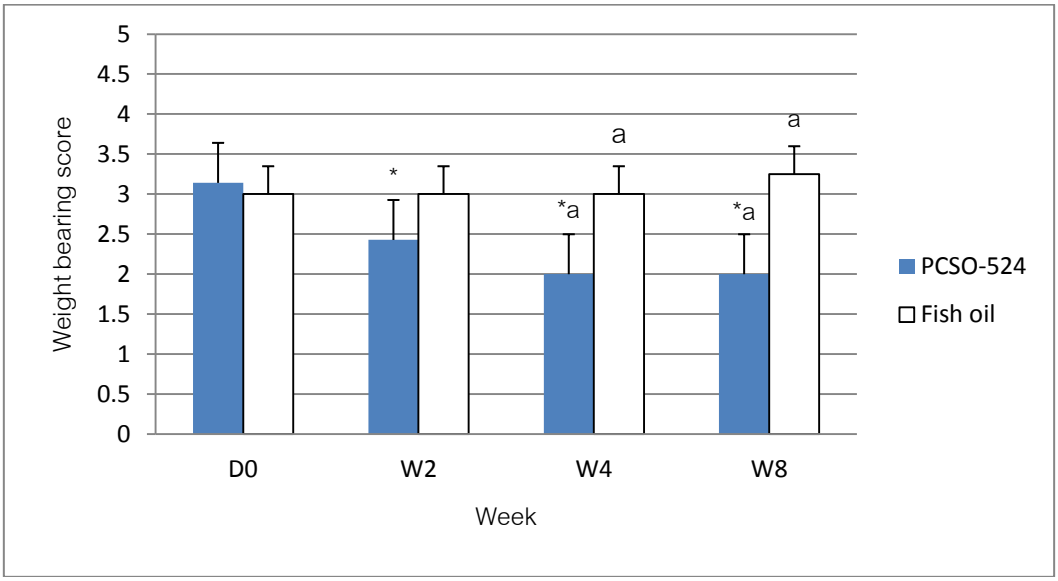


Figure 12. Mean of weight-bearing scores (shoulder joints). * Values were significantly different compared with the pre-treatment (D0) within the groups (p<0.05). [a] Values were significantly different between groups within the week (p<0.05).

Table 10. Mean of weight bearing scores of shoulder joints

Group (n)	Mean (SD) of weight bearing scores of shoulder joints (N=11)			
	D0	W2	W4	W8
PCSO-524 (7)	3.14 (0.37)	2.42* (0.78)	2.00*[a] (0.57)	2.00*[a] (0.57)
Fish oil (4)	3.00 (0.81)	3.00 (0.81)	3.00[a] (0.81)	3.25[a] (0.50)

* Values were significantly different compared with the pre-treatment (D0) within the groups (p<0.05). [a] Values were significantly different between groups within the same week (p<0.05).

Radiographic findings

Hip

All hip joints were taken radiographs 4 times (D0, W2, W4, W8) and the evaluation of the mean of the radiographic score was calculated (Figure13, Table 11).

There was no significant difference in the radiographic score improvement in each group; whereas, there was significant difference between two groups in the 8th week. The trend of radiographic findings was improved in some case of the dogs that had PCSO-524 administration (Figure 15-16).

Shoulder

In addition, there was not significantly different in the mean of radiographic score within group and between groups in eleven shoulder joints (Figure 14, Table 12).

In shoulder joints, although there was not significantly different in radiographic score, the trend of radiographic finding was improved in some of PCSO-524 administration for eight weeks (Figure 17-18).

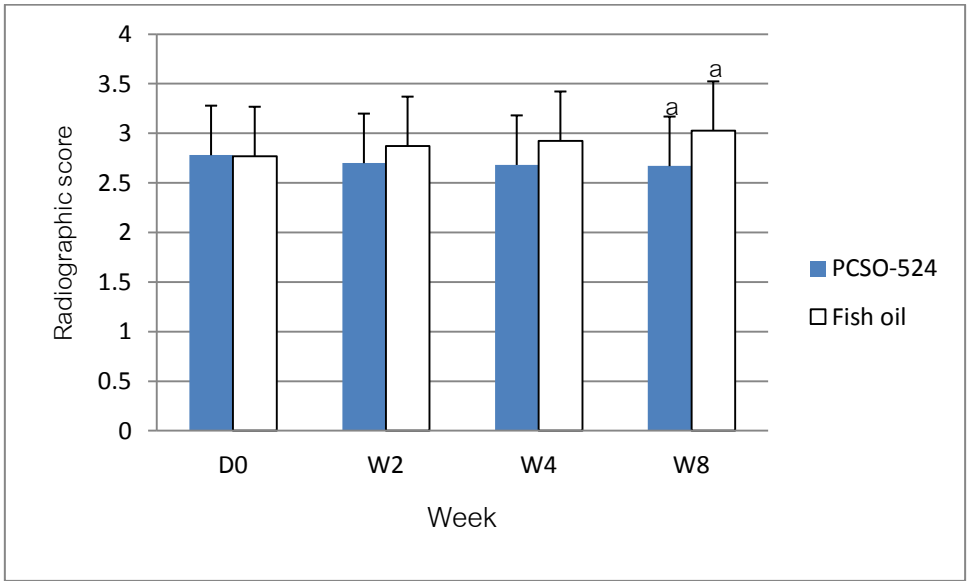


Figure 13. Mean of radiographic score (hip joints). [a] Values were significantly different between groups within the week ($p<0.05$).

Table 11. Mean of radiographic score of hip joints

Group (n)	Mean (SD) of radiographic score of hip joints (N=82)			
	D0	W2	W4	W8
PCSO-524 (42)	2.78 (0.72)	2.70 (0.74)	2.68 (0.78)	2.67 [a] (0.78)
Fish oil (40)	2.76 (0.98)	2.87 (0.83)	2.92 (0.87)	3.02 [a] (0.93)

[a] Values were significantly different between groups within the same week ($p<0.05$).

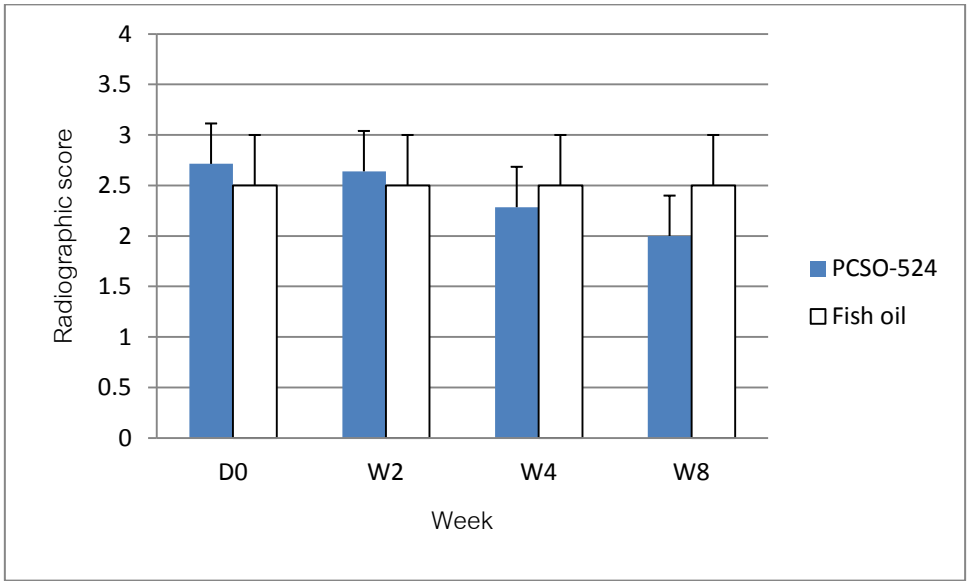
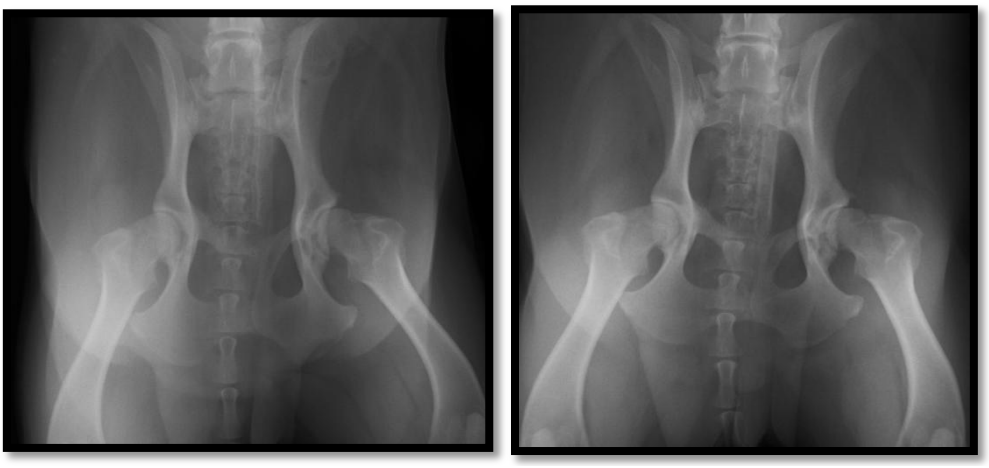


Figure 14. Mean of radiographic score (shoulder joints).

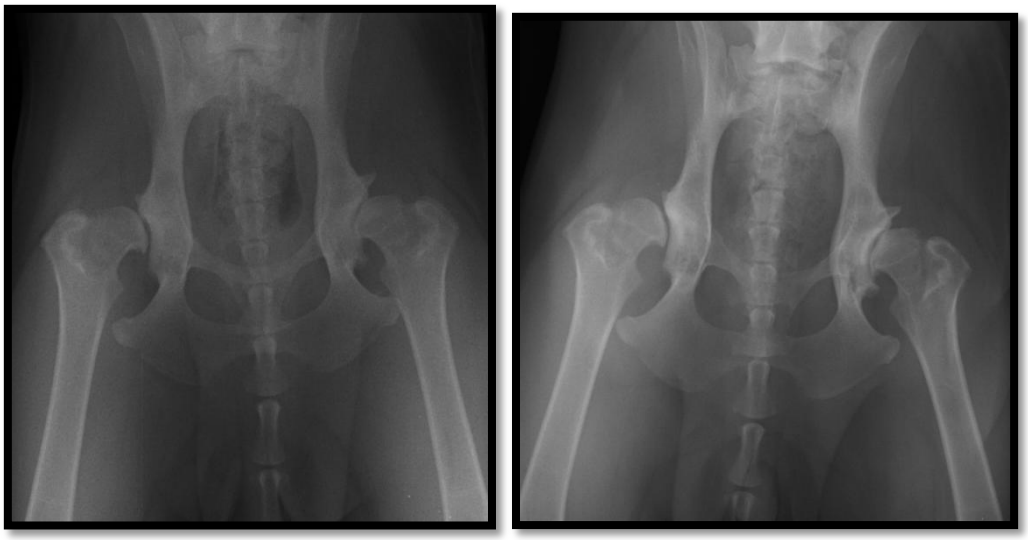
Table 12. Mean of radiographic score of shoulder joints

Group (n)	Mean (SD) of radiographic score of shoulder joints (N=11)			
	D0	W2	W4	W8
PCSO-524 (7)	2.71 (0.75)	2.64 (0.85)	2.28 (1.11)	2.00 (0.81)
Fish oil (4)	2.50 (0.57)	2.50 (0.57)	2.50 (0.57)	2.50 (0.57)



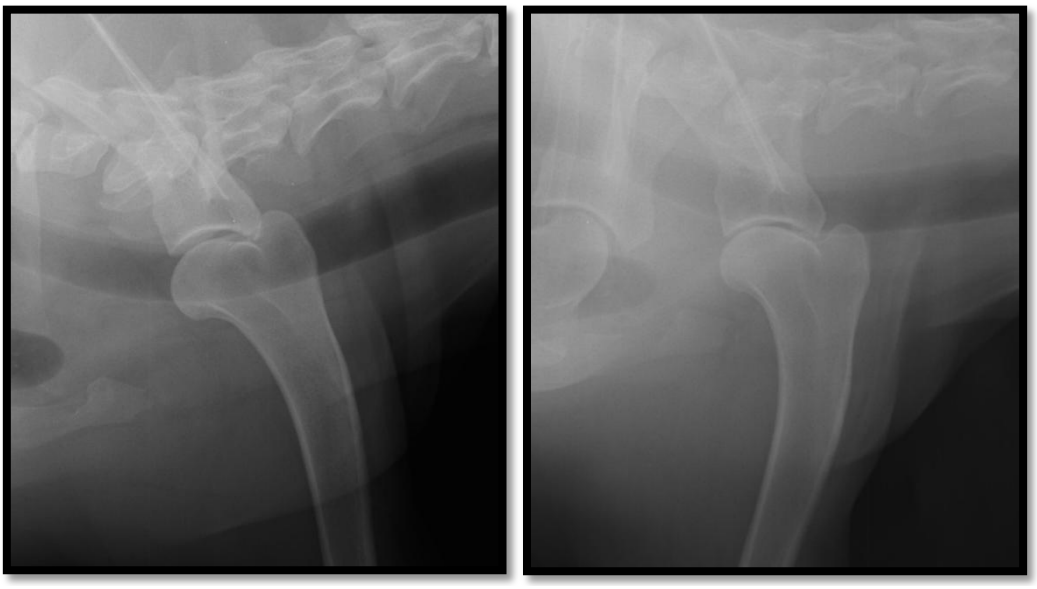
A B

Figure15. Radiographic findings (A) showed severe degree OA before the PCSO-524 administration. The radiographic OA findings seemed to be a bit better at the smooth surface at the hip joint after eight weeks of PCSO-524 use.



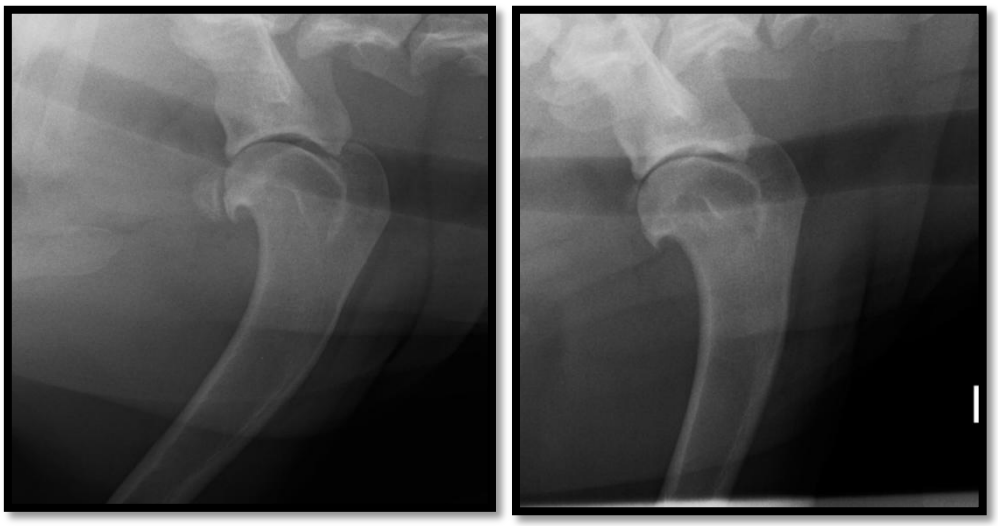
A B

Figure16. Radiographic findings before the administration of PCSO-524 (A) showed a severe degree OA (grade 3) that had marked narrowing of the joint space and some sclerosis. After the administration of PCSO-524 for eight weeks, the radiographic OA findings (B) seemed to have improved slightly at the smooth surface and there appeared to be a mild joint space improvement at the hip joint (grade 3).



A B

Figure 17. Radiographic findings (A) showed a moderate degree of OA (grade 2) before the administration of PCSO-524. After eight weeks of PCSO-524 use, the radiographic OA findings (B) seemed to be a bit better at the smooth surface of the left shoulder joint (grade 1).



A B

Figure 18. Radiographic calcification (large osteophytes; grade 4) at the caudal part of the left shoulder joint (A) became at the smooth surface after eight weeks of PCSO-524 use (B) (definite osteophytes; grade 2).

Evaluation the percentage of improvement in clinical outcomes

(hip and shoulder joints; N=93)

The OA dogs were evaluated for clinical outcomes (lameness and weight bearing and radiographic findings) by separately affected joints. Eighty-two hip joints and 11 shoulder joints that were assessed by clinical scoring and radiographic finding were categorized into three groups, which were: improvement, not improved and progression (worse) after eight weeks of PCSO-524 (Table 13) and fish oil administration (Table 14). The results revealed that a large percentage of the PCSO-524 group experienced improvements in clinical lameness (83.67%) and weight bearing (75.51%), although only a few had better radiographic findings (20.41%). In contrast, a large percentage of the fish-oil group did not improve in clinical lameness (54.44%), or weight bearing (79.55%). Some of the fish-oil group (22.73%) were shown by radiographic findings to have deteriorated and none of the group (0%) showed an improvement.

Table 13. The clinical outcome after eight weeks of PCSO-524 administration
(hip and shoulder joints; N=49)

Clinical outcomes N (%)	Improvement	Not improvement	Progression
Lameness score	41 (83.67%)	6 (12.25%)	2 (4.08%)
Weight bearing score	37 (75.51%)	10 (20.41%)	2 (4.08%)
Radiographic finding	10 (20.41%)	38 (77.55%)	1 (2.04%)

Table 14. The clinical outcome after eight weeks fish oil administration
(hip and shoulder joints; N=44)

Clinical outcomes N (%)	Improvement	Not improvement	Progression
Lameness score	8 (18.18%)	24 (54.55%)	12 (27.27%)
Weight bearing score	4 (9.09%)	35 (79.55%)	5 (11.36%)
Radiographic finding	0 (0%)	34 (77.27%)	10 (22.73%)

Range of motion (ROM)

ROMs were acquired from 46 dogs, which were divided into two groups. In the PCSO-524 group there were 42 hip joints and seven shoulders. In the fish-oil group there were 40 hip joints and four shoulder joints.

PCSO-524 group

Hip joints

In the PCSO-524 group, ROMs of hip joints were calculated. The means (SD) degree of flexions were 40.11°(7.50) (range, 25° - 54°), 34.57° (6.87) (range, 26° - 49°), 33.75° (5.33) (range, 27°- 46°) and 33.32 ° (4.78) (range, 27° - 45°) before treatment and at the second, the fourth, the eighth week after treatment, respectively. ROMs of hip joints revealed that the means (SD) (range) of extensions' degree in PCSO-524 group were 122.60 ° (10.74) (range, 105 ° - 142 °), 130.60 ° (11.74) (range, 113 ° - 146 °), 133.48 ° (10.76) (range, 117 ° - 151 °) and 135.91 ° (10.02) (range, 120 ° -150 °) before treatment and at the second, the fourth and eighth week after treatment, respectively (Table 15). There were significant improvements for hip's flexion and extension in dogs that were fed PCSO-524 at the second (W2), fourth (W4) and eighth (W8) compared with those before treatment (D0).

Shoulder joints

ROMs of shoulder joints revealed that the means (SD) of flexion's degree were 51.93 ° (9.32) (range, 41 ° - 64 °), 41.93 ° (8.99) (range, 29 ° - 49 °), 40.56 ° (7.88) (range, 29 ° - 46 °) and 38.43 ° (6.98) (range, 28 ° - 44 °) before treatment and at the second, fourth and eighth week after treatment, respectively. The means (SD) (range) of extensions' degree of shoulder joints were 121.75 (4.66) (range, 120 -124), 127.87(5.8) (range, 122 -135), 129.81(9.27) (range, 120 -140) and 132.31(10.74) (range, 119 -142) before treatment and at the second, fourth and eighth week after PCSO-524 administration, respectively. The results revealed that comparisons of the means (SD) of the ROMs were significantly different between pre-treatment and at the second (W2), fourth (W4) and eighth (W8) week after treatment commenced (Table 15).

Table15. Mean (SD) of the ROMs in PCSO-524 group

ROM	Hip joint (degree)		Shoulder joint (degree)	
	Flexion	Extension	Flexion	Extension
D0	40.11(7.50)	122.60(10.74)	51.93(9.32)	121.75(4.66)
Range	(25-54)	(105-142)	(41-64)	(120-124)
W2	34.57(6.87)*	130.60(11.74)*	41.93(8.99)*	127.87(5.8)*
Range	(26-49)	(113-146)	(29-49)	(122-135)
W4	33.75(5.33)*	133.48(10.76)*	40.56(7.88)*	129.81(9.27)*
Range	(27-46)	(117-151)	(29-46)	(120-140)
W8	33.32(4.78)*	135.91(10.02)*	38.43(6.98)*	132.31(10.74)*
Range	(27-45)	(120-150)	(28-44)	(119-142)

*Means (SD) of the ROMs were significantly different compared with day 0 within the PCSO-524 group ($p < 0.05$).

Fish oil group

Hip joints

In fish-oil group, ROMs of hip joints were calculated. The means (SD) (range) of the degree of flexions were 39.25° (6.63) (range, 25° - 49°), 38.51° (7.01) (range, 21° - 49°), 39.21° (6.59) (range, 22° - 50°) and 39.55° (5.69) (range, 26° -50°) before treatment and at the second, fourth and eighth week after treatment commenced. ROMs of hip joints revealed that the means (SD) (range) of the degree of extensions were 130.60° (11.41) (range, 111° - 143°), 132.21° (7.98) (range, 116° -142°), 133.57° (7.87) (range,120° -142°) and 135.05° (6.7) (range, 124° -142°) before treatment and the second, fourth and eighth week after treatment commenced. The results revealed that comparison of means (SD) of the ROMs were not significantly different between the pre-treatment and the second, fourth and the eighth weeks after treatment commenced. The mean (SD) of hip extensions at the eighth week were significantly different (Table 16).

Shoulder joints

ROMs of shoulder joints revealed that the means (SD) (range) of flexions' degree to extensions's degree were 53.16 ° (3.01) (range, 50 ° -56 °), 51.33 ° (1.89) (range, 50 ° -

54°), 53.25° (1.63) (range, 52° -55°) and 51.5° (2.78) (range, 48° -54°) before treatment and at the second, fourth and eighth weeks after fish-oil administration, respectively. ROMs of shoulder joints revealed that the means (SD) (range) of the degree of extensions were 136.66° (5.77) (range, 130° -140°), 136.33° (5.34) (range, 130° -141°), 136.75° (5.25) (range, 131° -140°) and 137.16° (4.9) (range, 132° -140°) before treatment and at the second, fourth and eighth weeks after treatment commenced.

The results revealed that comparison of means (SD) of the ROMs in shoulder joints were not significantly different among the pre-treatment and at the second, fourth and eighth weeks after treatment commenced (Table16).

Table16. Mean (SD) of the ROMs in fish-oil group

ROM	Hip joint (degree)		Shoulder joint (degree)	
	Flexion	Extension	Flexion	Extension
D0 Range	39.25(6.63) (25-49)	130..60(11.41) (111-143)	53.16 (3.01) (50-56)	136.66(5.77) (130-140)
W2 Range	38.51(7.01) (21-49)	132.21 (7.98) (116-142)	51.33(1.89) (50-54)	136.33(5.34) (130-141)
W4 Range	39.21(6.59) (22-50)	133.30(7.66) (120-142)	53.25(1.63) (52-55)	136.75(5.25) (131-140)
W8 Range	39.55 (5.69) (26-50)	134.80(6.48)* (124-142)	51.5(2.78) (48-54)	137.16(4.9) (132-140)

*Means (SD) of the ROMs were significantly different compared with day 0 within the fish-oil group ($p < 0.05$).

The means (SD) of flexion degree between both groups were not significantly different to those recorded pre-treatment (D0). Table 17 showed that the means (SD) of flexion degrees were significantly different between groups in the same week after treatment commenced. The means (SD) of flexion degrees in both joints improved

significantly in the PCSO-524 group, but not in the fish-oil group. The means (SD) of extension degree between both groups were significantly different in the pre-treatment (D0). In PCSO-524 group, the means (SD) of extension degree after treatment commenced (W2, W4, W8) for both joints increased significantly compared with the pre-treatment (D0) measurements. There were no significant differences in the means (SD) of extension degrees for in both hip and shoulder joints in the fish-oil group at W2 and W4 compared with D0. The means (SD) of ROMs improved significantly in the PCSO-524 group compared with the pre-treatment (D0) states.

Table 17. Mean (SD) of the ROMs in the PCSO-524 group and in the fish-oil group

Joint		Hip joints (Mean(SD))				Shoulder joints (Mean(SD))			
Time		Do	W2	W4	W8	D0	W2	W4	W8
Flex (degree)	PCSO-524	40.11 (7.50)	34.57*† (6.87)	33.75*† (5.33)	33.32*† (4.78)	51.93 (9.32)	41.93*† (8.99)	40.56*† (7.88)	38.43*† (6.98)
	Fish oil	39.25 (6.63)	38.51† (7.01)	39.2† (6.59)	39.55† (5.69)	53.16 (3.01)	51.33† (1.89)	53.25† (1.63)	51.5† (2.78)
Extend (degree)	PCSO-524	122.60† (10.74)	130.6* (11.74)	133.48* (10.76)	135.91* (10.02)	121.75† (4.66)	127.87*† (5.8)	129.81* (9.27)	132.31* (10.74)
	Fish oil	130..60† (11.41)	132.21 (7.98)	133.30 (7.66)	134.80* (6.48)	136.66† (5.77)	136.33† (5.34)	136.75 (5.25)	137.16 (4.9)

*Values were significantly different compared with the pre-treatment (D0) within the groups ($p < 0.05$). † Values were significantly different between groups within the week ($p < 0.05$).

Osteoarthritic biomarkers

Chondroitin sulfate WF6 epitope (Figure 19, Table 18)

The serum of 46 dogs was evaluated by the CS-WF6 epitope concentration. The levels of serum CS-WF6 epitope in the pre-treatment (D0) were not significantly different between groups. In PCSO-524 group, there was a significantly lower level of serum CS-WF6 epitope in the second week (W2), the fourth week (W4) and the eighth week (W8) compared with the levels in the pre- treatment (D0). In the fish-oil group the level of serum CS-WF6 epitope at the eighth week (W8) was significantly greater than that it was before treatment commenced (D0). There was a significant difference in CS-WF6 concentration between groups at the eighth week after treatment commenced (W8).

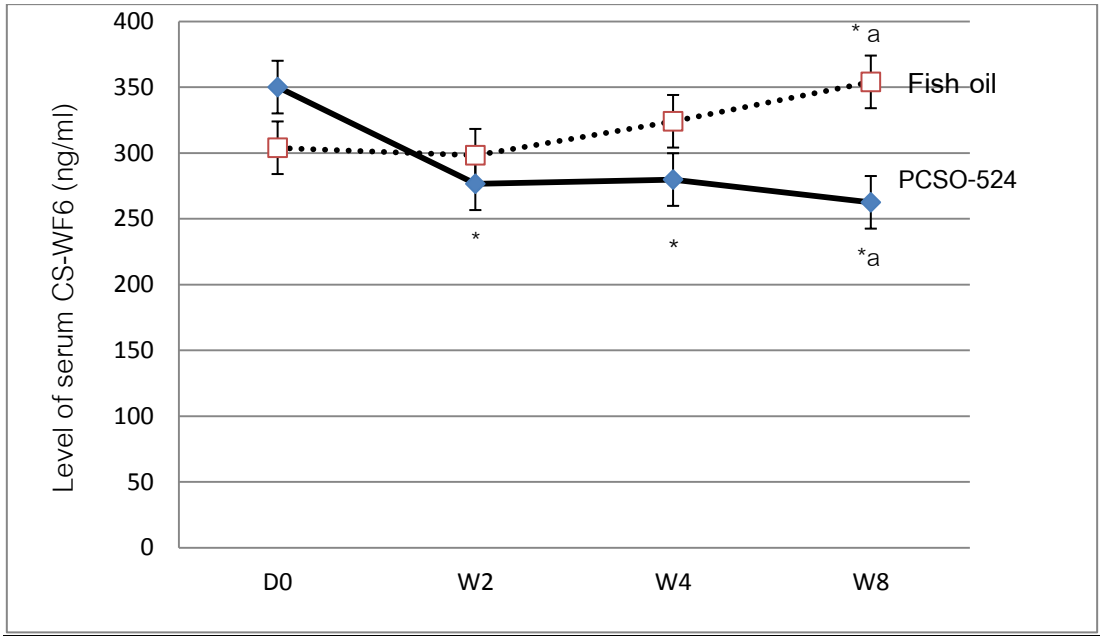


Figure 19. Mean of the levels of serum chondroitin sulfate epitope (CS-WF6; ng/ml). * Values were significantly different compared with the pre-treatment (D0) levels within the groups ($p<0.05$).[a] Values were significantly different between groups within the week ($p<0.05$).

Hyaluronan (HA) (Figure 20, Table 18.)

The result revealed that the level of HA was not significantly different within groups and between groups.

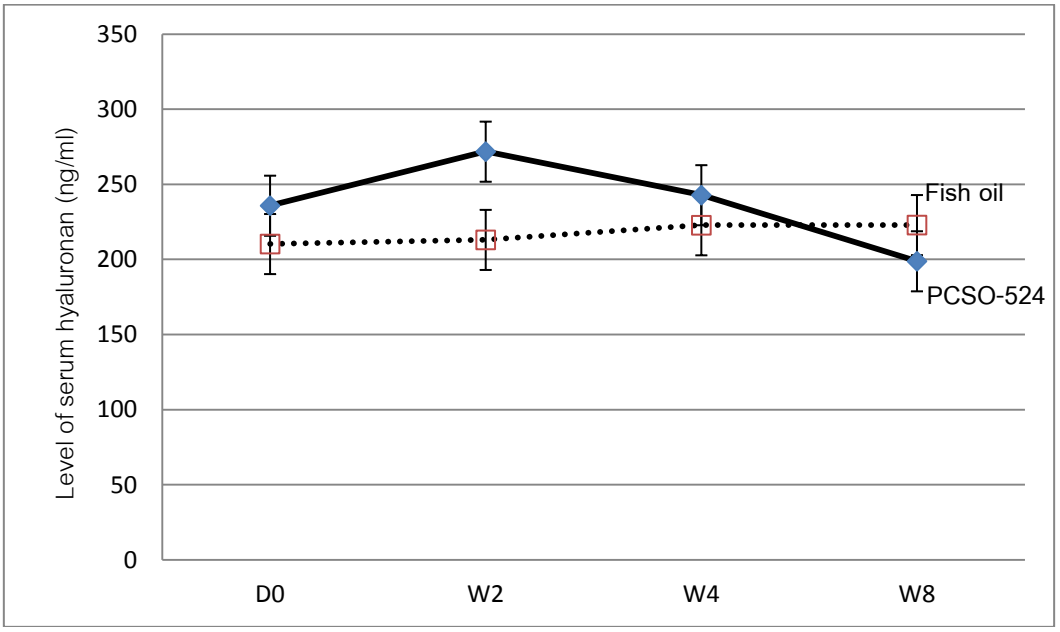


Figure 20. Mean of the levels of serum hyaluronan (ng/ml).

Table 18. Mean (SD) of the serum chondroitin sulfate epitpoe WF6 concentration and the serum hyaluronan concentration (ng/ml) in PCSO-524 group and in the fish-oil group

Week	Mean(SD) of CS-WF6 epitope (ng/ml)		Mean(SD) of HA (ng/ml)	
	PCSO-524	Fish oil	PCSO-524	Fish oil
D0	350.03 (174.78)	303.94 (135.69)	235.84 (241.87)	210.32 (90.33)
W2	276.58* (139.01)	298.24 (92.61)	271.81 (309.44)	213.07 (72.07)
W4	279.77* (151.07)	324.05 (109.10)	242.84 (285.16)	222.82 (87.45)
W8	262.46* [a] (162.24)	353.99* [a] (132.25)	198.86 (250.14)	222.82 (101.47)

* Values were significantly different compared with the pre-treatment (D0) within the groups ($p<0.05$). [a] Values were significantly different between groups within the week ($p<0.05$).

Chapter V
Conclusion, Discussion, Comment

Conclusion

This study was designed to investigate the effects of the use of PCSO-524 and fish oil on clinical outcomes (lameness score, weight-bearing score and radiographic score), ROM and OA biomarkers concentration (WF6 and HA) in OA dogs which were presented at Surgery Unit of the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. Forty-six dogs (82 hip joints and 11 shoulder joints) participated.

At week two, most of the patients that had been given PCSO-524 had significant improvements in their lameness scores, weight bearing scores and ROM ($p<0.05$). A large percentage of OA dogs showed improvement in both lameness (41/49, 83.67%) and weight-bearing scores (37/49, 75.51%) after eight weeks of PCSO-524 administration. The patients that had been given fish oil had a significantly greater lameness score and a significantly greater weight bearing scores than those that were given PCSO-524 at the fourth week and the eighth week after administration commenced ($p<0.05$). In the fish-oil group, the percentage of dogs that did not exhibit an improvement in their lameness score or in their weight-bearing score were 54.55 and 79.55, respectively. Although most of the fish-oil group did not improve significantly in their lameness and weight-bearing scores within eight weeks, the ROM was significantly increased at the eighth week ($p<0.05$).

There was no significant difference in the radiographic score improvement during the time of the study ($p>0.05$), a significant difference was presented between the two groups at the eighth week but only in the hip joints ($p<0.05$). The evaluation of radiographic findings after eight weeks of PCSO-524 administration revealed a small percentage (10/49, 20.41%) of improvement and a large percentage (38/49, 77.55%) of non improvement. 22.73% (10/44) and 77.27% (34/44) were detected as the

progression and the non improvement in radiographs after eight weeks of fish oil administration, respectively.

The OA dogs that received fish oil had a significantly greater concentration of WF6 epitope than the PCSO-524 group at the eighth week ($p<0.05$). The dogs that were administrated PCSO-524 had a significantly lesser concentration of WF6 epitope at the second, fourth and eighth weeks after treatment commenced compared with their pre-treatment ($p<0.05$). The HA concentration was not significantly different both within and between groups ($p>0.05$).

There were no proven side effects in either of the nutraceuticals that were administered during this clinical trial. The analyses of complete blood counts and blood chemistry were not different between the pre-treatment and the end of this study.

This study indicated that PCSO-524 had a therapeutic effect in OA dogs. The PCSO-524 administration can improve clinical signs, ROM and decrease the level of serum WF6 epitope in canine shoulder and coxofemoral osteoarthritis, but the fish-oil administration can't.

Discussion

According to pathogenesis of OA, the inflammatory pathway has a major role in the imbalance of cartilage metabolism. In previous studies, omega 3 fatty acids supplementation has been shown to decrease the production of inflammatory eicosanoids such as prostaglandin E2, leukotriene B4 and thromboxane B2 by inflammatory cells (Meydani, et al., 1991; Kelley et al.,1999; Trebble et al., 2003). Ecosanoids which are created from omega-3 fatty acids, are less potent inducers of inflammation than those created from arachidonic acid (Calder and Zurier, 2001). The actions of cyclooxygenase and lipoxygenase are inhibited by omega 3 (Brian, 2004). There are many types of omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA), docasahexaenoic acid (DHA) and eicosatetraenoic acid (ETA). Omega-3 fatty acid administration may also reduce the serum concentrations and activities of the

proteoglycan degrading enzymes, cyclooxygenase and inflammation-inducible cytokines (Curtis et al, 2000).

The results of this study revealed that the lameness score, the weight-bearing score and the ROM in the PCSO-524 group showed significantly more improvement than those in the fish-oil group. The level of the WF6 epitope was significantly lower than that of the fish-oil group. These results supported the improvement of OA signs and the slowed OA progression among the dogs that were given PCSO-524. This was in accord with previous studies, which found that the OA dogs had greater lameness scores and a greater concentration of WF6 epitope than that was found in normal dogs (Nganvongpanit et al, 2008; Trakulsantirat et al, 2010). Although most of the PCSO-524 group improved in clinical signs, the radiographic findings were not significant. The trend of OA lesions in radiographic findings was better and smoother in some of the dogs that had been given PCSO-524. Research in humans has revealed that clinical function of OA may not be related directly to radiographic evidence of osteoarthritis (Swagerty and Hellinger, 2001). Also, another study in dogs revealed that radiographic findings did not correspond with clinical signs in OA dogs (Gordon et al., 2003).

The normal of shoulder ROM is 45^{o}(±15) - 165^{o}(±5) and the normal range of hip ROM is 50^{o}(±2) – 162^{o}(±3) (Millis et al., 2004). ROM in this study was evaluated as passive joint motion, which was measured by a veterinarian (Millis et al., 2004). Dogs that were not sedated used active force to resist movement, which distorted the ROM findings, especially extensional measurement. So, the extensional ROM results were significantly different at the pre-treatment. A goniometer is used to measure the ROM, however it may be not have been precise. To avoid this effect, we recommended using the same technique, the same equipment and the same veterinarian as a way to reduce the size of the possible error. A study tried to evaluate the valid goniometry comparison between sedated dogs and dogs that were awake. Sedation did not influence the ROM of the evaluated joints (Jaegger et al., 2002).

Although subjective assessment of OA signs is inferior to objective assessment obtained from force platform gait analysis (Roush et al., 2010), clinical outcomes in the

present study were assessed by the same blinded veterinary surgeon who had extensive experience in OA treatment. This study found that the clinical lameness and weight bearing were improved in PCSO-524 group whereas those in fish oil group were worse at four weeks after administration commenced.

The percentage of clinical outcomes after eight weeks of PCSO-524 administration revealed only a 4.08% of progression of lameness and weight bearing scores. One dog required surgical intervention at the femoral head and neck excision. Rehabilitation and NSAID combined with omega 3 were applied for pain relief and to assist in the return of limb function. The veterinarian decided to give more treatment because the pain score evaluation was more than five points on the scale of Glasgow Composite Measure Pain Score-Short Form (Gaynor and Muir, 2009). The severely-high of lameness score and the small size of limb's muscle mass at the pre-treatment might cause this responsible case of the use of PCSO-524.

Five dogs in the fish-oil group were interchanged to use PCSO- 524 after the end of this study because it was indicated clinically and because of their advanced age. Because PCSO-524 has been shown to have no adverse side effects (Whitehouse et al., 1997), the nutraceutical was an ideal choice for the elderly dogs.

Previous studies investigated the serum WF6 epitope level and the alteration of the articular cartilage, which is more sensitive to joint cartilage degradation (Nganvongpanit et al., 2008a; Pruksakorn et al., 2008). Therefore, the released serum WF6 from extracellular matrix of joint cartilage is due to the destruction of cartilage and the assay should be proven for monitoring OA treatment or before and after traumatic arthritis detection (Pruksakorn et al., 2008). The results showed that the serum WF6 epitope concentration in the PCSO-524 group was significantly less than that in the fish-oil group at the end of study. It was interesting that PCSO-524 may decrease the cartilage destruction in OA dogs and the effect was detected after two weeks of administration. On the other hand, fish-oil cases continued to experience joint cartilage degradation, as indicated by an increase of the WF6 epitope concentration at the eighth week. The mean of age between groups was not significantly different, so the WF6 level

changes were directly as a result of the joint cartilage alteration. A previous study showed that this epitope was not significantly different in age groups (Trakulsantirat et al, 2010).

The level of serum HA increased in the chronic liver cases and the rheumatoid arthritis cases, which presented severely-inflamed joints.

Several factors such as liver problems, eating and morning activities influence the HA accumulations (Sakugawa et al., 2005; Isman et al., 2007). As an effect of HA levels in different conditions, none of the OA dogs had a liver problem of the CBC or had blood chemistry that would have cause them to be excluded. Abnormal hepatic signs were monitored during the period of the study. The serum from which OA biomarkers was determined was collected in the mornings, before the dogs were given anything to eat. Conditions confirmed that the results of HA levels were as a result of joint metabolism directly and that these levels were not influenced by others factors. Although the biomarker is not specific to osteoarthritis, the increase in the level can be used to track the inflammatory progression, especially in the case of rheumatoid arthritis (Louthrenoo et al., 2001). Although the results of HA levels that were not significantly different were known, we were somewhat surprised to find that the trend was for HA levels to decrease after eight weeks of PCSO-524 administration. The trend in the fish-oil group was for HA levels to increase slightly. The analysis of HA levels seemed to not be an ideal way to detect changes in the condition of joint cartilage, nor are the WF6 epitope levels (Pruksakorn et al., 2008).

As a condition of the clinical trial, the management of client owned dogs was important for the duration of the study. Thus, we selected carefully all participants by using strict criteria, which included taking the history and performing a physical examination of each dog. A researcher advised the owner of each dog to not exercise the dog extensively, to not give it snacks or treats, to feed it only prescribed food, how to administer the nutraceutical, how to look for abnormal signs and when to attend monitoring appointments. Owners were reminded of the appointments every two weeks by text and telephone.

Nutraceuticals have been become important in the prevention and treatment of disease. The word *nutraceutical* is a union of the words *nutrition* and *pharmaceutical.* The definition is a food or a part of a food that could prevent or treat a disease like health benefits or medical (Brower, 1997). The definition of the term *dietary supplement* (extracts and concentrates) is that it provides a dietary ingredient that is intended to supplement the diet. In veterinary practice, a nutraceutical is an essential component that is extracted or purified in order to provide a structural benefit or improve a normal function to provide better health and quality of life by oral administration (Boothe, 1997).

A systematic review of research was done on the efficacy of nutraceuticals for the relief of clinical signs of oateoarthritis examined 22 papers. Although the conclusion was that the efficacy of nutraceuticals was poor, omega-3 fatty acid in dogs was the exception (Vandeweerd et al., 2012). The efficacy of omega-3 use in OA dogs varies according to the source, the omega-3 to omega-6 ratio, the volume of EPA and DHA and also the extraction method and subsequent processing. The carrier elements and the anti-oxidant used to stabilize an active ingredient also have an effect on the efficacy (Whitehouse et al., 1997; Treschow et al., 2007; Fritsch et al., 2010). A study revealed that high EPA and DHA concentrations in food (approximately 2.94 % dry weight) and a high omega-3 to omega-6 fatty acid ratio (approximately 2.19) improved the clinical outcome significantly after 90 days (Fritsch et al., 2010). Another study found that the lameness and weight-bearing scores of 38 OA dogs improved after they received a diet that contained 3.5% omega-3 fatty acids (fish oil) (Roush et al., 2010). These results were the opposite of this study, however the period of the study was only 60 days. The result of a consistent dose of fish oil in dogs is not understood well because there have been only a small number of animal studies.

Although the data that pertains to the dosage is limited, veterinary clinics in small animal practice recommend DHA and EPA (from fish oil about 60 mg/kg/d) for arthritis patients (Beale, 2004). However, the efficacy of the dose of EPA and DHA in OA dogs should be investigated in a further study. The adverse effect is that EPA may inhibit blood clotting. There are no known adverse effects of DHA (Beale, 2004). The

impact on blood clotting was measured twice (monthly) during the term of a study, by recording mucosal bleeding. All the dogs experienced normal bleeding (less than 2.6 minutes) (Jergans et al., 1987). It should also be born in mind that the omega-3 and omega-6 content of each product is different because of source variation and different processes. The source of the omega-3 has been shown to have a great bearing on its efficacy.

The different omega-3 fatty acids between fish oil and PCSO-524 may cause the different clinical outcomes in this study. There were no of ETA and a low concentration of EPA in fish oil. These may be a cause of non improvement of clinical outcomes in the fish-oil group. A study differentiated omega-3 fatty acids by gas chromatography, then the anti-inflammatory essay revealed that the C20:4 can decrease more LTB4 than others (Treschow et al., 2007).

Omega-3 chains extracted from the New Zealand green-lipped mussel have been shown to be much more potent than fish oil as an anti-inflammatory (Whitehouse et al., 1997). Omega-3 fatty acids in fish oil include EPA and DHA (Fritsch et al., 2010) but those in PCSO-524 include compound fatty acids (ETA, EPA, DHA ,etc) (Treschow et al., 2007). Although PCSO-524 is a GLM extract, it is different from other GLM products. PCSO-524 is the result of patented extraction and stabilization process, as part of which a super-critical fluid process is used (Whitehouse et al., 1997, Treschow et al., 2007, Soontornvipart and Mongkhon, 2012). PCSO-524 is a lipid-rich extract that improved OA signs and delayed cartilage degradation at the second week after administration in the present study. A previous study, which used GLM powder, found that it provided a significant improvement by reducing the severity of arthritis in dogs after week six of the treatment (Bierer and Bui, 2002). Another study of an extract of GLM in OA dogs found that on day 56 the GLM treated dogs had improved in clinical signs (Pollard et al., 2006). These studies support the effective of GLM. The present study found that the unique composition of PCSO-524 provided a faster rate of improvement than did the GLM powder. Comparison study between different GLM extract should be further investigated.

Another advantage of nutraceutical is that it can be used in the long term without adverse side effects (Beale, 2004; Wang et al., 2004) to treat OA, provide pain relief and help the dog to regain limb function quickly (Nelson et al., 2006, Ettinger and Feldman, 2010). Non steroidal anti- inflammatory drugs (NSAIDs) are usually provided in cases of severe lameness because the onset time and the duration of action are well understood, in contrast with nutraceuticals. To avoid side effects, to decrease long term use and to reduce the dosage of NSAIDs, the combination of NSAIDs and omega-3 has been recommended for OA dogs (Fritsch et al., 2010).

Comment

The osteoarthritis is a chronic inflammatory condition. The goal of the treatment is improvement of quality of life by pain relief and help the dog regain limb function. The management of OA dogs should provide not only medication but also rehabilitation. Nutraceuticals seem to be a good choice for greater improvement in OA dogs; especially, PCSO-524 (stabilized GLM lipids ectracts) because it has been shown to slow the progression of the disease in OA dogs. Veterinarian should not confuse the different forms of GLM extract, the patented process used to create PCSO-524 extracts the rich lipids and preserves their efficacy by preventing oxidization.

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Biography

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