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# Anti-inflammatory efficacy of New Zealand green-lipped mussel extract in canine chronic stifle osteoarthritis monitoring by thermographic imaging

Supakit Vilaisuntornkiat
*Faculty of Veterinary Science*

# ANTI-INFLAMMATORY EFFICACY OF NEW ZEALAND GREEN-LIPPED MUSSEL EXTRACT IN CANINE CHRONIC STIFLE OSTEOARTHRITIS MONITORING BY THERMOGRAPHIC IMAGING

Mr. Supakit Vilaisuntornkiat



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# ประสิทธิภาพในการลดการอักเสบจากสารสกัดจากหอยแมลงภู่ปากเขียวนิวซีแลนด์ในสุนัขที่มีภาวะข้อเข่าเสื่อมเรื้อรังโดยใช้การถ่ายภาพแผนภูมิความร้อน

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| Thesis Title | ANTI-INFLAMMATORY EFFICACY OF NEW ZEALAND GREEN-LIPPED MUSSEL EXTRACT IN CANINE CHRONIC STIFLE OSTEOARTHRITIS MONITORING BY THERMOGRAPHIC IMAGING |
| By | Mr. Supakit Vilaisuntornkiat |
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| Thesis Advisor | Assistant Professor KUMPANART SOONTORNVIPART, D.V.M., Ph.D., D.T.B.V.S. |

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..... Dean of the FACULTY OF VETERINARY SCIENCE

(Professor SANIPA SURADHAT, D.V.M., Ph.D.)

THESIS COMMITTEE

..... Chairman

(Instructor Dr. NICOLE SIRISOPIT MEHL, D.V.M., Ph.D.)

..... Thesis Advisor

(Assistant Professor KUMPANART SOONTORNVIPART, D.V.M., Ph.D., D.T.B.V.S.)

..... Examiner

(Assistant Professor DAMRI DARAWIROJ)

..... External Examiner

(Professor Korakot Nganvongpanit, D.V.M., Ph.d.)

ศุภกิตต์ วิไลสุนทรเกียรติ : ประสิทธิภาพในการลดการอักเสบจากสารสกัดจากหอยแมลงภู่ปากเขียวนิวซีแลนด์ในสุนัขที่มีภาวะข้อเข่าเสื่อมเรื้อรังโดยใช้การถ่ายภาพแผนภูมิความร้อน. ( ANTI-INFLAMMATORY EFFICACY OF NEW ZEALAND GREEN-LIPPED MUSSEL EXTRACT IN CANINE CHRONIC STIFLE OSTEOARTHRITIS MONITORING BY THERMOGRAPHIC IMAGING) อ.ที่ปรึกษาหลัก : ผศ. ดร.กัมปนาท สุนทรวิภาต

การศึกษาครั้งนี้มีเป้าหมายเพื่อเปรียบเทียบผลของการลดอุณหภูมิในระหว่างกลุ่มของสารสกัดจากหอยแมลงภู่นิวซีแลนด์กับกลุ่มของยาหลอกในสุนัขที่มีภาวะโรคของเอ็นไขว้หน้าข้อเข่าโดยใช้กล้องถ่ายภาพแผนภูมิความร้อนเพื่อวัดอุณหภูมิที่เปลี่ยนไป การศึกษาครั้งนี้มีสุนัขเข้าร่วม 52 ตัวโดยคัดเลือกจากสุนัขที่มีภาวะเอ็นไขว้หน้าเข่ามีความผิดปกติร่วมกับมีการเคลื่อนของสะบ้าเข้าด้านใน สุนัขทั้งหมดในการทดลองถูกแบ่งออกเป็น 2 กลุ่ม กลุ่มเอกับกลุ่มบี อุณหภูมิในวันที่เริ่มต้นการทดลองของกลุ่มเอคือ 99.60 องศาฟาเรนไฮต์และของกลุ่มบีคือ 99.07 องศาฟาเรนไฮต์ ผลการวัดอุณหภูมิในสัปดาห์ที่ 4 ของการทดลองพบว่า อุณหภูมิของกลุ่มเอคือ 97.09 องศาฟาเรนไฮต์ และ ของกลุ่มบีคือ 99.02 องศาฟาเรนไฮต์ ผลการศึกษาพบว่าการใช้สารสกัดจากหอยแมลงภู่นิวซีแลนด์สามารถลดอุณหภูมิของข้อเข่าได้เมื่อเปรียบเทียบกับกลุ่มของยาหลอกโดยวิธีการวัดการใช้กล้องแผนภูมิความร้อน



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This study aimed to compare the anti-inflammatory effect of the NZGLM and placebo in patients with cranial cruciate ligament disease using thermographic imaging. This study evaluated the temperature from thermographic imaging to measure the reduce of inflammation. This study included 52 dogs with cranial cruciate disease and medial patellar luxation. All dogs were divided into 2 group that was group A and group B. The temperature at day 0 were 99.60 °F in group A and 99.07 °F in group B. The temperature at 4$^{th}$ week were 97.09 °F in group A and 99.02 °F in group B. The result revealed that NZGLM group can reduce the temperature of the stifle joint. In conclusion, based on temperature of the NZGLM group and placebo group in this study, The temperature of the stifle joint in this study by using thermographic imaging method can show the different of the NZGLM group.



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

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# CHAPTER I

# INTRODUCTION

## Importance and rationale

Osteoarthritis (OA) is the most common orthopedic problem in many species. This problem makes patients suffer from chronic pain all the time, leading to physical disability. The previous study showed that osteoarthritis patients suffer from pain and have a lower quality of life (Burnett et al., 2006; Ramírez-Flores et al., 2017; Schmidli et al., 2018). This disease can occur from various diseases. The pathogenesis of this disease was influenced by low grade inflammatory process in the joint. The inflammatory process can occur due to the instability of joint, abnormal force loading and other factors. These inflammatory pathways take responsible in subchondral bone and cartilage destruction. Inflammation induces synovial cell to produce cytokines such as Interlerkin-1 (IL-1), Interlerkin-6(IL-6), matrix metalloproteinase (MMP), and tumor necrosis factor alpha (TNF-$\alpha$). This disease can monitor by various methods. The common method that widely used in clinical practice were radiographic imaging and lameness scoring. The radiographic imaging can detect the pathological changes that occur in the joints, this method can only detect the late stage of the OA. The other methods that occasionally used such as biomarkers, CT scan, ultrasonography, and thermographic imaging were only used in the researching unit for OA monitoring because of the skill requirement and special equipment application. Thermographic imaging was the one of the monitoring methods, that detects infrared radiations that emitted from object to produce the infrared picture. This method is the non-invasive ways to detect heat that occur from increase of blood circulation in the inflammation process (Denoble et al., 2010; Jin et al., 2013). The thermographic imaging detect the temperature around the

inflammation area compare to the other area temperature (Jin et al., 2013). This method was used widely in the wildlife and horse clinical field.

In the past few years, there were many options to manage OA such as surgical, medicine, rehabilitation, and nutraceuticals. The effective treatment is aimed to slow the progression of the OA and reduce pain. Nowadays, there are many nutraceuticals that are available in veterinary medicine such as undenatured collagen type II, chondroitin sulfate, glucosamine, various antioxidant, and New Zealand green-lipped mussel extract products (Buddhachat et al., 2017a). These nutraceuticals can be used in the combination with other treatments option to increase the effect of the treatment plan and reduces the adverse effect of long-term medication (Kwananocha et al., 2016). The New Zealand green-lipped mussel extracted (NZGLM) were reported that can reduces inflammation and degradation without adverse effects. This active component was omega-3 polyunsaturated fatty acid (Ω-3 PUFAs), this component was the result from cold live extracted (Coulson et al., 2015). NZGL can also reduce the matrix metalloproteinases (MMP) enzymes that induce inflammation in multiple systems such as joint, central nervous system, and gastrointestinal system (Ahmad et al., 2018). Matrix metalloproteinase are the enzymes produced form chondrocyte after mechanical injury to the joint. This enzyme induces collagen destruction and responsible for osteoarthritis progression. The benefit of this product is less side effect compared to drugs that reduce inflammation, and profound the multiple effects on the other systems. From all, these benefit effects of nutraceuticals was the most selected choice from dog's owner more than drugs. This study aims to evaluate the clinical anti-inflammatory efficacy of the New Zealand green-lipped mussel extract product monitoring by thermographic imaging.

# CHAPTER II

# LITERATURE REVIEW

## 2.1 Osteoarthritis

Osteoarthritis (OA) is a chronic progressive disease that worsen the quality of life of the patients (Vijarnsorn et al., 2019). OA was one of the most founded orthopedics problems. OA can be occurred by many conditions. There were 20 percent of matures dogs that suffer from this disease (Johnston, 1997). This disease was induced by many factors such as genetics, breed, age, obesity, and trauma (Burnett et al., 2006). With the slow progression of disease, joint's function and structure is altered which lead to reveal the abnormality in gait and posture. The pathological change of this problem is irreversible such as destruction of articular cartilage, ligament damaged, narrowing in joint space, and thickened subchondral bone. These pathological changes leading to lameness problem and decrease in musculoskeletal abilities (ROY et al., 1992). OA was a chronic disease that progress worsen. The nociceptor has been stimulated all the time that problem was progress which stimulated the central nervous system to sensitization that led to overall pain (Knazovicky et al., 2016).

There are many factors that leading to OA problem both congenital and acquired. For congenital, the arthropathies of young dogs leading to secondary OA such as joint dysplasia, osteochondrosis dissecans, and patellar luxation (Martinez, 1997). For acquired musculoskeletal disorders as developmental abnormalities, progressive cartilage deterioration have played the major role (Martinez and Coronado, 1997). Direct trauma has also induced intra-articular lesion. Injuries of ligament or joint structure may form a consequence of joint instability that led to the onset of the OA. There are many factors that accelerate progression of the OA

problem. The abnormal mechanical loading via joint induced osteochondral microfracture, cartilages loss, bone sclerosis, and abnormal cartilage formation (Henrotin et al., 2005).

Pathophysiology of OA begins with the chondrocytes change their phenotypic modifications, leading to cartilage hypertrophy due to chondrocytes repair ability, mineralization, and degradation (Braunstein et al., 1990). Hypertrophic cartilage is characterized by increased matrix hydration, cell cluster formation, and accelerated matrix turnover (Henrotin et al., 2005). In normal condition, chondrocytes will produce collagen type I, IIA, III or X and tenascin for repair damaged chondrocytes (Poole et al., 2002). As the production of those molecules excess, the cartilage will become more hypertrophy for the repair process. The cartilages repair reaction is transient and inefficient when its repair process goes excessively (Sandell and Aigner, 2001). Chondrocytes are producing more matrix metalloproteinase which break the balance between catabolic and anabolic processes in the result of matrix degradation (Burnett et al., 2006). Chondrocytes are produce interleukin-1 (IL-1) and tumor necrosis factor (TNF) while decreasing in transforming growth factor (TGF) receptor (Wang et al., 2003). IL-1 and TNF-$\alpha$ play a major roles in activation of matrix metalloproteinase synthesis (Yasuda, 2006). Then IL-6 comes to complete the catabolic activity (Garvican et al., 2010). Cartilage matrix also have oxidative stress by reactive oxygen species (ROS) which generated by chondrocytes. Subchondral bone also takes a role in the OA, characterized by subchondral bone sclerosis but there is not clear that bone changes are the cause or consequence of cartilage lesion. Subchondral bone thickening was the result from increasing osteoid volume and a low mineralization (Henrotin et al., 2005). The osteophyte also produces more cytokines, the imbalance between cytokines lead to enhance bone matrix formation (Sandell and Aigner, 2001; Garvican et al., 2010). This result made the patients suffer from pain and worsen their quality of life (Burnett et al., 2006)

The clinical sign of OA was seem as pain, changes in daily behaviors, and joint stiffness (Slatter, 2003; Sandersoln et al., 2009). The synovial fluid of OA patients has increased in the number of mononuclear cells which are mainly macrophages, inflammatory cytokines, and T Lymphocyte (Kamekura et al., 2005). The structural

changes of synovial membrane are hyperplasia of synovial lining as an effect of inflammatory cells infiltration (Sandell and Aigner, 2001; Kamekura et al., 2005; Sandersoln et al., 2009). Most of the owner did not detect that their dogs have OA problem until there have lameness sign.

There are many treatments to reduce pain and inflammation of OA patients such as surgery, medicine, rehabilitation, and nutraceuticals which improve the quality of life of the patients. The treatment was aim for relieving the pain or discomfort and slow the progression of the OA, which could be served by pharmacologic treatment. There are two groups of medicine that commonly used to treat OA patients, which is steroids and non-steroidal anti-inflammatory drugs. Both of steroids and non-steroidal anti-inflammatory drugs have benefit and adverse effect when administration by any route (Monteiro-Steagall et al., 2013). The nutraceuticals have more benefit when compared to those 2 groups of medicine. The most type of nutraceuticals have more than one effect (Gregory and Fellner, 2014) and less adverse effect when compared to medicine. The most common treatment for OA patients was using the combination of medicine and nutraceutical to decrease the inflammation and reduce the inflammatory effect of the OA with minimize the adverse effect.



**2.2 Nutraceuticals**

Today we have a numerous nutraceutical that used to reduce the inflammation such as glucosamine, chondroitin sulfate, collagen, hyaluronic acid (HA), and New Zealand green-lipped mussel extract (NZGLM).

Previous research found that glucosamine and chondroitin sulfate can reduced inflammation in vitro and in vivo study (Vijarnsorn, 2003; Clegg et al., 2006; Huskisson, 2008; Gregory and Fellner, 2014). The effect of glucosamine in vitro laboratory was stimulated the chondrocyte to synthesize proteoglycans, to provide substrate for cartilage repair (Huskisson, 2008). However, in the clinical practice this supplement did not show the significant effect when compared to other (Vijarnsorn, 2003; Kongwut et al., 2015; Kwananocha et al., 2016; Buddhachat et al., 2017b). Chondroitin supplement have a vary of the clinical activity depends on origin of the

chondroitin sulfate, the molecular weight, and chain length (Adebowale et al., 2000; Barnhill et al., 2006).

The study if hyaluronic acid (HA) shown that there can reduce the inflammation without any adverse effect when intraarticular administration (Techaarpornkul et al., 2015). HA provides synovial fluid with its viscoelastic properties (Gerwin et al., 2006; Tsai et al., 2013). In vitro studies, HA demonstrated apparent protective effected in cultures chondrocytes (Marshall et al., 2000; Moreland, 2003). Injection HA performs a variety of anti-inflammatory effect. HA is believed to modulate synovial fibroblast metabolism and restore the rheological properties of synovial fluid, and interacting with proinflammatory mediators (Henrotin et al., 2013; Conrozier et al., 2016).

NZGLM combined with other substances, such as vitamin, mineral, and other nutraceutical were the most popular for the owner and have a highly effect when combined with medical treatment such as non-steroidal anti-inflammatory drugs (Soontornvipart et al., 2015; Kwananocha et al., 2016). NZGLM is the active ingredients of the product that use cold extraction from New Zealand green-lipped mussel. The active ingredients can reduce the matrix metalloproteinase activity that is the cause of pathological changes mechanism. These active ingredients show the good result in biological activity in laboratory environment (Coulson et al., 2015). The benefit of this ingredients was that it can use with other pharmaceutical medications such as non-steroidal anti-inflammatory drugs (NSAIDs) with synergistic effect. the research in the past shown that the extraction from mussel can alleviated the severity of lameness and joint pain without any adverse effect (Pollard et al., 2006; CAYZER et al., 2012; Coulson et al., 2015). The past clinical research shown that the extracted highly contain omega-3 polyunsaturated fatty acid that have been proven in its efficacy of reducing inflammatory and degradation pathways. Our current understanding points to omega-3 polyunsaturated fatty acid (Ω-3 PUFAs) was that it has highly potent anti-inflammatory properties. It decreases inflammatory mechanisms and inflammatory inducible cytokines. NZGLM shown that it can reduce the degrading of the extracellular matrix protein via reducing the MMP activity, resulting in reduction of the inflammation in the multiple systems and it can be used

with other methods to reduce the inflammation in the multiple pathways (Pollard et al., 2006; CAYZER et al., 2012; Gregory and Fellner, 2014; Coulson et al., 2015; Soontornvipart et al., 2015; Ahmad et al., 2018).

### 2.3 Thermographic imaging

Diagnostic methods of OA are commonly determined in clinical examination by orthopedic and radiographic examination. The inflammation of joint can be easily detected from lameness, physical examination. However, radiography can appear only when secondary changes was occurred. There are many changes in soft tissue structure prior to a detection of bony changes in radiographic finding (Johnston, 1997; Innes et al., 2004; Burnett et al., 2006; Ramírez-Flores et al., 2017). There are many different methods to detect osteoarthritis in the research field such as serum OA biomarkers, computed tomography (CT-scan), ultrasonography and thermographic imaging. The biomarker was used to detect the OA biomarker such as chondroitin sulfate (CS) and hyaluronan (HA) in the serum that was the result of the OA in every joint of the body (Hegemann et al., 2002; Nganvongpanit et al., 2008). The biomarker can be detected in the early stage of the inflammation. The ultrasonography was an addition method for orthopedics examination (Ramírez-Flores et al., 2017).

Thermographic imaging has been studied and used widely in human and various interested species of wild animals. This imaging method can be used to detect the inflammation both acute and chronic stages. The imaging can compare between body temperature and other areas, so this imaging showed that the area that has inflammation will have a higher temperature than body temperature (Jones, 1998; Igna et al., 2017). The pattern of the imaging can be used to detect the inflammation earlier than radiographic imaging (Varjú et al., 2004). The clinical researches showed that the temperature of the stifle joint was lower than diaphysis area (Innes et al., 2004). Due to the location, the stifle joint was suitable to represent thermal change detected by thermal camera (Denoble et al., 2010; Jin et al., 2013).

The thermal camera that was used in the fields must be at least 180x180 pixels with the factory calibrated (Vainionpää et al., 2012). The lower resolution cannot measure the temperature accurately. The common thermal camera that used in research was made by FLIR company. The thermal camera that produced and developed by FLIR company have the factory calibrated that make sure the temperature was correct and have a program that can use to process the picture after the imaging was done. The camera can detect -20℃ to 250℃ with accuracy ±2℃, with have a vary type of measurement analysis in the camera program.

Thermographic imaging is widely used in veterinary fields in horse and wildlife examination. There are not much of studied in small animal field. All the study used thermal camera for inflammation monitoring (Vainionpää et al., 2012; Igna et al., 2017). Thermal camera was one of the inflammation monitoring methods for most of species without invasive method.

Thermographic imaging used to be addition method to detect the inflammation of the many area in animals (Jones, 1998). The thermographic imaging has been proven that it can show the evidence of osteoarthritis (Jin et al., 2013). More than that, the thermographic imaging can use to detect acute phase of the osteoarthritis (Kwon, 2019). All the monitoring techniques for OA patients was done to detect the progression of the disease. Benefit of using thermographic imaging for monitoring joint disease are non-invasive method and high accuracy rate if detection in both early and late stage of the inflammation. The previous study showed that thermographic camera with at least 180x180 pixels could be used to detect the inflammation of the body area effectively (Vainionpää et al., 2012), which can divide the area of the study into multiple spots and that will make the measurement of the temperature in that area being more effectively. This early detection will help practitioners in managing the problem before it becomes more complicated.

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 Animals

This study included 11 dogs with cranial cruciate disease induces by medial patellar luxation detected by laxity in cranial drawer test, medial buttress, pain when fully extended, lameness score grade less than 3, patellar luxation combined with grade 2-3. They were divided as they were diagnosed into 2 groups which was group A and group B. Each group undergone by randomized sampling method. The same appearance of both samples was also applied to avoid any research bias. Dogs included in this study would be in any breed, ages, body condition score, and weight. The body condition score was determines based on nine-integers BCS scale system modified from (Laflamme, 1997; Burkholder, 2000). The inclusion criteria for this study were dogs diagnosed that had cranial cruciate disease with medial patellar luxation without any other orthopedic diseases and normal health condition. If the patients used anti-inflammatory drugs or nutraceutical, 2 weeks of wash-out must be done to decrease the interference effect. If the health condition was not in the normal condition of the dogs was worsen clinical signs from the start of the study or have a lameness score of 5 that dogs will be excluded from this study. The dogs that excluded from this study have a surgery treatment after excluded.

All patients were diagnosed and treated at surgery unit, small animal hospital, Faculty of veterinary, Chulalongkorn university, Thailand. This study followed the guidelines for the care and use of laboratory animals and approved by the animal care use committee of the faculty of Veterinary science, Chulalongkorn university,

Bangkok, Thailand, animal use protocol 2031080. All owner of the patients included in this study must sign consent form to attend tin this study.

### 3.2 Study designs

This study included 52 dogs for this study and divided into 2 groups by double blind random sampling method. The duration of treatment was 5 weeks consecutively. All dogs were recorded in video for evaluation of lameness score, gait, and posture before and after treatment. The video was evaluated by 3 veterinarians. Both groups were given nutraceutical A and B that have similar appearance in the first visit. Nutraceutical A and B was NZGLM and placebo. Both researcher and owner did not know which one was the NZGLM and placebo to minimize the bias for this study. The recommend dose was 10 kilograms per tablet. All dogs were recorded by thermographic imaging and radio graphic imaging before and after treatment. The thermographic imaging was done immediately after record the direct core temperature by using thermometer. The core temperature was not different more than 0.3°F every time of the record, if the temperature was too high, the patients must wait until the core temperature was back into range of the 1st visit temperature. The thermographic image was taken in 2 view, medial and lateral. The medial view used the patellar ligament and the tibia as a landmark. The lateral view applied at the space among the patella, the femur, and the tibia. Each view was taken 3 images to maximize the measurement more accurate. The background of the image was set into blue color to make the image easy to measure. The clinical evaluation including thermographic imaging and lameness score were accessed every 1 week. The unit of measurement was report in Fahrenheit unit. After image was taken, the image was measure by FLIR tools 2.0 to make the correct measurement. Some of the patients from group B was take a surgical treatment after the 4th week for correction of the disease and continued the supplement until complete 5 week of the study. Due to

the worsen in lameness score from the start of the study. Any dogs have taken medicine, other nutraceutical, rehabilitation, or other surgery was cut off from the study

### 3.3 Experimental designs

#### a. Radiographic imaging

All the patients were investigated by radiographic imaging in lateral view and craniocaudal view. Each dog was positioned in lateral and dorsal recumbency with the limb extended along the long axis of the femur and parallel to the long axis of the tibia. they were 2 observers for diagnosis the stifle OA. This OA score will be evaluated based on Widmer et al. (1994), Innes et al. (2004), and Wessely et al. (2017).

**Table 1** Osteoarthritis grading system and corresponding radiographic changes modified form Widmer et al. (1994), Innes et al. (2004), and Wessely et al. (2017).

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

**b. Lameness score**

All patients were evaluated lameness score by 3 veterinary and recorded in each visit with blind evaluation modified from Impellizeri at al., (2000) and Tinga et al. (2021)

**Table 2** Lameness score criteria modified from Impellizeri et al. (2000) and Tinga et al. (2021)

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



**c. Thermographic imaging**

The thermographic imaging in this study was done by using FLIR® E6-XT. The specification of the thermal camera has infrared resolution 240x180 pixels, minimum focus distance 0.5 meter, 6 type of measurement analysis, accuracy ±2% of reading

in the ambient temperature (10°C to 35°C), and after imaging measurement program. The thermographic imaging was done in cranial and lateral view. In the cranial view the marker was set between patellar ligament and tibia. In the lateral view, the marker was set between patellar, tibia, and femur. The patients must be in the standing position and the background must be the wall and table that did not expose to sunlight. The image was taking while the camera was far from stifle around 50 centimeters. The imaging was done 3 times each view. The image was done immediately after the core temperature was measure by direct method. After finished the imaging, image was saved for analysis between before and after treatment, the base temperature of the image was set to the core temperature of the patients to show the pattern of the inflammation in joints.

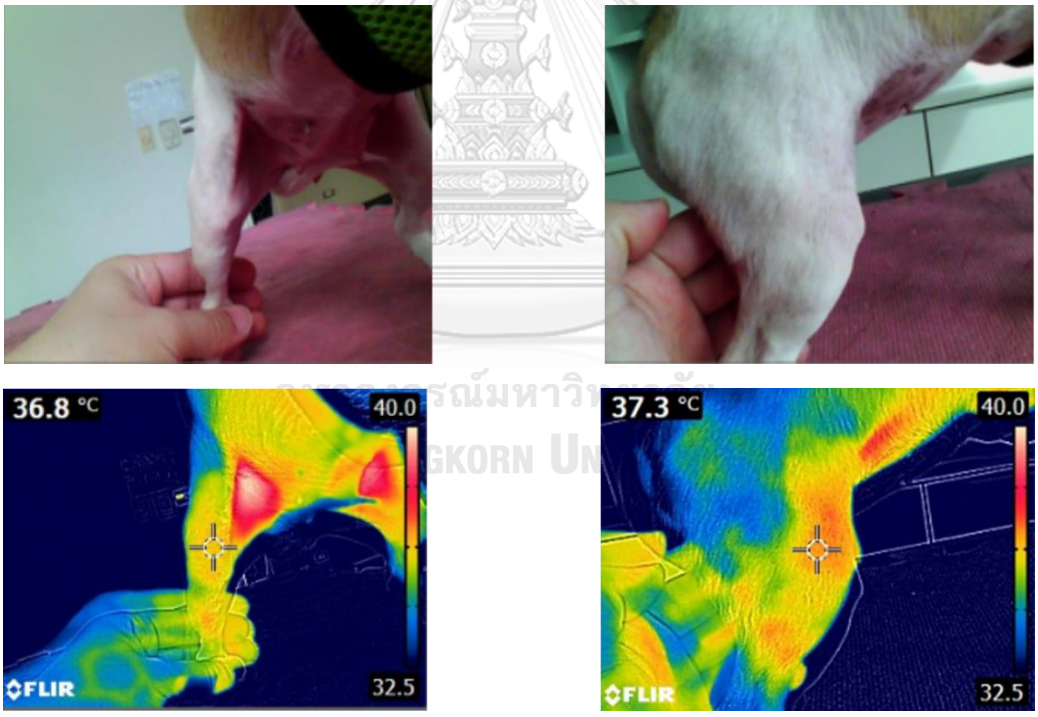


**Figure 1** showed the normal imaging and thermal imaging using thermal camera

**d. Statistical analysis**

The data were presented in inferential statistic of mean and standard deviation on each group. The parameter of the temperature from thermographic image between before and after treatment was calculated by Paired-t test. The parameter of lameness score and temperature between group were analyzed by using Wilcoxon matched paired test. All statistics analysis will be performed using SPSS statistic version 22. *P-value* <0.05 was considered as statistically significant.

# CHAPTER IV

## RESULTS

52 dogs were enrolled in this study. They were classified into 2 groups which were group A and group B. both groups consisted of 26 dogs in each group. There were 15 males and 11 female dogs in group A, 13 male and 13 female dogs in group B. The mean ± SD of age was 7.08 ± 3.316 in group A, and 6.69 ± 3.108 in group B. Mean ± SD of ages and gender among all groups were not significant difference shown in the table 4.

**Table 3** Mean ± SD of age and gender of dogs on group A and group B

| Data | | Group A (N=26) | Group B (N=26) | P-value |
|---|---|---|---|---|
| Age (year) | | 7.08 ± 3.316 | 6.69 ± 3.108 | 0.68 |
| Gender (N) | Male (N) | 15 | 13 | 0.4 |
| | Female (N) | 11 | 13 | |



**Table 4** Mean ± SD of body condition score of dogs on group A and group B

| Data | Group A (N=26) | Group B (N=26) | P-value |
|---|---|---|---|
| Body condition score | 3.84 ± 0.88 | 3.65 ± 1.12 | 0.5 |

**Table 5** show neutral status of dogs in both group

| | Group A (N=26) | | Group B (N=26) | | P-value |
|---|---|---|---|---|---|
| | Male | Female | Male | Female | |
| Neutral (N) | 8 | 6 | 8 | 7 | 0.66 |
| Non-Neutral (N) | 7 | 5 | 5 | 6 | |

**4.1 lameness score**

Lameness score was evaluated at the first every visit for 5 weeks by 3 veterinarians. Lameness score was observed and evaluated for all dogs. Most of the lameness score were improved when compared by raw data when treated with nutraceutical A. Wilcoxon matched pairs test was calculated for pre-treatment and post-treatment. There were significant different in group A in pre- and post-treatment ($P<0.05$). there were not significant differences in group B ($P=0.5$).



**Table 6** showed lameness score of Group A and B

| | Group A | | | Group B | | |
|---|---|---|---|---|---|---|
| | Pre | Post | P-value | Pre | Post | P-value |
| Sample | 2.92 ± 0.55 | 1.73 ± 0.46 | 0.01 | 3.06 ± 0.5 | 2.97 ± 0.57 | 0.5 |

**4.2 Thermographic examination**

In thermographic imaging, they were done in 2 views, cranial and lateral view. In cranial view the marker was place between patellar ligament and tibia. In lateral view the marker was place between patellar, tibia, and femur. All image was done in

the same thermal camera setting. The core temperature was measuring every time, and not different more than ± 0.3 °F from the first visit. Thermographic was shown in the figure below of pre-treatment and post-treatment in each group.

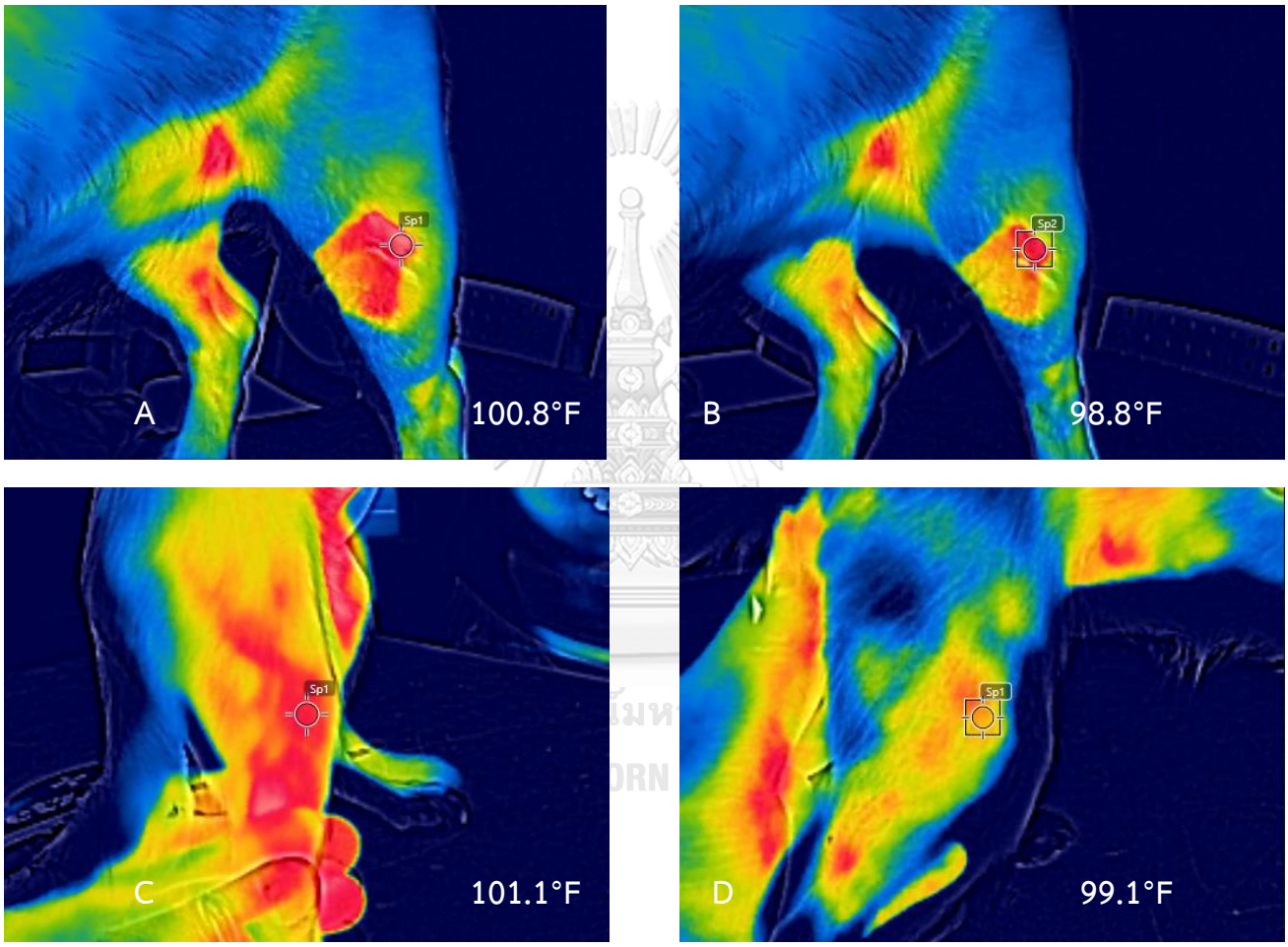


**Figure 2** showed lateral view in group A, A and C showed before treatment of nutraceutical A. B and D showed after 4 weeks of treatment by nutraceutical A. the marker in this picture showed the area of temperature that was measure.

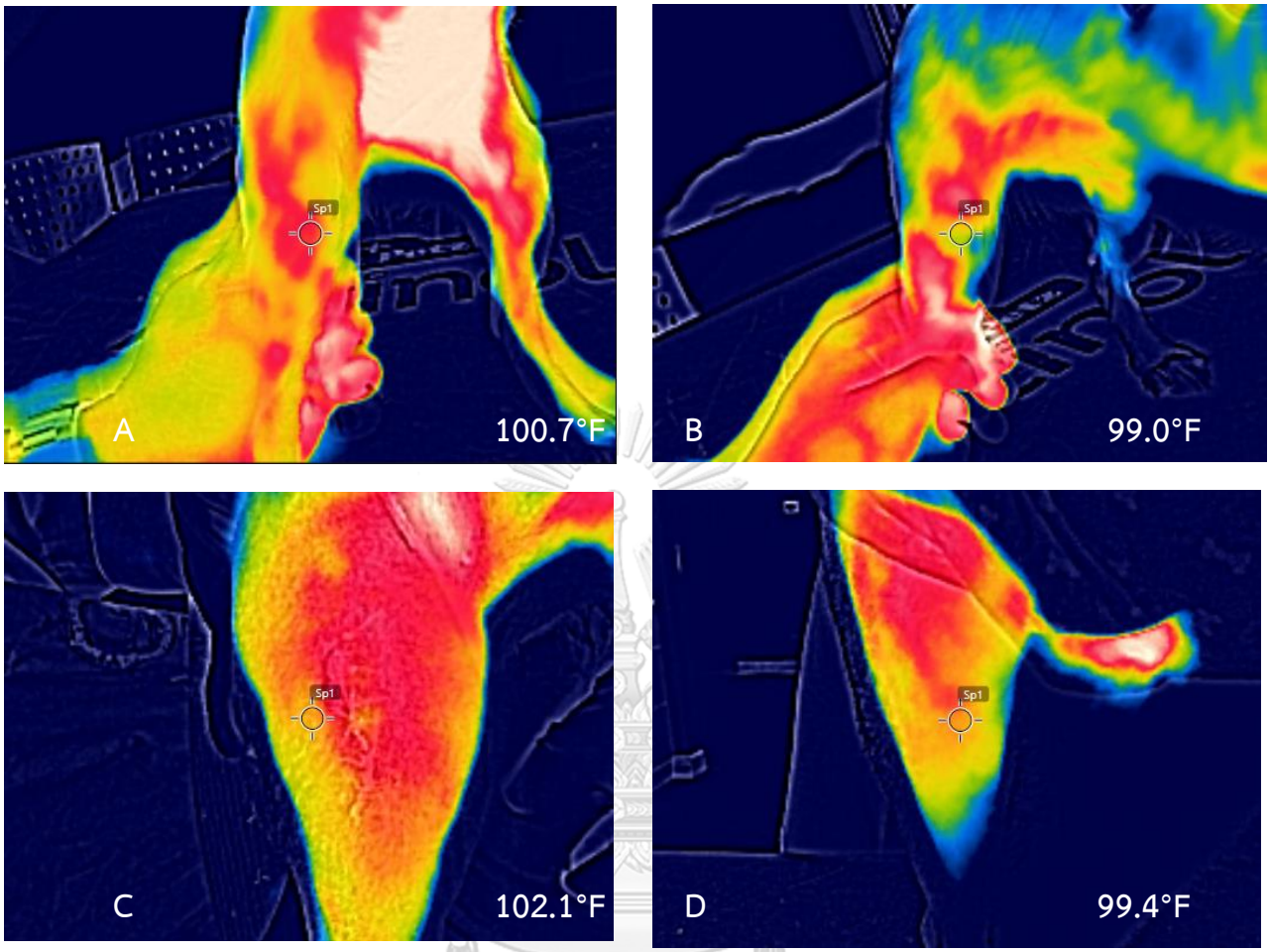


**Figure 3** showed cranial view in group A. A and C showed before treatment of nutraceutical A, B and D showed after 4 weeks of treatment by nutraceutical A. The marker was place in the measurement area.

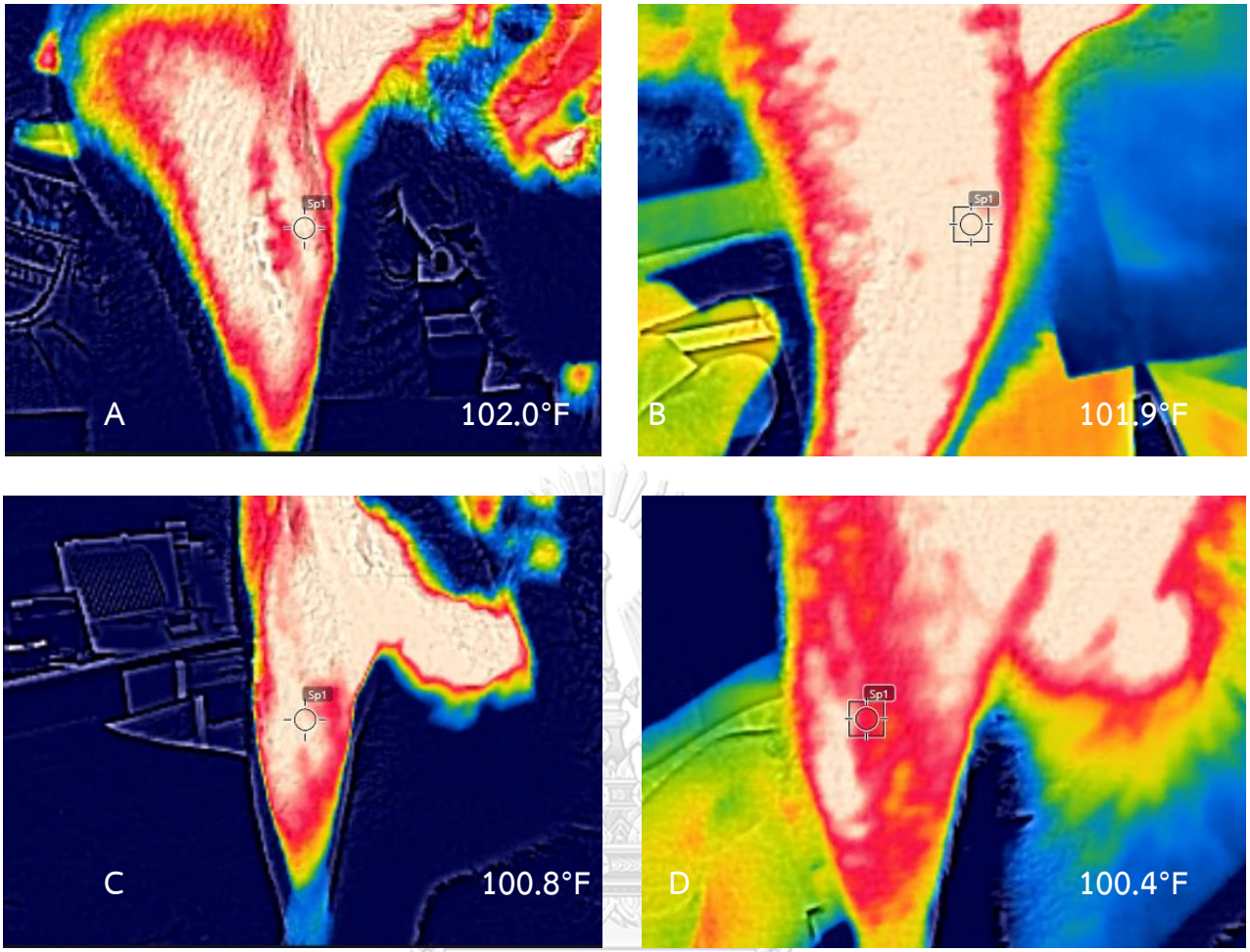


**Figure 4** showed cranial and lateral view in group B. A and B showed before and after treatment with nutraceutical B in the lateral view. C and D showed before and after treatment with nutraceutical B in the cranial view.



Evaluation of temperature in both views of thermographic images was obtained in pre-treatment and post-treatment in both groups. The temperature focuses in our statistical analysis was the different temperature between stifle temperature and core temperature. The temperature was shown in the minus value because the core temperature was higher than stifle temperature. In group A pre- and post-treatment was statistically significant in the P-value of 0.01. While P-value

of group B was 0.1 which considered not statistically significant as shown. There was statistically significant between group A and B in the P-value of 0.05

**Table 7** showed different between averaged area temperature and averaged core temperature of both group in weeks

| Week | Group A (°F) | Group B (°F) |
|---|---|---|
| 1 | -0.94 | -1.085 |
| 2 | -1.69 | -1.07 |
| 3 | -2.28 | -1.02 |
| 4 | -2.87 | -1.02 |
| 5 | -3.505 | -1.05 |

In order to fulfill the study, the unknown sample secret was revealed after finishing the study, the final blind samples were informed that the sample A was NZGLM, and the sample B was placebo.



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# CHAPTER V

# DISCUSSION

Canine osteoarthritis is a common orthopedics disease in all dogs. It can occur in any breed, age, and gender. When they have abnormality of the joints. Then osteoarthritis was begun. The recommendation for canine osteoarthritis is both medical and surgical treatment to correct the main problem. dogs include in this study are delaying in the surgical treatment, and the owner want to treat with only by medical. In theoretically, nutraceutical have potential in reduce pain and inflammation, so it can use to treat with other medicine before the surgical treatment to increase the anti-inflammatory effect when combined with other medicine and reduce the side effect from taking medicine in the long term. Therefore, nutraceutical become the treatment of choice for improve the quality of life and reduce inflammation and pain from osteoarthritis.

In this study, age, gender, breed, and body condition score among group were no significant different, most breed are small breed which Pomeranian dogs have the most number around 38% of all dogs in this study, follow by Chihuahua that was 27%. There is 44% that have OA in the right stifle joint and 56% that have OA in the left stifle joint. The age of the study is age range 1-12 years (mean is 6.88 years). This result can be said that dogs at the ages around 7 years and more are likely to encounter with OA just as the study of Comerford shown that the mean age of small breed dog was 7.4 years

The body condition score of group A was 3.84 ±0.88, in group B was 3.65 ± 1.12. From this result, the body condition score from both groups have not statistically significant. Most of the dogs have body condition score more than 3. The previous study showed that dogs with body condition score more than 3 were likely

to encounter with cranial cruciate disease just as the study of Lampman et al. (2003) shown that the peak prevalence was more than 3 in body condition score.

The result of lameness score was found statistically significant in group A. Most of the data from group A shown improvement, in other word the score in group B was found not have statistically significant. The lameness score of group A was reduce according to the temperature of the joint. The clinical signs of group A were improved and the inflammation was reduced. The owner comment that their dogs have a better clinical sign at week 3 of the study.

The neutral status result was not significantly different in each group. The previous study show that neutral status can be the one of the factor that affect the body condition score (Kluess et al., 2021). The neutral dogs have a higher chance to have a body condition score higher than normal due to many factors such as decrease activity, decrease in hormonal activity, and increase appetite (Dorn and Seath, 2018). From this result the neutral status was the one important factor of the OA progression.

The normal temperature of the normal stifle dog is 95.3 $\pm$ 2.11 from the measurement of normal dogs in hospital with the same condition of the patients in this study. The temperature of the osteoarthritis patients in 100.3 $\pm$ 1.43. the temperature of the osteoarthritis patients is higher than normal group due to increase blood flow from inflammation process



The thermographic image resulted in significantly reduced temperature in group A while there was no significant result in group B. In group A, there was also a trend in reducing lameness score. For those dogs in group A with the same lameness score might be the effect of highly potential on reducing inflammation and pain, so this group have a higher chance that dogs can use their limbs better than group B. Once the inflammation was occurred it goes on repeatedly in the vicious cycle. The

pathological changed of the stifle joints are the result of osteoarthritis. The lesion of osteoarthritis is irreversible and have no treatment to return to the normal joint.

The temperature of group A was reduced in week 3 of treatment around 76%, and week 2 of treatment around 24%. The temperature was reduced to the maximum effect of nutraceutical at week 4 of treatment around 53%, and week 5 of treatment around 47%. The effective dose was 10 kilograms per tablet was the dose we choose in this study (should showed in the results in every week) or shown in diagram

The thermal camera used in this study can measure the temperature of the limb accurately by using the program FLIR tools 2.0. The measure method that was done by program can measure multiple spots at the same time and can convert the temperature to the Fahrenheit. The distances between target and thermal camera were one of the factors that effect the measure. Too far from the target will make the measurement different from the real temperature because of the measurement area in the camera was fix area, if the target was smaller than measurement area, the thermal camera will measure all area including the background. The temperature would be the mean of that area. if the target was too close, the measurement will happen in the target but only the normal camera cannot focus the image. Thermal camera can use to measure the temperature of multiple joint but in clinical field the temperature can be vary due to the temperature of the environment, the condition of hair coat, and the core temperature of the patient. Thermographic imaging can be used to detect the temperature if the patient has the same condition in every visit to properly measure the target temperature.



The comment from the owner in this study, 65% was told that the tablet size was too big for the small breed. 40% of the owners have to force feed their pets.

6 dogs from group B have a worsen clinical sign at week 4 of the study. Those dogs had a surgical treatment and NSAIDs was given. The temperature after given the

NSAIDs was reduced statistically because of the NSAIDs. So, the temperature after the surgery treatment and NSAIDs should be performed the further study to evaluate the result. All the patients were given surgery treatment after finishing the study.

From this study, the dogs with patellar disease more likely have combine with cranial cruciate disease without detection of the veterinary. The problem of this cranial cruciate disease was undetected by normal radiographic but can detected by orthopedic examination. The small breed dogs may improve in clinical sign due to natural thickening of the joint capsule from inflammatory process.

# Appendix

**Appendix 1** Classification of nine-integer body condition score scale system modified from Laflamme (1997) and Burkholder (2000)

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

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|---|---|
| **NAME** | Supakit Vilaisuntornkiat |
| **DATE OF BIRTH** | 20 August 1993 |
| **PLACE OF BIRTH** | Bangkok |
| **HOME ADDRESS** | 865 Senavilla84 village, Soi 25, Happy land road, Klongjan, Bangkapi, Bangkok 10240 |

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