A blinded, randomized, placebo-controlled prospective study of the impact of the effect of photobiomodulation therapy in dogs with cranial cruciate ligament rupture after TPLO surgery.

by

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D.V.M., University of Guadalajara, Mexico 2011

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Clinical Sciences
Veterinary Medicine College

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2023

Approved by:

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Abstract

Objective: Effect of photobiomodulation therapy (PBMT) in patients with CCLR after TPLO surgery by measuring C-reactive protein, percentage weight bearing, lameness using a short form of a composite measure pain scale, evaluated by the clinician and owners, and surgical site infection.

Sample population: 54 client-owned dogs with CCLR undergoing unilateral TPLO surgery were enrolled in this study between April 5, 2021 – April 10, 2022**.**

Methods: The study population was randomly assigned to either a treatment group receiving PMBT (24 dogs) or control group (30 dogs). PMBT was performed on the treatment group immediately after induction, and 6 hours, 24 hours, 48 hours and 8 weeks post-operatively. The control group received sham PMBT (device turned off) at the same time. Evaluation of CRP, CMPS-SF, evidence of SSI, and %WB were evaluated for all dogs 24 hours pre-operatively, and then 24 hours, 48 hours, and 8 weeks post-operatively. Owners completed CMPS-SF and subjective evaluations weekly for 8 weeks post-operatively.

Results: No statistically significant differences were found between treatment groups when evaluating CRP, %WB and CMPS-SF by clinician and weekly evaluation of the CMPS-SF by owners. Although no statistically significant differences were found on patients developing surgical site infections between treatment groups, SSI were only observed in patients in the control group (5/30, 16.6%). Most were minor/superficial infections (4/30 13.3%), and a single dog (1/30, 3.3%) had a major/deep surgical site infection**.**

Clinical relevance: Although with promising but not statistically significant differences between groups, surgical site infections may be reduced after PBMT application.

List of abbreviations

TPLO – Tibial plateau leveling osteotomy

NSAID – Non-steroidal anti-inflammatory drug

PBMT – Photobiomodulation therapy

LASER – Light amplification by stimulated emission of radiation

CCLR – Cranial cruciate ligament rupture

SF-CMPS – Short form Glasgow composite measure pain scale

SSI – Surgical site infection

CRP - C reactive protein

ATP – Adenosine Triphosphate

cAMP – Cyclic adenosine monophosphate

MAPKs – mitogen activated protein kinases

FGF_2 – Fibroblastic growth factor 2

EFG – Epidermal growth factor

NFG – Nerve growth factor

FOXOs – Forkhead box Os

AP-1 – Activator protein - 1

Table of Contents

List of Tables

No table of figures entries found.

Acknowledgements

I would like to acknowledge my co-authors and Master Committee: Walter Renberg for the support and mentorship during this project and Natalia Chernichero for her contributions to the statistics of this project.

Also, to Christina Marquez and Grace Calo who helped me to perform all the after surgery PBMT.

A great thanks to the surgery nurses Deborah Cobb, Tiffany Copeland, Tonia Gilliam, Natalie Uranga that were collaborating along with this project.

Thanks to Kris Richardson for assistance in coordinating the clinical trial.

The study was supported by an institutional grant from the Department of Clinical Sciences of the College of Veterinary Medicine, Kansas State University.

Dedication

This thesis is dedicated to my Fiancé and future wife Erica Chavez-Peon Berle for the support, motivation, and encouragement every day during the development of this project.

Her motivational words after a long journey of working in the hospital and after the evaluation of the patients of this project. Those motivational words were sparks of amazing energy to keep working.

Also, to all my Parents Oscar and Hilda for the education and effort that they put on me during all my personal life and professional career.

To my brother Esau and my sister Xochitl for making me laugh every time that my soul needed to breathe after a long journey of work.

Finally, to my mentors and friends Walter Renberg and Emily Klocke for always believing in me without hesitation and yelling at me when I needed to be focused during this project.

Chapter 1 - Photobiomodulation Therapy

The principles of the laser date to 1916 when Albert Einstein first described the theory of stimulated emission, but it was not until 1958 that Charles Townes and Arthur Schalow wrote a paper on the proof of concept for creating one.[81] The first working laser was created by Theodore Mainman at Hughes Aircraft Research Laboratory in Malibu, California in 1960.[8]

The word LASER is an acronym for Light Amplification by Stimulated Emission of Radiation. A laser generates light through a process of optical amplification based the stimulated emission of electromagnetic radiation.[4]

The process of light emissions begins with activation of electrons, generally from either helium-neon (HeNe), gallium arsenide (GaAs), or gallium-aluminum-arsenide (GaA1As) to an excited state.[82]

A laser is created when light or energy is transmitted to an optical material like glass, crystal, or gas. The light, or another source of energy excites the electrons to move from low energy to a high energy around the nucleus of the atoms.[6]

Inside a Laser, there are commonly two mirrors forming the laser chamber that bounce the photon back towards the electrons, cloning, amplifying and aligning them in the same direction and speed to emit coherent energy.[6]

Light from a therapeutic laser must be monochromatic, having the same energy, wavelength and a single color, in contrast to sunlight or white light that may be broken in different wavelengths.[82] Placing the two mirrors of the laser in the same plane to emit the laser light in the same direction with a minimal divergence will produce a collimated and coherent laser light.[6]

These last 2 properties allow the light to be focused precisely on small areas of the body and, at a

therapeutic dose, these properties allow PBMT to penetrate the surface of the skin with no heating effect, no damage to the skin, and few or no side effects[82]

To understand the movement of the laser beam it is useful to think about any visible light like a lightbulb. The light of the bulb light moves in different wavelengths and in different directions meaning the bulb light is incoherent.

Laser light may be reflected, scattered, transmitted or absorbed by the tissues. Reflected photons have no clinical effect and may be dangerous to tissues such as the eyes. In order to reduce the reflection, the laser beam should be directed as close to perpendicular to the skin as possible. In addition, when photons pass through the tissues, some will be scattered, thus reducing the amount of energy delivered to the tissue.[82] Longer wavelengths will penetrate deeper tissues reducing scattering of the beam. Transmitted photons pass completely through the tissues without being absorbed. This is rarely a problem in rehabilitation because tissues are thick enough to complete transmission. Finally, the beam can be absorbed by chromophores such as water, hemoglobin, melanin, amino acids, and proteins. The absorption of the wavelengths are measured in nanometers (nm) which determine the biologic effect of the tissues. [11] For example ultraviolet light (100-400nm) is absorbed primary by melanin, proteins, and nucleic acid; visible light (400-760nm) is scattered and absorbed by melanin, hemoglobin, and myoglobin; with near infrared light (760nm -1400nm), photons are mainly scattered but a great variety of chromophores absorb these photons. Wavelengths of >1400 are absorbed almost entirely by water; these wavelengths are found in surgical lasers. Therefore, the optimal window to have tissue penetration with less scattering and surface absorption is likely to be in the 600-1200nm range.[86]

In addition to wavelength, power is also a characteristic that is important to measure while using laser. Power is a measure of energy per unit time and is expressed in watts (W) or milliwatts (mW). For example 1 watt = 1 joule/second.[86]

The power density or intensity indicates the power per surface area. Larger spots result in more homogenous passage of laser light through the tissues with less photon scattering and light dispersion. Another measurement reported for therapeutic laser is the dosage, which isthe power emitted multiplied by the time of treatment. For example, a 50-mW laser delivers 1 J of energy in 20 seconds of treatment time. A 500 mW laser takes 2 seconds of treatment to deliver 1 J of energy. A 1-W laser takes only 1 second , and 10 watts laser takes 1/10 of a second. Therefore, from a treatment standpoint, a higher power laser delivers the same treatment in a shorter time than a lower power laser.[86]

There are four main classes of lasers as defined by the international Engineering Consortium (IEC) standard 60825 based on their power output, measured in milliwatts (mW), and the potential for ocular damage. The types of lasers are Class 1/1M -, Class 2/2M , Class 3A/3B, Class 4.[5]

Class 1 and 2 include everyday devices such as laser pointers, laser printers, grocery scanners and CD players. Their power level is very low: < 0.05mW and low < 1mW, respectively. The power level of Class 3 laser devices is low (<5mW for 3A lasers used as laser pointers, and low power PBMT devices) and medium for 3B(<500mW that the majority of the PBMT devices use). The class 4 laser devices are considered surgical lasers (hot lasers) which can cut over have a high power level of >500mW.

When lasers are used for PMBT, they are typically class 3B devices. The main characteristic of this type of laser is that its radiation is athermic, meaning that the biological effects are caused by

biostimulation rather than perceptible heat or cell damage, and they are limited to <500mW.[4] The light triggers the cells to stimulate biochemical changes.[5]

PBMT has several synonyms such as cold laser therapy, low intensity laser therapy, and low level laser therapy. In 2014, at an international conference of the North American Association for Light Therapy and the World Association for Laser Therapy, a consensus was reached that photobiomodulation therapy was a more accurate name for the action of transmitting light to the tissues and stimulating healing.[7]

PBMT is now defined as the application of light from 10mW to 500mW with a wavelength in the red to near infrared region of the spectrum (660nm to 905nm).[5]

Chapter 2 - Mechanisms of PBMT

The mechanism of PBMT is poorly understood. There are several theories focused on the modulation of the inflammatory process and direct stimulation of regeneration in a damaged cell to. [9,10] The optimal wavelength for penetration of light into the tissue is around 810nm.[10]

The wavelengths in this optimal window allow the light to be absorbed by cytochrome C oxidase on mitochondria, thus increasing the availability of electrons for the reduction of molecular oxygen in the catalytic center of cyclooxygenase (COX). This in turn increases the mitochondrial membrane potential and thus the levels of adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP) and reactive oxygen species (ROS).[83]

At the shorter wavelengths, 600-800nm, a significant quantity of photonic energy is absorbed by melanin, hemoglobin, and oxyhemoglobin chromophores. Melanin has a very high absorption, so dark skin absorbs light to a greater extent, especially for wavelengths less than 830 nm. In contrast, wavelengths longer than 1300 are absorbed strongly by water and therefore penetration into tissues is decreased.[11]

PBMT devices should emit in the 800 – 1000nm range in order for the chromophores mentioned above to absorb the light energy.[10,11.] When there is an injury to tissue, nitric oxide (NO) produced by the mitochondria can inhibit respiration in the cell by binding to COX and displacing oxygen.[12] One of the targets of PBMT is the chromophore, cytochrome C (CcO), also known as Complex IV, which is the last enzyme in the electron transport chain.[13,14] It is theorized that once the laser light has targeted CcO in the injured cell, it will photo-dissociate the NO leading to increase activity of the electron transport chain, greater mitochondrial membrane potential, and increased adenosine triphosphate (ATP) production.[15]

ATP provides energy to the cell and regulates many biochemical pathways such as cyclic adenosine monophosphate (cAMP)- and ATP-driven carriers for ions such as Na^+/K^+ ATPase and calcium ion pumps. ATP also helps with the stimulation of intracellular pathways such as mitogen-activated protein kinases and can act in conjunction with growth factors such as fibroblastic growth factor (FGF) 2, epidermal growth factor, and nerve growth factor.[15,16]

In addition, PBMT of certain wavelengths (632.8nm, 812nm and 820nm) has shown to produce and modulate reactive oxygen species (ROS).[83,84] High concentrations of reactive oxygen species (ROS) produced by chronic inflammatory processes, toxic chemical exposure or environmental stresses can cause oxidative cell damage.[17,18] On the other hand, lower concentrations of ROS have been appreciated as an important signaling molecules. Finally, the stimulation and modulation of ROS have been shown to promote cell proliferation.[19,20.]

Cells respond to ROS in different ways resulting in production of scavenger antioxidants, protein modification and gene expression.[17] Some of those transcription factors are redox factor 1, dependent activator protein 1, nuclear factor Kappa, hypoxia inducible factor and HIF-like factor. Activation of those factors can lead to the synthesis of proteins induced in cell proliferation, tissue oxygenation, and cytokine modulation.[17]

Another effect of PBMT is on NO, which is a well-known mediator that helps with vascular homeostasis as a vasodilator and antithrombic factor, inducing disaggregation of pre-aggregated platelets by enhancing endogenous fibrinolysis.[21,22,23] NO synthase (eNOS) suppression with concomitant NO deficiency, results in vasoconstriction, inflammation, platelet activation, hypercoagulation, and cardiovascular diseases.[21]

In hypoxic cells or stressed cells, mitochondria generate NO, which binds to CcO and displaces oxygen.[24] The result of this binding leads to inhibited cellular respiration, reduced ATP

generation and increased oxidative stress.[25] This state activates various intracellular signaling pathways and transcription factors such as redox factor-1, hypoxia-inducible factor (HIF)-1, and HIF-like factor 17, activator protein-1, nuclear factor-kB, p53, activating transcription factor/cyclic adenosine monophosphate (cAMP)-response element-binding protein (ATF/CREB), inducing the downstream production of both inflammatory mediators like interleukins IL-1 and IL-6, tumor necrosis factor-alpha, COX-2, and prostaglandin E2 and anti-inflammatory mediators like Transforming Growth Factor-beta and IL-10.[25]

Evidence indicates that administering PBMT with appropriate parameters to stressed cells can dissociate NO from its competitive binding to CcO, increase ATP production, and restore the balance between pro and antioxidant mediators, reducing oxidative stress.[25]

PBMT has shown several benefits in studies in vivo and ex vivo in humans and animal cells. However, there is still controversy about its clinical use. Some authors believe that this controversy stems from the lack of standardized treatment protocols.[27,28]

Variations in respond to PBMT have been reported based on dose, with too low of a laser dose inducing no response and too much potentially being toxic.[27] In 2020, Hochman et al, examined the effects of laser power, wavelength, coat color, and coat length on tissue penetration using PBMT in healthy dogs, concluding that hair length and coat color affected tissue penetration with longer and darker coats having less penetration.[27] Also, another study by Piao and colleagues in 2019 showed that PBMT light could penetrate the spinal cord of a cadaver after hemilaminectomy using a multichannel spinal probe, concluding that being in contact with skin increased transmission 67% compared with the cadaver that did not have contact.[29]

[8] Endre Mester became the first to explore the healing effects of lasers while working at Semmelweis University in Budapest, Hungary, by discovering that applying laser to the back of

a shaved mice can induce faster growth of hair than in the untreated mice.[1] After that discovery, photobiomodulation therapy (PBMT) became a topic of research to treat skin wounds.[2,3]

The most common uses of PBMT in veterinary medicine in dogs, cats, and horses are to treat postoperative pain, chronic pain due to osteoarthrosis,[34] enhance wound healing and to treat neurological disorders.[30,31,32,33,35,36,37,38,39] Proposed mechanisms to treat the different conditions mentioned above have been discussed by Hochman et al in a review of the use of PBMT. PBMT acts through four different processes to treat pain: Transduction, transmission, modulation, and perception. During transduction, mechanical and chemical heat stimulus can activate pain depolarizing A-delta (A^{δ}) and C fibers. PBMT can decrease bradykinins and kinin receptor activity, proinflammatory interleukin-1, and proinflammatory prostaglandins and can increase NO, thereby causing vasodilatation and removal of inflammatory mediators.[9,40,41,42,43,44,] During transmission, the depolarization of C fibers will be blocked and will increase the nerve cell action potential.[45,46] During modulation, the signal of pain is inhibited to the dorsal horn prior to ascent to the brain, producing beta-endorphins and decreasing acetylcholine leading to to decreased discharge of excitatory neurons. Finally, during perception, PBMT can increase levels of serotonin for mood augmentation.[86]

PBMT has been studied during wound healing in laboratory studies on mice, but other studies have shown negative effects in dogs and horses. [2,30,31,49,50] The mechanisms behind the enhancement of wound healing are postulated to be regeneration of the injured cell by increasing ATP and modulation of ROS and NO. This leads to the activation of several growth factors such as platelet derived growth factor, FGF, vascular endothelial growth factor, keratinocyte growth

factor and transforming growth factor, thus accelerating fibroblast proliferation and re-epithelization of the wounds.[51,52]

Neurological disorders such as intervertebral disk disease and spinal fractures will cause primary and secondary injury in the spinal cord by decreasing blood supply thus leading to alterations of local ion concentrations, production of free radicals and release of cytotoxic neurotransmitters, resulting in necrosis and apoptosis of the spinal cord tissue.[53,54,55] PBMT is also reported to be beneficial for peripheral nerve regeneration, increasing axonal growth and myelinization, reducing degeneration of corresponding motor neurons and increasing Schwann cell proliferation.[48,56]

Chapter 3 - PBMT in Veterinary Medicine

PBMT has been used in *in vitro* studies to enhance angiogenesis, leading to a faster wound healing process.[2]

Wardlaw et, al 2019, during the evaluation of wound healing, based on digital photographs, using a scar scale 0 – 5 of the skin incisions, within a post operative period of 24 hours and 3,5,7 and 21 days in twelve Dachshunds that underwent thoracolumbar hemilaminectomies for intervertebral disk disease (IVDD). The authors reported that all scar scores significantly improved with increasing time from surgery (<0.001). Good agreement was achieved for inter-rater reliability ($p = 0.9$). Laser therapy increased the scar scale score, leading to improved cosmetic healing by day seven, and continued to show an increase on day 21 compared to control dogs ($p < 0.001$). The scar scale was developed based on the first three dogs after surgery with (0) being a fresh surgical incision, (1) fresh incision but no hemorrhage present, (2) incision with some scabbing, (3) skin remodeling but resolving bruising or inflammation, (4) healing progressing but visible scar present and (5) healed incision with epithelization, contraction and hair regrowth. The authors concluded that application of laser therapy for seven days at 8J/cm2 hastened wound healing and improved the cosmetic appearance in 4 dogs compared to the other 5 dogs who were in the control group.[76]

In contrast, Gammel et al, in 2018, evaluated primarily closed incisions and full thickness open wounds in 10 dogs and reported no difference between groups for subjective assessment of healing time and wound measurements ($P = .7$). There was no difference in histopathologic assessment except that the control group had more necrosis and perivascular lymphocytes and macrophages at day 7 ($P = .03$). The treatment group had more perivascular lymphocytes and macrophages at day 14 ($P = .01$). The closed incisions were bilateral for flank ovariectomy

procedures, and open wounds were created bilaterally with a punch biopsy. Each side of the dog (incision and open wound) was randomly assigned to the treatment group or the control group. The treatment group received PMBT once daily for 5 days with a 980-nm laser and a total energy density of 5 J/cm^2. The control group received a sham treatment (laser turned off) for an identical amount of time each day. The wounds were assessed visually, measured, and photographed at postoperative days 3, 7, 11, and 14; and biopsied on postoperative days 7 and 14. A 2-way repeated measures multivariate analysis of variance was used to analyze differences between groups.[77]

There are 3 studies in veterinary medicine evaluating the effect of PBMT in dogs with CCLR that underwent TPLO surgery. Rogatko et al 2017, used a single pre-operative treatment dose of 3J/cm^2 (6 watts,800-970nm dual wavelength, continuous and pulsed) vs a sham group in 27 dogs. Evaluation of lameness score, response to manipulation, force plate evaluation, and radiographic healing found increased weight bearing of treated dogs on force plate at 8 weeks after TPLO surgery compared to the sham group. The limitations of this study include that variables such as age, diet, and activity level were not controlled. An additional limitation was the small sample size that may have led to type 2 error.

In 2018, Renwick et al used 95 dogs in three post-operative treatments in a four-course design using several dosages from 660nm, 800nm, 905nm and 970 nm, with 10 phases of different pulse frequency vs ‘placebo’ red light at 660nm. During the study they assessed the Liverpool Osteoarthritis in Dogs , adjusted Canine Orthopedic Index (ACOI), radiographic healing index of osteotomy, time to cessation of NSAID administration, and wound healing. The only significant

result was found in the evaluation of gait section of the ACOI, where the treated group had better scores compared to the control group. Limitations were not mentioned in this study.

A 3rd study from Kennedy et al 2018, using 12 dogs evaluated dosages of PBMT at 2.25 J/cm2 during hospital treatment and 1.5 J/cm2 during at-home treatments (class 2 laser 635 nm) vs. control group treated with the same laser units, with the 5-mW diodes replaced with red LED light-bulbs. The study found better ground reaction forces during recovery for the control group vs PBMT group.

Even though these studies are randomized controlled studies, the variability of the dosages and frequency of PBMT given prevent a definitive conclusion on the effect of PBMT. Lack of established protocol of PBMT for dogs and small sample size were the major limitations of these studies.

Chapter 4 - Variables to measure

In our study we evaluated the following objective measurements: CRP using IDEXX Catalyst® CRP Test (IDEXX Laboratories, Westbrook, Maine, USA), percent of weight bearing on a PetSafe Stance Analyzer (LiteCure LLC, Companion Animal Health 38" L x 24" W x 1.75" H), incidence of surgical site infections based on the CDC guideline. Subjective measurements were also using CMPS-SF.

CRP

CRP is the major positive protein of the acute phase proteins (APPs) in dogs. This species can show up to an increase of 10-1000-fold increase reaching a peak 24-48h after the insult. It is the most sensitive APP in dogs.[57] Its molecular weight is approximately 100kDa. It is mostly produced after proinflammatory stimulation by cytokines such as IL-1, IL-6, and a tumor necrosis factor -α (TNF-α).[58]

CRP has been monitored after surgery in dogs. In one study, CRP levels were not changed after ovariohysterectomy (OHE).[59] In a second study, the authors compared the concentration of CRP levels preoperative and at different times post-operative in dogs that went under vasectomy, OHE laparotomy or OHE laparoscopy. The vasectomy group had the lowest post-operative CRP scores, followed by the laparoscopy group. The laparotomy group had the higher CRP scores confirming that CRP can be used as a quantitative marker of systemic inflammation and can help differentiate the degree of the inflammatory process in the body after surgery. Overall, the CRP concentration started to drop 24 hours after surgery.[60] Another study evaluated amyloid A and CRP after TPLO for early detection of SSI. Löfqvist K et al, 2018 found that patients who maintained high levels of CRP on day 6 post operatively were more likely to have an SSI.[65] There are no reports to the investigator's knowledge of the effect of PBMT on CRP in patients

after TPLO surgery. There is one report of PBMT in human patients with pneumonia due to COVID-19 where values of CRP were slightly lower than the control group but there was not statistically significant difference.[61]

Percentage of weight bearing

The current study also measured percentage of weight bearing using a PetSafe Stance Analyzer (LiteCure LLC, Companion Animal Health) both pre-operatively and post-operatively.

Since the late 1970s, force plates have been used to evaluate lameness at walk, trot or jumping. These force plates were operating using strain gauges or piezolectric sensors[66.67,68] In dogs this methodology has been used to measure ground reactions forces (GRF) before and after stabilization of the stifle and to compare affected limbs and normal hind limbs after surgery as well another applications.[63,64] Static weight distribution is defined as the percentage of weight distributed to each limb during standing. In dogs, 30% is distributed to each thoracic limb and 20% to each pelvic limb.[70] In a study measuring static percentage weight distribution on a weight distribution force platform the highest sensitivity and specificity to detect lameness and orthopedic disease was a cut off 2 points below the normal value.[69]

These weight distribution force plates are comprised of four individual plates that measure each leg independently. Patients must be in a standing position with their head forward and each limb must be on an individual plate, as the percentage of total weight will be calculated for each plate. Weight distribution platforms have previously been verified as an accurate and repeatable measure through comparison with static standing on a pressure-sensitive walkway.[70]

Short form of the Glasgow composite measure pain scale (CMPS-SF)

The third variable measured in this study is a modified short form of the Glasgow composite measure pain scale (CMPS-SF).[71]

Pain is an unpleasant multi-dimensional experience with sensory and emotional components, which, by its nature, is not directly measurable in animals as they are unable to self- report. Some simple tools used frequently include simple descriptive scales, numerical rating scales and visual analog scales.[72] These tools are associated with high levels of inter-observer variation and their unidimensional nature may not adequately capture complex constructs like pain.[71,72] CMPS-SF was developed for routine clinical use and is comprised of six behavioral changes with associated descriptors: vocalization, attention to wound, mobility, response to touch, demeanor and posture/activity.[71] It has been validated for the assessment of acute post -operative pain and the score is linked to an intervention level, which guides the requirement for additional analgesia.[71]

The CMPS-SF was evaluated in hospital by the clinician pre-operatively, 24-, and 48 hours post-operatively and weekly by the owner for 8 weeks. On the same sheet of paper as the pain scale, two questions were added to identify potential surgical site infection. The first question asked the owners if they noticed any changes to the surgical site such as redness, discharge (sero-sanguinous or purulent), if the site was warm to the touch, or more painful to touch compared to the previous days. If the response to this question was yes to one of those changes, the owners were asked to come for a recheck or to go to their closest veterinarian as soon as possible for assessment and to receive antibiotics if needed. The second question asked was what type of medication the patients were receiving at that moment to confirm any use of antibiotics.

Surgical site infections after TPLO surgery

TPLO surgery is one of the most common surgeries in dogs in veterinary medicine and has a high risk of SSI (reported as 2.9%-25.9%)[73] compared to other sterile elective orthopedic procedures.[74]

It seems to be related to thermal bone necrosis, increased surgery time, inexperienced surgeons, obesity, or the use of non-locking plates in patients >50kg,[79,80]

Several strategies have been implemented to decrease the prevalence of SSI such as antibiotic impregnated suture, extended postoperative antimicrobials, and implementation of preventative intraoperative and postoperative protocols without showing significant positive results. The additional cost of SSI can be 50% of the price of the original surgery and, in some cases, double the original cost.[75]

In addition, the increase of multidrug resistant bacteria is a therapeutic challenge in both veterinary and human medicine. The most common bacteria isolated from these surgical sites is S*taphylococcus intermedius,* with 20% - 40% of this bacterial isolate showing multidrug resistance.[75] For humans, the Centers for Disease Control and Prevention guidelines for an implant associated infection recommend the use of long-term of antibiotics, implant removal and surgical debridement if possible.[76]

Chapter 5 - Conclusions and future research

PBMT is a noninvasive therapy that has been used as an adjunctive treatment to treat pathologies in veterinary medicine such as pain after trauma, tendon, or peripheral nerve injuries, and to enhance wound healing. PBMT is becoming more popular, and it has been used in veterinary medicine for a few years, however standard protocols to treat different pathologies are lacking in the literature.[35,46,50]

As mentioned before, studies in humans and animals are still controversial. Specifically, studies in dogs after TPLO surgery are inconclusive as to the benefits of PBMT. One of the studies cited above showed positive results while measuring GRF at 8 weeks, and another found improved scores during gait evaluation using the Canine Orthopedic Index. In contrast, a 3rd study found better GRF in the control group than the treatment group.[35,36,37]

Dosages, length, and number of treatments varied between those studies also compared to the study presented here. In the present study, a CTX SmartCoat™ type IV laser (Companion Animal Health, New Castle, Delaware) was utilized for all treatments (Power 0.5 to 15 watts, Modes CW or pulsed, wavelengths 980/810nm). A profile for each patient was entered based on species, body weight, area to treat, coat color and length, skin color and condition to treat. The laser software then generated a protocol of power and duration of application. The options entered for each patient in the treatment group were as similar as possible for each patient. The recommendations of the manufacturer were followed due to ease of repeatability.

The number of sessions were based on an effort to precondition tissues before surgery and to modulate the inflammation phase in tissue after surgery.[35]

The variable factored into the software species, body condition score, condition treated, area treated, coat color and coat length (because the animals were shaved, “light” and “short” were always selected).

The elaboration of future studies in dogs with larger populations and repeatability will be helpful to conclude a definitive answer of the use of PBMT before and after TPLO surgery.

Even though TPLO is one of the most common procedures in dogs, it carries a higher risk of SSI. Finding a positive treatment effect of PBMT could benefit many animals and decrease owners, cost, and the use of antibiotics.

In addition, a negative effect would be difficult to find because PBMT lacks side effects in dogs.

References

1.- Mester E, Szende B, Gärtner P. Die Wirkung der Lasstrahlen auf den Haarwuchs der Maus [The effect of laser beams on the growth of hair in mice]. Radiobiol Radiother (Berl). 1968;9(5):621-6. German. PMID: 5732466.

2.- Mester E, Nagylucskay S, Döklen A, Tisza S. Laser stimulation of wound healing. Acta Chir Acad Sci Hung. 1976;17(1):49-55. PMID: 970061.

3.- Mester E, Spiry T, Szende B, Tota JG. Effect of laser rays on wound healing. Am J Surg. 1971 Oct;122(4):532-5. doi: 10.1016/0002-9610(71)90482-x. PMID: 5098661.

4.- Schindl A, Schindl M, Pernerstorfer-Schön H, Schindl L. Low-intensity laser therapy: a review. J Investig Med. 2000 Sep;48(5):312-26. PMID: 10979236.

5.- Cotler HB, Chow RT, Hamblin MR, Carroll J. The Use of Low Level Laser Therapy (LLLT) For Musculoskeletal Pain. MOJ Orthop Rheumatol. 2015;2(5):00068. doi: 10.15406/mojor.2015.02.00068. Epub 2015 Jun 9. PMID: 26858986; PMCID: PMC4743666.

6.- Bachmann, L., & ZEZELL, D. M. (2006). Laser physics and laser tissue interactions.

7.- Anders JJ, Lanzafame RJ, Arany PR. Low-level light/laser therapy versus photobiomodulation therapy. Photomed Laser Surg. 2015 Apr;33(4):183-4. doi: 10.1089/pho.2015.9848. PMID: 25844681; PMCID: PMC4390214.

8.- Riegel RJ. The history of laser therapy. In: Laser therapy in veterinary medicine. John Wiley & Sons, Inc; 1017. P. 29-35.

9. Hamblin, MR.; Demidova-Rice, TN. Cellular chromophores and signaling in LLLT. In: Hamblin, MR., et al., editors. Mechanisms for Low-Light Therapy II. The International Society for Optical Engineering; Bellingham, Washington, USA: 2007.

10. Hamblin, MR.; Demidova, TN. Mechanisms of low level light therapy - an introduction. In: Hamblin, MR., et al., editors. Mechanisms for Low-Light Therapy I. Vol. 61001. The International Society for Optical Engineering; Bellingham, Washington, USA: 2006. p. 1-12

11.-Wong-Riley MT, Liang HL, Eells JT, Chance B, Henry MM, et al. Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase. J Biol Chem. 2005; 280(6):4761–4771. [PubMed: 15557336]

12.- Brown GC. Nitric oxide regulates mitochondrial respiration and cell functions by inhibiting cytochrome oxidase. FEBS Lett. 1995; 369(2–3):136–139. [PubMed: 7649245]

13.-Anders JJ, Ketz AK, Wu X. Basic principles of photobiomodulation and its effects at the cellular, tissue, and system levels. In: Laser therapy in veterinary medicine. John Wiley & Sons, Inc; 2017. p. 36–51.

14.- Hamblin MR. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. AIMS Biophysics 2017;4(3):337–61.

15.- de Freitas LF, Hamblin MR. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. IEEE J Sel Top Quantum Electron. 2016 May-Jun;22(3):7000417. doi: 10.1109/JSTQE.2016.2561201. PMID: 28070154; PMCID: PMC5215870.

16.- Karu T. Mitochondrial mechanisms of photobiomodulation in context of new data about multiple roles of ATP. Photomed Laser Surg. 2010 Apr;28(2):159-60. doi: 10.1089/pho.2010.2789. PMID: 20374017.

17.- Chung H, Dai T, Sharma SK, Huang YY, Carroll JD, Hamblin MR. The nuts and bolts of low-level laser (light) therapy. Ann Biomed Eng. 2012 Feb;40(2):516-33. doi: 10.1007/s10439-011-0454-7. Epub 2011 Nov 2. PMID: 22045511; PMCID: PMC3288797.

18.- Farivar S, Malekshahabi T, Shiari R. Biological effects of low level laser therapy. J Lasers Med Sci. 2014 Spring;5(2):58-62. PMID: 25653800; PMCID: PMC4291815.

19.- Tafur J, Mills PJ. Low-intensity light therapy: exploring the role of redox mechanisms. Photomed Laser Surg. 2008 Aug;26(4):323-8. doi: 10.1089/pho.2007.2184. PMID: 18665762; PMCID: PMC2996814.

20.- Rhee SG. Redox signaling: hydrogen peroxide as intracellular messenger. Exp Mol Med. 1999 Jun 30;31(2):53-9. doi: 10.1038/emm.1999.9. PMID: 10410302.

21.- Kashiwagi S, Morita A, Yokomizo S, Ogawa E, Komai E, Huang PL, Bragin DE, Atochin DN. Photobiomodulation and nitric oxide signaling. Nitric Oxide. 2023 Jan 1;130:58-68. doi: 10.1016/j.niox.2022.11.005. Epub 2022 Nov 30. PMID: 36462596; PMCID: PMC9808891.

22.- D.N. Atochin, P.L. Huang, Role of endothelial nitric oxide in cerebrovascular regulation, Curr. Pharmaceut. Biotechnol. 12 (2011) 1334–1342.

23.-J. Loscalzo, Nitric oxide insufficiency, platelet activation, and arterial thrombosis, Circ. Res. 88 (2001) 756–762.

24.- G.C. Brown, C.E. Cooper, Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase, FEBS Lett. 356 (1994) 295–298.

25.- M.G. Mason, P. Nicholls, M.T. Wilson, C.E. Cooper, Nitric oxide inhibition of respiration involves both competitive (heme) and noncompetitive (copper) binding to cytochrome c oxidase, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 708–713.

26.- M.R. Hamblin, Mechanisms and mitochondrial redox signaling in photobiomodulation, Photochem. Photobiol. 94 (2018) 199–212.

27.- Hochman-Elam LN, Heidel RE, Shmalberg JW. Effects of laser power, wavelength, coat length, and coat color on tissue penetration using photobiomodulation in healthy dogs. Can J Vet Res. 2020 Apr;84(2):131-137. PMID: 32255908; PMCID: PMC7088515.

28.- Bunch J. Photobiomodulation (Therapeutic Lasers): An Update and Review of Current Literature. Vet Clin North Am Small Anim Pract. 2023 Jul;53(4):783-799. doi: 10.1016/j.cvsm.2023.02.010. Epub 2023 Mar 22. PMID: 36964028.

29.- Piao D, Sypniewski LA, Dugat D, Bailey C, Burba DJ, DeTaboada L. Transcutaneous transmission of photobiomodulation light to the spinal canal of dog as measured from cadaver dogs using a multi-channel intra-spinal probe. Lasers Med Sci. 2019 Oct;34(8):1645-1654. doi: 10.1007/s10103-019-02761-0. Epub 2019 Mar 16. PMID: 30879228.

30.- Wardlaw, J.L.; Gazzola, K.M.; Wagoner, A.; Brinkman, E.; Burt, J.; Butler, R.; Gunter, J.M.; Senter, L.H. Laser Therapy for Incision Healing in 9 Dogs. Front. Vet. Sci. 2019, 5, 349.

31.- Kurach, L.M.; Stanley, B.J.; Gazzola, K.M.; Fritz, M.C.; Steficek, B.A.; Hauptman, J.G.; Seymour, K.J. The Effect of Low-Level Laser Therapy on the Healing of Open Wounds in Dogs.

32.- de Lima, F.J.C., de Oliveira Neto, O.B., Barbosa, F.T. *et al.* Is there a protocol in experimental skin wounds in rats using low-level diode laser therapy (LLDLT) combining or not red and infrared wavelengths? Systematic review. *Lasers Med Sci* **31**, 779–787 (2016). https://doi.org/10.1007/s10103-016-1893-z

33.- Figurova M, Ledecky V, Karasova M, et al. Histological assessment of a combined low-level laser/light-emitting diode therapy (685 nm/470 nm) for sutured skin incisions in a porcine model: a short report. Photomed Laser Surg 34:53–55, 2016

34.- Looney, A.L.; Huntingford, J.L.; Blaeser, L.L.; Mann, S. A randomized blind placebo-controlled trial investigating the effects of photobiomodulation therapy (PBMT) on canine elbow osteoarthritis. Can. Vet. J. 2018, 59, 959–966.

35.- Rogatko, C.P.; Baltzer, W.I.; Tennant, R. Preoperative low level laser therapy in dogs undergoing tibial plateau levelling osteotomy: A blinded, prospective, randomized clinical trial. Vet. Comp. Orthop. Traumatol. 2017, 30, 46–53.

36.- Renwick, A.I.; Brodbelt, D.C.; Ferguson, J.; Abreu, H. Influence of class IV laser therapy on the outcomes of tibial plateau leveling osteotomy in dogs. Vet. Surg. 2018, 47, 507–515.

37.- . Kennedy, K.C.; Martinez, S.A.; Martinez, S.E.; Tucker, R.L.; Davies, N.M. Effects of low-level laser therapy on bone healing and signs of pain in dogs following tibial plateau leveling osteotomy. Am. J. Vet. Res. 2018, 79, 893–904.

38.- Bruno, E.; Canal, S.; Antonucci, M.; Bernardini, M.; Balducci, F.; Musella, V.; Mussoni, M.; Spinella, G. Perilesional photobiomodulation therapy and physical rehabilitation in post-operative recovery of dogs surgically treated for thoracolumbar disk extrusion. BMC Vet. Res. 2020, 16, 120.

39.- Bennaim, M.; Porato, M.; Jarleton, A.; Hamon, M.; Carroll, J.D.; Gommeren, K.; Balligand, M. Preliminary evaluation of the effects of photobiomodulation therapy and physical rehabilitation on early postoperative recovery of dogs undergoing hemilaminectomy for treatment of thoracolumbar intervertebral disk disease. Am. J. Vet. Res. 2017, 78, 195–206.

40.- Institute of Medicine (US) Committee on Pain, Disability, and Chronic Illness Behavior; Osterweis M, Kleinman A, Mechanic D, editors. Pain and Disability: Clinical, Behavioral, and Public Policy Perspectives. Washington (DC): National Academies Press (US); 1987. 7, The Anatomy and Physiology of Pain. Available from: https://www.ncbi.nlm.nih.gov/books/NBK219252/

41.- Jimbo K, Noda K, Suzuki K, et al. Suppressive effects of low-power laser irradiation on bradykinin evoked action potentials in cultured murine dorsal root ganglion cells. Neurosci Lett 240:93–96, 1998

42.- Bortone F, Santos HA, Albertini R, et al. Low level laser therapy modulates kinin receptors mRNA expression in the subplantar muscle of rat paw subjected to carrageenan-induced inflammation. Int Immunopharmacol 8:206–210, 2008

43.- Lopes-Martins RA, Albertini R, Martins PS, et al. Spontaneous effects of low-level laser therapy (650 nm) in acute inflammatory mouse pleurisy induced by carrageenan. Photomed Laser Surg 23:377–381, 2005

44.- Mizutani K, Musya Y, Wakae K, et al. A clinical study on serum prostaglandin E2 with low-level laser therapy. Photomed Laser Surg 22:537–539, 2004

45.- Wakabayashi H, Hamba M, Matsumoto K, et al. Effect of irradiation by semiconductor laser on responses evoked in trigeminal caudal neurons by tooth pulp stimulation. Lasers Surg Med 13:605–610, 1993

46.- Cambier D, Blom K, Witvrouw E, et al. The influence of low intensity infrared laser irradiation on conduction characteristics of peripheral nerve: a randomised, controlled, double blind study on the sural nerve. Lasers Med Sci 15:195–200, 2000

47.- 7. Labajos M. b-endorphin levels modification after GaAs and HeNe laser irradiation on the rabbit. Comparative study.. Investigacion clinica laser 1–2, 1988

48. Rochkind S, Shainberg A. Protective effect of laser phototherapy on acetylcholine receptors and creatine kinase activity in denervated muscle. Photomed Laser Surg 31:499–504, 2013

49.- Kaneps, A.J.; Hultgren, B.D.; Riebold, T.W.; Shires, G.M. Laser therapy in the horse: Histopathologic response. Am. J. Vet. Res. 1984, 45, 581–582.

50.- Petersen, S.L.; Botes, C.; Olivier, A.; Guthrie, A.J. The effect of low level laser therapy (LLLT) on wound healing in horses. Equine Vet. J. 1999, 31, 228–231.

51.- G al P, Vidinský B, Toporcer T, et al. Histological assessment of the effect of laser irradiation on skin wound healing in rats. Photomed Laser Therapy 24:480–488, 2006

52.- Stadler I, Lanzafame RJ, Evans R, et al. 830nm irradiation increases the wound tensile strength in a diabetic murine model. Lasers Surg Med 28:220–226, 2001

53.- Webb AA, Ngan S, Fowler JD. Spinal cord injury I: A synopsis of the basic science. Can Vet J. 2010 May;51(5):485-92. PMID: 20676289; PMCID: PMC2857426.

54.- Kwo S, Young W, Decrescito V. Spinal cord sodium, potassium, calcium, and water concentration changes in rats after graded contusion injury. J Neurotrauma. 1989 Summer;6(1):13-24. doi: 10.1089/neu.1989.6.13. PMID: 2754736.

55.- Juurlink BH, Paterson PG. Review of oxidative stress in brain and spinal cord injury: suggestions for pharmacological and nutritional management strategies. J Spinal Cord Med. 1998 Oct;21(4):309-34. doi: 10.1080/10790268.1998.11719540. PMID: 10096045.

56.- Hochman L. Photobiomodulation Therapy in Veterinary Medicine: A Review. Top Companion Anim Med. 2018 Sep;33(3):83-88. doi: 10.1053/j.tcam.2018.06.004. Epub 2018 Jul 5. PMID: 30243364.

57.- McClure, V.; van Schoor, M.; Thompson, P.N.; Kjelgaard-Hansen, M.; Goddard, A. Evaluation of the use of serum C-reactive protein concentration to predict outcome in puppies infected with canine parvovirus. J. Am. Vet. Med. Assoc. 2013, 243, 361–366.

58.- Paul, C.; Hansson, L.-O.; Seierstad, S.L.; Kriz, K. Canine C-Reactive Protein—A Clinical Guide, 1st ed.; LifeAssays AB: Lund, Sweden, 2011; p. 3.

59.- Ahn, S.; Bae, H.; Kim, J.; Kim, S.; Park, J.; Kim, S.K.; Jung, D.I.; Yu, D. Comparison of clinical and inflammatory parameters in dogs with pyometra before and after ovariohysterectomy. Can. J. Vet. Res. 2021, 85, 271–278.

60.- Kjelgaard-Hansen, M.; Strom, H.; Mikkelsen, L.F.; Eriksen, T.; Jensen, A.L.; Luntang-Jensen, M. Canine serum C-reactive protein as a quantitative marker of the inflammatory stimulus of aseptic elective soft tissue surgery. Vet. Clin. Pathol. 2013, 42, 342–345.

61.- Vetrici MA, Mokmeli S, Bohm AR, Monici M, Sigman SA. Evaluation of Adjunctive Photobiomodulation (PBMT) for COVID-19 Pneumonia via Clinical Status and Pulmonary Severity Indices in a Preliminary Trial. J Inflamm Res. 2021 Mar 19;14:965-979. doi: 10.2147/JIR.S301625. PMID: 33776469; PMCID: PMC7989376.

62.- Corr SA, McCorquodale CC, McGovern RE, et al: Evalu-ation of ground reaction forces produced by chickenswalking on a force plate. Am J Vet Res 64:76–82, 2003

63.- Budsberg SC, Verstraete MC, Soutas-Little RW, et al: Forceplate analyses before and after stabilization of canine stiflesfor cruciate injury. Am J Vet Res 49:1522–1524, 19883.

64.- McLaughlin RM Jr, Miller CW, Taves CL, et al: Force plateanalysis of triple pelvic osteotomy for the treatment of ca-nine hip dysplasia. Vet Surg 20:291–297, 1991

65.- Löfqvist K, Kjelgaard-Hansen M, Nielsen MBM. Usefulness of C-reactive protein and serum amyloid A in early detection of postoperative infectious complications to tibial plateau

leveling osteotomy in dogs. Acta Vet Scand. 2018 May 21;60(1):30. doi: 10.1186/s13028-018-0385-5. PMID: 29784055; PMCID: PMC5963089.

66.- Budsberg SC, Verstraete MC, Soutas-Little RW. Force plate analysis of the walking gait in healthy dogs. *Am J Vet Res*. 1987;48(6):915-918.

67.- Budsberg SC, Verstraete MC, Brown J, Reece L. Vertical loading rates in clinically normal dogs at a trot. *Am J Vet Res*. 1995;56(10):1275-1280.

68.- Yanoff SR, Hulse DA, Hogan HA. Measurements of vertical ground reaction force in jumping dogs. Vet Comp Orthop Traumatol 1992;5:44–50.

69.- Clough WT, Canapp SO Jr, Taboada L, Dycus DL, Leasure CS. Sensitivity and Specificity of a Weight Distribution Platform for the Detection of Objective Lameness and Orthopaedic Disease. *Vet Comp Orthop Traumatol*. 2018;31(6):391-395. doi:10.1055/s-0038-1667063

70.- Bosscher G, Tomas A, Roe SC, Marcellin-Little DJ, Lascelles BD. Repeatability and accuracy testing of a weight distribution platform and comparison to a pressure sensitive walkway to assess static weight distribution. *Vet Comp Orthop Traumatol*. 2017;30(2):160-164. doi:10.3415/VCOT-16-09-0128

71.- Testa B, Reid J, Scott ME, Murison PJ, Bell AM. The Short Form of the Glasgow Composite Measure Pain Scale in Post-operative Analgesia Studies in Dogs: A Scoping Review. Front Vet Sci. 2021 Sep 30;8:751949. doi: 10.3389/fvets.2021.751949. PMID: 34660773; PMCID: PMC8515184.

72.- Holton LL, Scott EM, Nolan AM, Reid J, Welsh E, Flaherty D. Comparison of three methods used for assessment of pain in dogs. J Am Vet Med Assoc. (1998) 212:61–6.

73.- Weese JS. A review of post-operative infections in veterinary orthopaedic surgery. *Vet Comp Orthop Traumatol*. 2008;21(2):99-105. doi:10.3415/vcot-07-11-0105

74.- Eugster S, DrMedVet PS, Gaschen FDR, Ecvim-Ca D, Boerlin P, A. prospective study of postoperative surgical site infections in dogs and cats. Vet Surg. (2005) 33:542–50. doi: 10.1111/j.1532-950X.2004.04076.x

75.- Budsberg SC, Torres BT, Sandberg GS. Efficacy of postoperative antibiotic use after tibial plateau leveling osteotomy in dogs: A systematic review. *Vet Surg*. 2021;50(4):729-739. doi:10.1111/vsu.13603

76.- Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant S. aureus infections in adults and children: executive summary. Clin Infect Dis Off Publ Infect Dis Soc Am. (2011) 52:285–92. doi: 10.1093/cid/ cir034

77.- Wardlaw JL, Gazzola KM, Wagoner A, Brinkman E, Burt J, Butler R, Gunter JM, Senter LH. Laser Therapy for Incision Healing in 9 Dogs. Front Vet Sci. 2019 Jan 29;5:349. doi: 10.3389/fvets.2018.00349. PMID: 30761311; PMCID: PMC6362418.

78.- . Gammel, J.E.; Biskup, J.J.; Drum, M.G.; Newkirk Kim Lux, C.N. Effects of low-level laser therapy on the healing of surgically closed incisions and surgically created open wounds in dogs. Vet. Surg. 2018, 47, 499–506.

79.- Fitzpatrick N, Solano MA. Predictive variables for complications after TPLO with stifle inspection by arthrotomy in 1,000 consecutive dogs: predictive variables for TPLO complications. Vet Surg. (2010) 39:460–74. doi: 10.1111/j.1532-950X.2010.00663.x

80.- Bergh MS, Rajala-Schultz P, Johnson KA. Risk factors for Tibial tuberosity fracture after Tibial Plateau Leveling Osteotomy in dogs. Vet Surg. (2008) 37:374– 82. doi: 10.1111/j.1532-950X.2008.0039

Chapter 6 - Photobiomodulation therapy in dogs undergoing TPLO after cranial cruciate ligament rupture shows promise but no statistically significant difference in a randomized trial.

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Keywords:

PBMT, TPLO, CRP, CCLR, LASER.

Abstract

Objective: Effect of photobiomodulation therapy (PBMT) in patients with CCLR after TPLO surgery by measuring C-reactive protein, percentage weight bearing, lameness using a short form of a composite measure pain scale, evaluated by the clinician and owners, and surgical site infection.

Sample population: 54 client-owned dogs with CCLR undergoing unilateral TPLO surgery were enrolled in this study between April 5, 2021 – April 10, 2022**.**

Methods: The study population was randomly assigned to either a treatment group receiving PMBT (24 dogs) or control group (30 dogs). PMBT was performed on the treatment group immediately after induction, and 6 hours, 24 hours, 48 hours and 8 weeks post-operatively. The control group received sham PMBT (device turned off) same time. Evaluation of CRP, CMPS-SF, evidence of SSI, and %WB were evaluated for all dogs 24 hours pre-operatively, and then 24 hours, 48 hours, and 8 weeks post-operatively. Owners completed CMPS-SF and subjective evaluations weekly for 8 weeks post-operatively.

Results: No statistically significant differences were found between treatment groups when evaluating CRP, %WB and CMPS-SF by clinician and weekly evaluation of the CMPS-SF by owners. Although no statistically significant differences were found on patients developing surgical site infections between treatment groups, SSI were only observed in patients in the control group (5/30, 16.6%). Most were minor/superficial infections (4/30 13.3%), and a single dog (1/30, 3.3%) had a major/deep surgical site infection**.**

Clinical relevance: Although with promising but not statistically significant differences between groups, surgical site infections may be reduced after PBMT application.

Introduction

Photobiomodulation therapy (PBMT), also known as low-level laser therapy, cold laser therapy, or low intensity laser therapy is increasingly popular in human and animal rehabilitation. It involves the use of a device designed to deliver photochemical rather than thermal energy.[1] PBMT has been used to address inflammation, edema, and chronic joint disorders[2], to promote healing of wounds[3], and to treat neurological disorders and pain[4]. The leading theory explaining the basic mechanism of PBMT in the tissues implicates the cytochrome C oxidase as the primary

photoreceptor. Once cytochrome C oxidase is stimulated by light, an electron transport is accelerated, leading to increased ATP production and thus hastened recovery of injured tissues.[5,6] Few studies have been done in veterinary medicine evaluating the influence of PBMT before or after surgery.[10,11,12,13] The results of the existing studies are controversial.

It is likely that some of the controversies are due to variability in the dosages used to treat the different conditions. The dose recommended to treat tissue in animals ranges from 3 to 10 J/cm^2. However, the dose used will depend on manufacturers' recommendation based on the therapeutic goal, skin color, coat color, and surface area.[7,8]

Additionally, PBMT has been used to control inflammatory processes and analgesia without definitive results. Measuring some serum proteins like C – reactive protein (CRP) in blood may help obtain more definitive results. C- reactive protein is an acute phase protein for inflammation; it increases six hours after tissue injury and eventually will decrease around 48 hours post-injury following a decrease ininflammation.[9]

Some studies have been conducted in dogs with cranial cruciate ligament rupture (CCLR) before and after tibial plateau leveling osteotomy (TPLO) surgery, to evaluate bone healing, percentage weight bearing (%WB), pain evaluation, and wound healing.[10,11]

TPLO surgery is one of the most common surgical techniques to correct CCLR in dogs. Despite good to excellent outcomes in most reports, some patients required prolonged time to recover from lameness or pain. Those patients have longer hospitalization, longer duration of pain medication, or require physical therapy to resolve lameness.

Based on human and veterinary studies, PBMT therapy has been used for preconditioning tissues before surgery to decrease inflammation and increase analgesia, vascularization, and tissue healing.[3,4,12] Rogatko et al. (2017) administered a single dose of PBMT to precondition the

tissues before TPLO surgery, showed an improvement in peak vertical force (PVF) at eight weeks compared to the control group.[13]

One of the most common complications after TPLO surgery is the high rate of surgical site infections (SSI; reported as 2.9%-25.9%).[14] The use of antibiotics after a TPLO surgery is still controversial since historical evidence does not support antibiotic use after a clean procedure.[15,16] On the other hand, some studies have found that postoperative antibiotics showed some protection against SSI.[17,18,19] The use of prophylactic antibiotics can lead to resistant strains of bacteria. To the authors' knowledge no studies have evaluated SSI in TPLO after PBMT.

The objective of the present study is to determine the effect of PBMT in adult, client-owned dogs of any breed or sex, with a diagnosis of cranial cruciate ligament rupture undergoing TPLO surgery, on measurement of C-reactive protein in blood (CRP), percentage of weight bearing (%WB), a short form of a composite Glasgow pain score[20] (CMPS-SF) in-hospital and in-home, , , and incidence of surgical site infections.

Our null hypothesis was that there would not be statistically significant differences post operative between groups in %WB, CRP, CMPS-SF by owner or clinician, and risk of SSI.

Materials and Methods

Study design: A blinded, randomized, placebo-controlled prospective study was conducted at the Veterinary Health Center, College of Veterinary Medicine, at Kansas State University from April 2021 to April 2022. All study procedures were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC #4500). Between the dates of April 2021 and April 2022, a consent form was provided to the dog owners and signed before enrolling their animals in the study. All patients had confirmed cranial cruciate ligament rupture

(CCLR) as determined by physical examination by the attending orthopedic surgeon or small animal surgery resident.

Eligibility: Inclusion criteria consisted of client-owned dogs of any breed or sex, older than one year of age, with diagnosis of CCLR, enrolled during their initial visit at the Veterinary Health Center. Standard lateral and craniocaudal radiographs were performed under sedation followed by TPLO surgery with any of the following variations: arthrotomy, arthroscopy, partial meniscectomy, meniscal release.

Exclusion criteria included results of a pre-operative complete blood count and serum chemistry demonstrating systemic illness or any other reason to avoid NSAID medication such as historical gastrointestinal signs or inability of the owners to give oral NSAIDs. Aggressive or anxious temperament that might interfere with stance analysis and subjective pain scoring, or neurologic disease confirmed by one of the clinical investigators were also grounds for exclusion.

Sample size determination:

Rogatko et al 2017,[13] using a single dose of PBMT preoperative, reported better peak vertical force (PVF) at 8 weeks; the mean static Percentage weight bearing (%WB) was 39.6% (± SD 4.7%) in patients that received laser treatment compared 28.9% ± 2.6% for patients in the sham group ($P < 0.01$). In another study evaluating PVF at 8 weeks after TPLO and PBMT, authors reported a mean of 52% ± 11% on the PBMT group and 48% ± 7% on the control group resulting in a not statistically significant difference.[10] Considering this data from the literature, and assuming a mean difference in static %WB of 5% between treatment groups (40% static %WB for the control and 45% static %WB for the treatment group),a standard deviation of 5 and 7% for the control and treatment groups, respectively, a power of 80%, and alpha of 0.05, the

number of patients needed per group was predicted to be 25, for a total of 50 patients.[10,13] Sample size was calculated using a two sample means test assuming unequal variances in standard software (Stata17.0 SE; StataCorp LLC).

Enrollment, treatment groups and outcome measurements: After enrollment, a randomization technique was performed using Microsoft Excel (2018) to assign patients to the treatment or control groups. For statistical analysis, the animal was deemed the experimental unit.

A Class IV, 980/810 nm gallium-aluminum-arsenide diode laser (CTX SmartCoat TM, Companion Animal Health, New Castle, Delaware) was used for all treatments.
A profile for each patient was entered into the software included with the device to note species, body weight, area to treat, coat color and length, skin color and condition to treat. The software then generated a protocol defining power and duration of application. Animals in the control group had a sham treatment with the device turned off but moved in a similar pattern for the calculated duration. Patients were treated after shaving their fur for surgery.
PBMT was performed on the medial and lateral aspect of the distal femur and proximal tibia using back and forth movements in contact with the skin from proximal to distal and distal to proximal in a constant movement for the predetermined time of the treatment in all patients assigned to the treatment group.. The laser energy was administered through a handpiece with a circular 4.7 cm diameter (2.21 cm2) spot size using a power setting of 10 W and passed in continuous motion over an area of 12.7 cm x 20.3 cm for 2.1 minutes delivering a total of 1300 J (5 J/cm2) to the medial and then repeated on the lateral aspect of the knee.

Evaluations of the patients occurred pre-operatively, at 24 and 48 hours post-operatively, and again at eight weeks post-operatively. The data was obtained by the same clinician who was blinded as to treatment group. The data was gathered as follows.

The IDEXX Catalyst® CRP Test (IDEXX Laboratories, Westbrook, Maine, USA) utilizing gold nanoparticles to measure antigen was used to measure C-reactive protein in blood. A lithium heparin container with 600-800 µL of whole blood was utilized in-house using a Catalyst one (IDEXX Laboratories, Westbrook, Maine, USA). A PetSafe Stance Analyzer (LiteCure LLC, Companion Animal Health) 38" L x 24" W x 1.75" H, was used to measure the percentage of weight bearing in all four limbs on all the patients.. Five measurements were collected at each time point and the highest and lowest value were eliminated to yield an average for the 3 remaining values.

A modified short form of a Composite Measure Pain Scale (CMPS-SF)[20] was used for all the patients . The same scale was used by one member of the owner's family, at home, weekly for 8 weeks. The forms evaluated by the owner were returned to the hospital at the 8-week recheck. Two questions were added to the CMPS-SF regarding the appearance of the surgical site, and which medications the patient was taking during the time of the evaluation. The owners were instructed that the same individual was to complete the evaluations during the next 8 weeks to minimize variability in measurements.

Surgical site infections (SSI) were evaluated based on the CDC guidelines.[21] The CDC defines SSI as present when there is purulent discharge from the surgical site or patients are showing clinical signs such as fever, localized pain, or tenderness. SSI are divided into superficial, where only skin and subcutaneous tissue are involved, and deep where fascia, muscle organ/space are involved.[21,22]. If the owners noticed discharge from the surgical site, they were asked to visit

their primary care veterinarian for a complete evaluation. When the veterinarian confirmed purulent material coming from the surgical site, antibiotics were prescribed.

Day 0 baseline evaluations

During intake, patients underwent a complete physical and orthopedic examination. A baseline CMPS- SF was performed by the primary author (OCZ), who was blinded as to treatment group, objective stance data was obtained, and blood was drawn from the jugular vein for a complete blood count, chemistry, and C-reactive protein. Radiographs were also obtained.

Day 1 (Day of surgery) evaluations

Patients were premedicated and then placed under general anesthesia. Anesthetic protocols were at the discretion of the boarded anesthesiologist in charge. A standard surgical preparation protocol for a TPLO surgery was performed. The first treatment of PBMT was done after clipping the surgical area. After PBMT was completed, patients received an ultrasound-guided femoral and sciatic nerve block with ropivacaine HCl 1mg/kg (Somerset Therapeutics, LLC, Hollywood, FL). A standard TPLO was performed either by a ACVS diplomate surgeon or supervised small animal surgery resident according to a previously described technique.[23] A partial meniscectomy was performed if a meniscal tear was found during medial parapatellar mini-arthrotomy; no meniscal releases were performed. For post-operative pain control, hydromorphone 0.08 mg/kg (hydromorphone HCL by Hykma Berkeley Heights, NJ) was administered subcutaneously every 4 hours for the first 24 hours along with a single dose of carprofen 2.2mg/kg SC once (Rymadil; Zoetis Kalamazoo,), After the first day, opioids and NSAID were switched to oral codeine 1.4 mg-2.0 mg/kg PO every 8 hours (West-Ward, Eatontwon, NJ), carprofen 2.2 mg/kg PO every 12 hours (Rymadil; Zoetis Kalamazoo,). Six

hours after surgery, a second PMBT was performed. The individuals performing the laser or placebo treatments did not participate in any evaluations of the study subjects..

Day 2 and 3 (24- and 48-hours post-operative) evaluations

Twenty four and 48 hours following surgery, the dogs received either PBMT or placebo treatment, C-reactive protein blood test, CMPS-SF, and stance analysis. The patients were discharged from the hospital 48 hours following the surgery. A modified short form of a composite measure pain scale (CMPS-SF) was given to the owners with the addition of two questions to evaluate the surgical site.

Eight-week evaluations

Owners of all the study patients were asked to return at 8 weeks after TPLO surgery for a recheck. This evaluation consisted of a complete physical and orthopedic examination, stance analysis, blood drawn for a CRP blood test, and a pain evaluation using the CMPS-SF, all performed by the original clinician (OCZ). Radiographs of the stifle were performed utilizing the same sedation as during the pre-operative study. After radiographs were finished, patients received either PBMT or placebo treatment and the study was deemed completed.

Masking: The investigator (OCZ) measuring and recording all outcome measurements as well as the statistician (NC) were blinded to treatment allocation status.

Statistical analysis: Descriptive statistics (mean, median, SD, range) for age, weight, and a frequency table for reproductive status were computed by treatment group.To confirm whether randomization balanced out signalment factors, age and weight were compared between treatment groups using a t-test, and reproductive status by treatment group using a Chi-square test.

Descriptive statistics or frequency tables were also computed for all outcome measurements (C reactive protein (CRP) values, % weight bearing, pain based on clinician, and based on owner's assessment, and surgical site infections) by treatment group and by time of measurement (pre-op, 24 hours, 48 hours and 8 weeks; for pain assessed by the owner time was categorized as follows: 1, 2, 3, 4, 5, 6, 7, and 8 weeks).

The effect of treatment group on CRP values was estimated in a linear mixed effects model which included fixed effects of treatment group (control vs treatment), time measurement (pre-op, 24 hours, 48 hours, 8 weeks) and the two-way interaction between treatment group and time of measurement. The dependent variable consisted of logarithmic-base 10 CRP values (CRP values were log-transformed to meet the normality assumption). A Newton-Raphson Optimization procedure and residual pseudolikelihood estimation were fitted. An unstructured covariance structure was included to account for repeated measures. A Tukey's *P* -value adjustment for multiple comparisons was implemented. Residual diagnostics were assessed graphically. Outcome values were back-transformed for reporting and interpretation (model-adjusted mean CRP values and standard error of the means (SEM) are depicted along with corresponding *P*-values).

The effect of treatment group on % weight bearing values was estimated in a generalized linear mixed effects model. Fixed effects of treatment group (control vs treatment), time measurement (pre-op, 24 hours, 48 hours, 8 weeks) and the interaction between treatment group and time were included. The outcome consisted of % weight bearing values divided by 100, to transform it into a continuous proportion. A beta distribution, logit link, Newton-Raphson Optimization procedure, residual pseudolikelihood estimation, and Kenward Roger degrees of freedom

adjustment were fitted. An unstructured covariance structure was included to account for repeated measures. A Tukey's *P*-value adjustment for multiple comparisons was implemented. The frequency (number and %) of surgical site infections by treatment group, and overall, was computed. An exact logistic regression was fitted to compare the proportion of surgical site infections by treatment group; odds ratio (OR) and corresponding *P*-value were reported.

Pain as evaluated by the clinician, and by the owner was recorded using the CMPS-SF. There are no reports on grading pain such as mild, moderate, or severe with this form. The pain score is the sum of the rank scores for the test's 6 categories; the maximum pain score possible is 24. The total CMPS-SF score has been shown to be a useful indicator of analgesic requirement and the recommended analgesic intervention level is 6/24; as such, pain scores were categorized into two categories; observations when patients had a pain score equal or lower than 6 (as in "analgesia not required"), and observations when patients had scores equal or higher than 7 ("analgesia is required"). Similarly, given all pain measurements as assessed by the clinician at 8 weeks were zeros, and all pain measurements as assessed by the owner after 4 weeks were zeros, for data analyses purposes, time measurements at 48 hours and 8 weeks were combined when evaluating pain recorded by the clinician, and observations from weeks 4 to 8 were combined when evaluating pain recorded by the owner.

The effect of treatment group on the probability of patients requiring analgesia (with pain assessed by the clinician) was estimated in a generalized linear mixed effects model. Fixed effects of treatment group (control vs treatment), time measurement (pre-op, 24 hours, 48 hours to 8 weeks) and the two-way interaction between treatment group and time of measurement were included. The outcome consisted of the probability of patients requiring analgesia (those with pain scores equal or greater than 7, as per clinician's assessment, compared to those with scores

less than 7 (not requiring analgesia)). A binary distribution, logit link, Newton-Raphson Optimization procedure, residual pseudolikelihood estimation, and Kenward Roger degrees of freedom adjustment were fitted. A first-order autoregressive (ar(1)) covariance structure was included to account for repeated measures. A Tukey's *P*-value adjustment for multiple comparisons was implemented.

Similarly, the effect of treatment group on the probability of patients requiring analgesia (with pain assessed by the owner) was estimated in a generalized linear mixed effects model. Fixed effects of treatment group (control vs treatment), time measurement (1 wk, 2 wks, 3 wks, and 4 to 8 weeks) and the two-way interaction between treatment group and time of measurement were included. The outcome consisted of the probability of patients requiring analgesia (those with pain scores equal or greater than 7, as per owner's assessment, compared to those with scores less than 7 (not requiring analgesia)). This model was fitted as described above.

An alpha level of 0.05 defines statistical significance.

Results

A total of 54 client-owned dogs completed the study. The overall mean (SD) for age was 5.54 (2.41) years old; age did not significantly ($P = 0.18$) vary by treatment group; the mean age was 6.03 years (2.54 years) for individuals in the treatment group and 5.14 years (2.27 years) for individuals in the control group. Overall mean body weight was 35.17 kg (9.86 kg). There were no statistically significant differences in body weight between treatment groups ($P = 0.22$); mean body weight was 33.33 kg (8.39 kg) for individuals in the treatment group and 36.64 kg (10.81 kg) for patients in the control group. Thirty-one patients were spayed females, two were intact females, and 21 were castrated males. There was a statistically significantly higher proportion of castrated males (54.2%, 95% CI = 34.2 – 71.1%) than females in the treatment group, compared

to the control group, where the proportion of castrated males was 26.7% (95 % CI = 10.8 – 42.5%) ($P = 0.04$). The treatment PBMT group consisted of 24 patients and the control group included 30 patients.

Percentage weight bearing and C-reactive protein

Descriptive statistics for CRP values and % weight bearing, overall, by treatment group, by time measurement, and by both treatment group and time measurement are presented in Table 1. Overall, the model-adjusted mean CRP values were considered normal pre-operatively on both treatment and control groups, with its highest value at 24 hours, followed by a decrease at 48 hours post-surgery. The treatment-by-time interaction was not statistically significant ($P = 0.21$), treatment group was not significant ($P = 0.13$), but CRP values significantly varied ($P < 0.001$) by time measurement. Specifically, mean CRP values were statistically significantly higher at 24 hours than pre-operatively ($P < 0.001$), and compared to 8 weeks ($P < 0.001$), and mean CRP values at 48 hours were statistically significantly higher than preoperatively ($P < 0.001$) and at 8 weeks ($P < 0.001$) (Table 2)

As observed with CRP values, for model-adjusted mean % weight bearing, the treatment-by-time interaction was not statistically significant ($P = 0.26$), treatment group was not significant ($P = 0.78$), but time measurement was significant ($P < 0.001$). Mean % weight bearing was significantly higher preoperatively than at 48 hours ($P = 0.04$), and mean % weight bearing at 8 weeks was statistically significantly higher than at 24 hours ($P < 0.001$), and at 48 hours ($P = 0.0001$) (Table 3).

Pain scale based on clinician and owners' evaluations.

Table 1 (supplemental) include a frequency table of proportion (and %) of patients where analgesia was required (pain scores $\geq$ 7) as per clinician's and owner's assessments, by both treatment group and time measurement.

When estimating model-adjusted mean percentage of patients when analgesia was required (for those patients with pain scores equal or greater than 7), as per the clinician's evaluation, the treatment-by-time interaction was not statistically significant ($P = 0.59$), treatment group was not statistically significant ($P = 0.97$), but time measurement was statistically significant ($P = 0.03$). Mean percentage of patients when analgesia was required was statistically significantly higher at 24 hours post-operatively than at 48 hours or 8 weeks post-operatively ($P = 0.04$; Table 4).

For model-adjusted mean percentage of patients when analgesia was required (for those patients with pain scores equal or greater than 7), as per the owner's evaluation, the treatment-by-time interaction was not statistically significant ($P = 0.99$), treatment group was not significant ($P = 0.41$) but time of measurement was statistically significant ($P < 0.001$). The mean percentage of patients when analgesia was required was statistically significantly higher at 1 week compared to 4 weeks or greater ($P = 0.001$), and at 2 weeks compared to 4 weeks or greater ($P = 0.01$) (Table 5).

Surgical site infections

There were 5 (5/30; 16.6%) patients with surgical site infections on the control group; four of those patients had minor/superficial site infections who responded to one round of antibiotics. One of the patients developed a major surgical site infection for which the TPLO plate had to be removed 6 months after the surgery. There were no infections reported on the treatment group at the moment of the study. The odds of patients experiencing surgical site infections in the control

group were 6.08 times greater than the odds of patients experiencing surgical site infections in the treatment group, however, this association was not statistically significant (OR (median unbiased estimate) = 6.08; 95% CI = 0.78 - +Inf; *P* = 0.09).

Discussion

In the present study, we failed to reject our null hypothesis and no statistically significant differences were found between treatment groups when evaluating gait analysis, C-reactive protein, or pain scores evaluated by the clinician or the owners. These measurements, however, significantly varied over time. Renwick et al. (2018),[11] when evaluating the influence of PBMT after TPLO surgery did not find differences between groups on osteotomy healing on a radiographic scale, time cessation of NSAID, and wound healing by owner questionnaires. They only found differences in terms of improvement in the gait section of the adjusted Canine orthopedic Index (COI).[11]

Results after PBMT are still controversial. Many of the published studies have been *in-vitro* or have been experimental, making it harder to extrapolate results into meaningful clinical outcomes.[5,6]

As mentioned on the introduction the dosages recommended for PBMT to treat tissue in animals ranges from 3 to 10 J/cm^2.[7,8] During this study we decided to follow the recommendations of the manufacturer entering the specifications for each patient such as body weight, skin color, coat color and the option short for the length of the fur. However, during the revision of the administered doses to the patients the author found that all the patients received the same dose of PBMT. The reason why the device decided to give the same dose for each patient remains unknown.

In the present study we rejected our null hypothesis while evaluating SSI between groups. 5 (16.6%) patients in the control group developed a surgical infection compared to no patients in the treatment group. Based on the CDC guidelines, four of those patients developed a superficial SSI infection where antibiotics were prescribed resolving clinical signs. One of those 5 patients presented a deep SSI where the plate had to be removed 6 months after TPLO surgery. Upon bacterial culture of the site and implant we were able to isolate *Staphylococcus Pseudointermedius*.

SSI were analyzed in this study due to a historically high rate after TPLO surgery in several reports. Surgical site infections rates after TPLO range from 2.9% up to 25.9%.[14.] Theories about the development of SSI after TPLO surgery include thermal bone necrosis, prolonged surgery, and anesthesia times, micromotion at the osteotomy site, and limited soft tissue coverage.[24,25]. If we analyzed those theories, we could see that in general the probable cause of SSI is the diminished or interruption of blood supply to the surgical site during the surgery or recovery time. PBMT is purported to help reduce healing times photoactivating cellular mechanisms, reducing edema, promoting fibroblast proliferation and collagen synthesis.[25,26,27] Therefore, it is possible that PBMT minimized the risk of SSI. Although no statistically significant (or borderline significant) differences were found on patients developing surgical site infections between treatment groups, surgical site infections were only observed in patients in the control group. Likely, significance was not achieved given the small effective sample size (n = 5); nonetheless this finding is promising from a clinical and prognostic standpoint and warrants further research.

No statistically significant changes were found when evaluating C-reactive protein (CRP) between treatment groups; however, CRP significantly varied over time. CRP is an acute phase

protein (APPs) that can be used as a marker of systemic inflammation. All the patients in the study had a normal preoperative CRP between 0 to 1.0 mg/dL. To report the influence of CRP in this study, preoperative values and postoperative values were taken. All the patients had a normal preoperative CRP indicating that none of the patients had a significant systemic inflammation that could affect the outcome of the surgery. The maximum value of CRP was at 24 hours after surgery. CRP values started to decrease at 48 hours post-surgery. This behavior was first reported by Löfgvist et al. (2018), where serum CRP was run in patients after TPLO and a maximum peak was recorded at 24 hours after TPLO to then start declining. Patients who maintained abnormal (higher) levels of CRP at 6 days post-surgery where consistent of having a surgical site infection.[9] On a study in humans by Zwiri MA et al.2022, no statistically significant differences were found on patients with temporomandibular disorder in the groups treated with PBMT vs traditional conservative treatment such as diet and stress counseling and a hot towel therapy.[27]

Freitas et al. 2001, when evaluating the effect of 830nm LASER light using CRP levels did not found any difference between treatment group and control groups after removing the lower wisdom tooth.[28]

Based on the knowledge of the author (OCZ) there are no reports of PBMT and the evaluation of CRP in Veterinary Medicine. Therefore, this may be the first study evaluating it, finding a lack of significance in patients which are treated with PBMT after TPLO surgery.

While measuring % of weight bearing Rogatko et al,[13] found that a single dose of preoperative PBMT was associated with a statistically significant improvement in PVF for dogs undergoing TPLO on the operated limb eight weeks postoperative. During the present study we did not find

statistically significant difference while evaluating %WB between groups at the different times. These results are similar than Kennedy et al, which showed not statistically significant differences between LLLT group and the control group on PVF at 8 weeks after TPLO surgery.[10] During evaluation of pain by the clinician and by the owners no statistically significant difference was found in our study similar at the same study from Kennedy et al, mentioned above during the evaluation of pain using a modified Glasgow composite scoring system by the clinician and the owners using the CBPI scale where no statistically significant difference was found between groups.

Study limitations include the variability in surgical outcomes arising from several surgeons performing the TPLO surgeries. Even though TPLO is a well-described and commonly performed surgery, there are some differences on the surgical technique inherent to each surgeon. Similarly, it is possible that misclassification of some of the outcomes occurred, however, because the clinician (as well as the owners) conducting all measurements was blinded to treatment allocation, bias would likely be non-differential. Schnidl et al. (2001), in a review of low intensity laser studies, noted that one of the most common limitations in the literature is the lack of double-blinded protocols.[29] In our study, not only the clinician but the statistician was completely blinded to minimize bias. Although this study involved only client-owned animals, we consider this study group is representative of adult client-owned dogs elsewhere, as it included dogs of different breeds, reproductive status, and ages (older than 1 year of age).

In conclusion, although not conclusive, PBMT may reduce surgical site infections after TPLO surgery. The use of PBMT is still controversial as this study showed there are no significant differences in outcomes between PBMT and control group after TPLO surgery. We reject our null hypothesis finding not statistically significant differences when evaluating %WB, CRP or

pain scale by owners and clinician. There is still a long path to show efficacy of PBMT on clinical outcomes.

Acknowledgments: Grace Calo and Christina Marquez for their support performing the post operative PBMT to the patients.

Disclosures:

No conflict of interest

Funding:

This project was funded by the department of Clinical Sciences at Kansas State University

References

1.- Kitchen, S.S. and Partridge, C.J. (1991). A review of low level laser therapy. Physiotherapy 77(3): 161-168.

2.- Bjordal JM, Couppé C, Chow RT, Tunér J, Ljunggren EA. A systematic review of low level laser therapy with location-specific doses for pain from chronic joint disorders. *Aust J Physiother*. 2003;49(2):107-116. doi:10.1016/s0004-9514(14)60127-6

3.- Posten W, Wrone DA, Dover JS, Arndt KA, Silapunt S, Alam M. Low-level laser therapy for wound healing: mechanism and efficacy. *Dermatol Surg*. 2005;31(3):334-340. doi:10.1111/j.1524-4725.2005.31086

4.- Chow RT, Johnson MI, Lopes-Martins RA, Bjordal JM. Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials [published correction appears in Lancet. 2010 Mar 13;375(9718):894]. *Lancet*. 2009;374(9705):1897-1908. doi:10.1016/S0140-6736(09)61522-1

5. Karu T, Pyatibrat L, Kalendo G. Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. *J Photochem Photobiol B*. 1995;27(3):219-223. doi:10.1016/1011-1344(94)07078-3

6. Pastore D, Di Martino C, Bosco G, Passarella S. Stimulation of ATP synthesis via oxidative phosphorylation in wheat mitochondria irradiated with helium-neon laser. *Biochem Mol Biol Int*. 1996;39(1):149-157. doi:10.1080/15216549600201151

7.- Hochman-Elam LN, Heidel RE, Shmalberg JW. Effects of laser power, wavelength, coat length, and coat color on tissue penetration using photobiomodulation in healthy dogs. *Can J Vet Res*. 2020;84(2):131-137.

8.- Chow R, Armati P, Laakso EL, Bjordal JM, Baxter GD. Inhibitory effects of laser irradiation on peripheral mammalian nerves and relevance to analgesic effects: a systematic review. *Photomed Laser Surg*. 2011;29(6):365-381. doi:10.1089/pho.2010.2928

9.- Löfqvist K, Kjelgaard-Hansen M, Nielsen MBM. Usefulness of C-reactive protein and serum amyloid A in early detection of postoperative infectious complications to tibial plateau leveling

444 osteotomy in dogs. *Acta Vet Scand*. 2018;60(1):30. Published 2018 May 21. doi:10.1186/s13028-
445 018-0385-5
446
447 10.- Kennedy KC, Martinez SA, Martinez SE, Tucker RL, Davies NM. Effects of low-level laser
448 therapy on bone healing and signs of pain in dogs following tibial plateau leveling
449 osteotomy. *Am J Vet Res*. 2018;79(8):893-904. doi:10.2460/ajvr.79.8.893
450
451 11.- Renwick SM, Renwick AI, Brodbelt DC, Ferguson J, Abreu H. Influence of class IV laser
452 therapy on the outcomes of tibial plateau leveling osteotomy in dogs. *Vet Surg*. 2018;47(4):507-
453 515. doi:10.1111/vsu.12794
454
455 12.- Agrawal T, Gupta GK, Rai V, Carroll JD, Hamblin MR. Pre-conditioning with low-level
456 laser (light) therapy: light before the storm. *Dose Response*. 2014;12(4):619-649. Published 2014
457 Sep 22. doi:10.2203/dose-response.14-032.Agrawal
458
459 13.- Rogatko CP, Baltzer WI, Tennant R. Preoperative low level laser therapy in dogs
460 undergoing tibial plateau levelling osteotomy: A blinded, prospective, randomized clinical
461 trial. *Vet Comp Orthop Traumatol*. 2017;30(1):46-53. doi:10.3415/VCOT-15-12-0198
462
463 14.- Weese JS. A review of post-operative infections in veterinary orthopaedic surgery. *Vet*
464 *Comp Orthop Traumatol*. 2008;21(2):99-105. doi:10.3415/vcot-07-11-0105
465

15.- Aiken MJ, Hughes TK, Abercromby RH, Holmes MA, Anderson AA. Prospective, Randomized Comparison of the Effect of Two Antimicrobial Regimes on Surgical Site Infection Rate in Dogs Undergoing Orthopedic Implant Surgery. *Vet Surg*. 2015;44(5):661-667. doi:10.1111/vsu.12327

16.- Brown G, Maddox T, Baglietto Siles MM. Client-assessed long-term outcome in dogs with surgical site infection following tibial plateau levelling osteotomy [published correction appears in Vet Rec. 2016 Nov 5;179(18):461]. *Vet Rec*. 2016;179(16):409. doi:10.1136/vr.103688

17.- Hagen CRM, Singh A, Weese JS, Marshall Q, Linden AZ, Gibson TWG. Contributing factors to surgical site infection after tibial plateau leveling osteotomy: A follow-up retrospective study. *Vet Surg*. 2020;49(5):930-939. doi:10.1111/vsu.13436

18.- Nazarali A, Singh A, Moens NM, et al. Association between methicillin-resistant Staphylococcus pseudintermedius carriage and the development of surgical site infections following tibial plateau leveling osteotomy in dogs. *J Am Vet Med Assoc*. 2015;247(8):909-916. doi:10.2460/javma.247.8.909

19.- Nazarali A, Singh A, Weese JS. Perioperative administration of antimicrobials during tibial plateau leveling osteotomy. *Vet Surg*. 2014;43(8):966-971. doi:10.1111/j.1532-950X.2014.12269.x

20.- Reid J, Nolan A, Hughes J, Lascelles D, Pawson P, Scott E. Development of the short-form Glasgow Composite Measure Pain Scale (CMPS-SF) and derivation of an analgesic intervention score. *Anim Welfare.* (2007) 8:97–104.

21.- Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. *Infect Control Hosp Epidemiol*. 1992;13(10):606-608

22.- Borchardt RA, Tzizik D. Update on surgical site infections: The new CDC guidelines. *JAAPA*. 2018;31(4):52-54. doi:10.1097/01.JAA.0000531052.82007.

23.- Slocum B, Slocum TD. Tibial plateau leveling osteotomy for repair of cranial cruciate ligament rupture in the canine. *Vet Clin North Am Small Anim Pract*. 1993;23(4):777-795. doi:10.1016/s0195-5616(93)50082-7

24.- Gallagher AD, Mertens WD. Implant removal rate from infection after tibial plateau leveling osteotomy in dogs. *Vet Surg*. 2012;41(6):705-711. doi:10.1111/j.1532-950X.2012.00971.x

25.- Solano MA, Danielski A, Kovach K, Fitzpatrick N, Farrell M. Locking plate and screw fixation after tibial plateau leveling osteotomy reduces postoperative infection rate in dogs over 50 kg. *Vet Surg*. 2015;44(1):59-64. doi:10.1111/j.1532-950X.2014.12212.x

26.- Simunovic Z, Ivankovich AD, Depolo A. Wound healing of animal and human body sport and traffic accident injuries using low-level laser therapy treatment: a randomized clinical study of seventy-four patients with control group. *J Clin Laser Med Surg*. 2000;18(2):67-73. doi:10.1089/clm.2000.18.67

27.- Zwiri AM, Ahmad WMAW, Asif JA, et al. A Randomized Controlled Trial Evaluating the Levels of the Biomarkers hs-CRP, IL-6, and IL-8 in Patients with Temporomandibular Disorder Treated with LLLT, Traditional Conservative Treatment, and a Combination of Both. *Int J Environ Res Public Health*. 2022;19(15):8987. Published 2022 Jul 23. doi:10.3390/ijerph19158987

28.- Freitas AC, Pinheiro AL, Miranda P, Thiers FA, Vieira AL. Assessment of anti-inflammatory effect of 830nm laser light using C-reactive protein levels. *Braz Dent J*. 2001;12(3):187-190.

29.- Schindl A, Schindl M, Pernerstorfer-Schön H, Schindl L. Low-intensity laser therapy: a review. *J Investig Med*. 2000;48(5):312-326.

Table 1 Descriptive statistics for CRP values and % weight bearing by both treatment group and time of measurement.

Variable, unit	Statistic	Treatment group by time measurement						
		Treatment	Control	Treatment	Control	Treatment	Control	Tr
		Preoperative		24 hours		48 hours		
CRP, mg/dL	Mean	0.48	0.29	7.03	7.30	6.75	6.60	
	Median	0.40	0.20	7.00	7.20	7.55	7.00	
	SD	0.34	1.14	1.75	1.67	1.95	2.05	
	Range	0.10-1.20	0.10-0.70	3.90-9.30	2.70-9.50	2.30-9.00	1.90-10.00	0.
Weight bearing, %	Mean	11.67	10.80	5.92	8.90	8.57	7.81	
	Median	12.00	10.50	3.50	9.50	7.00	8.00	
	SD	4.83	5.48	6.09	5.83	6.12	6.17	
	Range	0.00-20.00	2.00-22.00	0.00-23.00	0.00-22.00	0.00-21.00	1.00-26.00	1.0

Table 2 Model-adjusted means (+/- SEM) for CRP values by treatment group and time of measurement.

Treatment group	Time measurement				
	Preoperative	**24 hours**	**48 hours**	**8 weeks**	**Overall**
Treatment	0.38 (1.11)	6.80 (1.10)	6.40 (1.11)	0.27 (1.12)	1.45 (1.05)
Control	0.27 (1.10)	7.06 (1.10)	6.21 (1.10)	0.25 (1.11)	1.30 (1.05)
Overall	0.32 (10.7)[a]	6.93 (1.07)[b]	6.30 (1.07)[b]	0.26 (1.08)[a]	

Linear mixed model included fixed effects for treatment group ($P = 0.13$), time measurement ($P < 0.001$), and the treatment group-by-time measurement interaction ($P = 0.21$), and an unstructured covariance structure to account for repeated measures. Means with different superscripts indicate significant differences (comparisons between columns).

Table 3 Model-adjusted % weight bearing (+/- SEM) by treatment group and time of measurement.

Treatment group	Time measurement				
	Preoperative	**24 hours**	**48 hours**	**8 weeks**	**Overall**
Treatment	12.17 (1.34)	7.10 (1.13)	8.96 (1.20)	13.65 (1.41)	10.18 (0.65)
Control	10.80 (1.11)	9.89 (1.13)	7.82 (1.02)	7.82 (1.02)	11.62 (0.58)
Overall	$11.47\ (0.87)_{ac}$	$8.39\ (0.82)_{b}$	$8.37\ (0.78)_{ab}$	$13.83\ (0.97)_{c}$	

Generalized linear mixed model (beta distribution and logit link) included fixed effects for treatment group ($P = 0.78$), time ($P < 0.001$), and the treatment-by-time interaction ($P = 0.26$), and an unstructured covariance structure to account for repeated measures. Means with different superscripts indicate significant differences (comparisons between columns)

Table 4 Model adjusted mean percentage (+/- SEM) of patients when analgesia was required (pain scores > 7) as per clinician's assessment by treatment group and time measurement.

Treatment group	Time measurement			
	Preoperative	**24 hours**	**48 hours & 8 weeks**	**Overall**
Treatment	4.17 (4.14)	20.83 (8.41)	6.68 (3.72)	8.56 (3.39)
Control	10.00 (5.55)	16.67 (6.90)	3.79 (2.62)	8.73 (2.84)
Overall	6.50 (3.66)[ab]	18.66 (5.40)[a]	5.04 (2.24)[b]	

Generalized linear mixed model (binary distribution and logit link)included fixed effects for treatment group (P = 0.97), time (***P* = 0.03**) and the treatment-by-time interaction (P = 0.59), and an ar(1) covariance structure to account for repeated measures. Means with different superscripts indicate significant differences (comparisons between columns).

Table 5 Model adjusted mean percentage (+/- SEM) of patients when analgesia was required (pain scores > 7) as per owner's assessment by treatment group and time measurement.

Treatment group	Time measurement				
	1-wk	**2-wk**	**3-wk**	**> 4-wk**	**Overall**
Treatment	16.67 (8.89)	11.11 (7.50)	5.56 (5.46)	1.12 (1.13)	6.01 (2.49)
Control	26.92 (8.80)	16.00 (7.42)	8.00 (5.49)	1.61 (1.14)	9.09 (2.60)
Overall	21.35 (6.56)[a]	13.37 (5.43)[a]	6.68 (3.99)[ab]	1.35 (0.83)[b]	

Generalized linear mixed model included fixed effects for treatment group (P = 0.41), time (P < 0.001) and the treatment-by-time interaction (P = 0.99), and an ar(1) covariance structure to account for repeated measures.. Means with different superscripts indicate significant differences (comparisons between columns).