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Effect of Class IV Therapeutic LASER on Post-Surgical Wound Healing Process in *Canis familiaris* and *Felis catus*: Preliminary Study

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Simple Summary

It is vitally important that scientists can describe their work simply and concisely to the public, especially in an open-access on-line journal. The simple summary consists of no more than 200 words in one paragraph and contains a clear statement of the problem addressed, the post-surgical wound healing is a critical aspect of veterinary recovery. This study evaluated the use of Class IV laser therapy to improve healing in dogs and cats after surgery. Each animal acted as its own control, with half of the surgical wound treated with laser and the other half untreated. Over eight days, the laser-treated areas showed reduced inflammation, less fluid buildup, faster resolution of bruising, improved elasticity, and more vibrant skin colour. These effects indicate better tissue repair and vascularization. The treatment was effective across all ages, sexes, and species, suggesting that Class IV laser therapy can help animals recover faster and more comfortably after surgery. This non-invasive tool may offer significant value in everyday veterinary practice.

Abstract

Class IV laser therapy has emerged as a promising non-invasive approach to promote tissue repair, but its use in small animal post-surgical wound healing remains underexplored. This preliminary study evaluated the effects of Class IV laser therapy on the healing of surgical wounds in 49 dogs and cats. Each incision was split into two anatomical zones: one treated with laser (CL) and the other left untreated (SL). Wound healing was assessed at three timepoints (T0, T1, T2) using validated clinical parameters. Laser-treated areas exhibited significantly reduced skin thickness, improved vascular coloration, faster hematoma resolution, enhanced elasticity, and decreased fluid accumulation compared to untreated areas. These effects were consistent across species, sex, and age. Class IV laser therapy significantly accelerated the wound healing process in both dogs and cats, offering a valuable adjunct to standard post-surgical care. Further studies are needed to confirm these findings and explore long-term outcomes.

Keywords: Dog; Cat; Surgical Wound; Class IV Therapeutic LASER; Wound Healing

1. Introduction

The skin is the largest organ of mammals, accounting for approximately 15% of total body weight. It plays a fundamental role in maintaining homeostasis, protecting against external

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aggressors, thermoregulation, sensory perception, and immune defense [1,2]. Structurally, the skin is composed of three main layers—epidermis, dermis, and hypodermis—which provide a physical and biochemical barrier and support wound repair [3,4]. In veterinary medicine, wound healing is of relevance in post-surgical care, as it directly impacts patient recovery, infection risk, and overall prognosis [5].

Wound healing is a dynamic and tightly regulated biological process that unfolds in three overlapping phases: inflammation, proliferation, and remodelling [5,6]. Each stage is coordinated through interactions between inflammatory cells, fibroblasts, endothelial cells, cytokines, and extracellular matrix components [7,8]. Several factors influence the quality and speed of healing, including tissue oxygenation, vascularization, pH, comorbidities, and hormonal status [9–11]. Notably, recent studies have highlighted the importance of maintaining an acidic skin pH in preventing pathogen colonization and modulating enzyme activity essential for epidermal renewal [17].

Photobiomodulation therapy (PBMT), particularly using Class IV therapeutic LASERs, has emerged as a promising tool in regenerative medicine. Class IV LASERs emit high-power infrared light (typically 780–980 nm), which penetrates deep into tissue, stimulating mitochondrial cytochrome C oxidase and enhancing ATP production, cellular proliferation, collagen synthesis, and angiogenesis [5,12,13]. These effects have been shown to positively modulate all stages of the healing process: reducing inflammation and oxidative stress in the initial phase, promoting fibroblast and keratinocyte migration and proliferation in the proliferative phase, and contributing to organized extracellular matrix remodelling during the final maturation phase [6,14–16].

Despite the growing body of literature supporting PBMT in both human and veterinary applications, studies focusing specifically on its use in companion animals, especially cats, remain limited. Moreover, there is a lack of controlled intraindividual comparisons assessing the differential healing response within the same subject and surgical site [8–11].

Therefore, the present study aimed to evaluate the effect of Class IV therapeutic LASER application on surgical wound healing in *Canis familiaris* and *Felis catus*. Using a split-wound design, this preliminary study investigates intraindividual differences in healing parameters—such as suture thickness, skin colour, regional fluids, local temperature, and presence of hematoma—over an 8-day postoperative period. The findings contribute to the growing understanding of PBMT's clinical utility in veterinary wound management and may support the development of standardized protocols for its use in small animal practice.

2. Materials and Methods

2.1. Study Design and Animal Selection

This prospective, intra-individual, controlled study included 49 animals (n = 49), comprising 25 dogs (*Canis familiaris)* and 24 cats (*Felis catus*) of both sexes (27 females and 22 males) and various breeds. All animals were hospitalized at the "Centro de Medicina Veterinária Anjos de Assis" and underwent surgical procedures across multiple specialties. Each patient had a surgical incision evaluated, with one half of the wound treated with Class IV therapeutic laser (cranial section) and the other half left untreated (caudal section), serving as intra-subject control [8,9,25].

Inclusion criteria were clinical stability, absence of active oncologic disease (current or past), and the ability to complete an 8-day postoperative follow-up. Written informed consent was obtained from all animal owners. The study protocol was approved by the institutional ethics committee (Ref. 015/2022).

2.2. Laser Treatment Protocol

Laser therapy was performed using a Class IV diode laser system (Doctor Vet Therapy Laser, LAMBDA®) with a combination of wavelengths (660, 808, and 915 nm) in continuous wave (CW)

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mode. The cranial portion of the incision was irradiated based on the estimated treatment area, as follows:

- Post-op S: 5 cm² area, 25 seconds, 2 W output, total energy 50 J;
- Post-op M: 25 cm² area, 2 min 5 s, 2 W output, total energy 250 J;
- Post-op L: 50 cm² area, 4 min 10 s, 2 W output, total energy 500 J.

Frequencies used included CW, 1, 2, 10, and 25 kHz, with distinct purposes:

- 1 kHz: epithelialization;
- 2 kHz: fibroblast stimulation;
- 10 kHz: infection control;
- 25 kHz: antimicrobial effect.

Each area was irradiated with at least two passes of the laser beam to ensure homogeneous energy delivery.

2.3. Evaluation Parameters and Timeline

The surgical suture line of each patient was divided into two equal parts:

- LASER-treated zone (CL): cranial segment;
- Control zone (SL): caudal segment (no LASER application).

Three postoperative timepoints were used for evaluation:

- T0: immediately after surgery;
- T1: 48 hours post-surgery;
- T2: 8 days post-surgery.

Wound healing was assessed using a validated scoring system adapted from Vitor & Carreira (2015) [23], which included the following clinical parameters: temperature, skin colour, hematoma, fluid presence, and suture thickness. All evaluations were performed by the same investigator to minimize variability and ensure consistency.

2.4. Statistical Analysis

Data was recorded in Microsoft Excel and analysed using IBM SPSS Statistics version 29 (Windows). Descriptive statistics included means, standard deviations, and frequencies. Data normality was tested using the Shapiro–Wilk test and homogeneity of variance via Levene's test. Inferential statistics included:

- Repeated Measures ANOVA, to assess intra-group changes over time for normally distributed variables;
- Student's t-test for independent samples: used when assumptions of normality and homogeneity were met;
- Mann–Whitney U test, applied for non-normally distributed variables;
- Fisher's exact test, used for categorical associations with low frequency outcomes;
- Cochran's Q test for repeated binary outcomes across time.

Categorical variables were converted to binary (dummy) variables for statistical modelling. A p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Sample Characterization

The total sample consisted of 49 animals, of which 51% (n = 25) were *Canis familiaris* (dogs) and 49% (n = 24) were *Felis catus* (cats). Female animals represented 55.1% of the total sample. Most of the animals were neutered (67.3%), with higher prevalence in cats (83.3%) compared to dogs (52.0%). The mean age was 6.90 ± 4.30 years across all animals, 6.82 ± 4.64 years in dogs, and 7.13 ± 4.18 years in cats. Regarding body weight, dogs showed a higher average (17.50 ± 12.57 kg) than cats (4.27 ± 1.20

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kg). The most frequent body condition score, based on the 9-point LaFlamme scale, was 6 in both species. Full descriptive data is presented in Table 1.

Table 1. Characterization of the Total Sample (N = 49) and the two species groups considered in the study — Dog (*Canis familiaris*, n = 25) and Cat (*Felis catus*, n = 24) — regarding Age, Body Weight, Sex, Body Condition Score, and Reproductive Status.

Parameters	Total Sample (N = 49)	Dog (n = 25)	Cat (n = 24)
Age (years)	6.90 ± 4.3%	6.82 ± 4.6%	7.13 ± 4.2%
Body Weight (Kg)	12.00 ± 11.6%	17.50 ± 12.6%	4.27 ± 1.2%
Sex (Female)	27 (55.1%)	15 (60.0%)	12 (50.0%)
Sex (Male)	22 (44.9%)	10 (40.0%)	12 (50.0%)
Body Condition Score (1-9)	Score 1: 2 ± 4.7%	Score 1: 1 ± 4.5%	Score 1: 1 ± 4.8%
	Score 2: 4 ± 9.3%	Score 2: 2 ± 9.1%	Score 2: 2 ± 9.5%
	Score 3: 3 ± 7.0%	Score 3: 1 ± 4.5%	Score 3: 2 ± 9.5%
	Score 4: 7 ± 16.3%	Score 4: 5 ± 22.7%	Score 4: 2 ± 9.5%
	Score 5: 10 ± 23.3%	Score 5: 5 ± 22.7%	Score 5: 5 ± 23.8%
	Score 6: 11 ± 25.6%	Score 6: 6 ± 27.3%	Score 6: 5 ± 23.8%
	Score 7: 4 ± 9.3%	Score 7: 1 ± 4.5%	Score 6: 6 ± 27.3%
	Score 8: 2 ± 4.7%	Score 8: 1 ± 4.5%	Score 6: 6 ± 27.3%
Reproductive Status (Neutered)	33 (67.3%)	13 (52.0%)	20 (83.3%)
Reproductive Status (Intact)	16 (32.7%)	12 (48.0%)	4 (16.7%)

N – Sample size; **n** – Subsample.

3.2. Wound Healing Parameters

All parameters were assessed at five timepoints: T0 (immediate post-surgery), T1 SL (48 h without LASER), T1 CL (48 h with LASER), T2 SL (8 days without LASER), and T2 CL (8 days with LASER). The analysis was performed on the total sample, and separately for dogs and cats.

3.2.1. Skin Thickness

Statistically significant differences in skin thickness were observed across all timepoints in the Total Sample ($F(4.192) = 80.008$; $p < 0.001$), in dogs ($F(4.21) = 17.756$; $p < 0.001$) and cats ($F(4.20) = 37.707$; $p < 0.001$), as analysed by repeated-measures ANOVA. Post-op comparisons (Cochran's test) revealed multiple significant differences between treatment conditions and timepoints. Full data are summarized in Table 2 and Figure 1.

Table 2. Characterization of the Total Sample (N = 49) and the two species groups considered in the study — Dog (*Canis familiaris*, n = 25) and Cat (*Felis catus*, n = 24) — regarding Skin Thickness.

Evaluation Moment	Total Sample (N = 49)	Dog (n = 25)	Cat (n = 24)
T0	2.93 ± 1.16%	2.92 ± 1.22%	2.92 ± 1.11%
T1 SL	4.82 ± 1.52%	4.49 ± 1.26%	5.15 ± 1.71%
T1 CL	2.91 ± 0.74%	2.75 ± 0.52%	3.07 ± 0.89%
T2 SL	3.82 ± 0.91%	3.73 ± 0.69%	3.91 ± 1.10%
T2 CL	2.38 ± 0.72%	2.38 ± 0.76%	2.36 ± 0.68%

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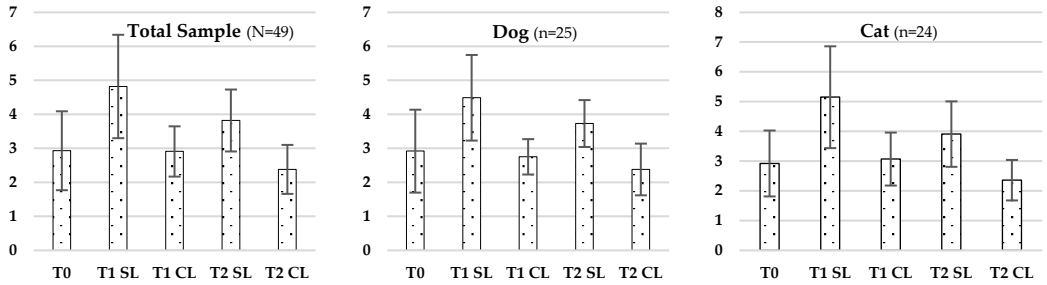

Figure 1. Evolution of Skin Thickness in the Total Sample (N = 49), Dogs (n = 25), and Cats (n = 24) across Different Evaluation Timepoints (T0, T1, T2), Considering LASER-Treated (CL) and Untreated (SL) Regions.

3.2.2. Skin Colour

Significant changes in skin colour were also detected (Cochran's Q test), both in the Total Sample (χ^2 (4) = 59.535; p < 0.001) and by species. Improved normalization of skin colour (pinkish hue) was notably more frequent at T2 CL. Detailed results are illustrated in Table 3 and Figure 2.

Table 3. Characterization of the Total Sample (N = 49) and the two species groups considered in the study — Dog (*Canis familiaris*, n = 25) and Cat (*Felis catus*, n = 24) — regarding Skin Coloration (%Normal / %Altered).

Evaluation Moment	Total Sample (N = 49)	Dog (n = 25)	Cat (n = 24)
T0	22.4 / 77.6	24.0 / 76.0	20.8 / 79.2
T1 SL	26.5 / 73.5	20.0 / 80.0	33.3 / 66.7
T1 CL	69.4 / 30.6	76.0 / 24.0	62.5 / 37.5
T2 SL	79.6 / 20.4	72.0 / 28.0	87.5 / 12.5
T2 CL	95.9 / 4.1	96.0 / 4.0	95.8 / 4.2

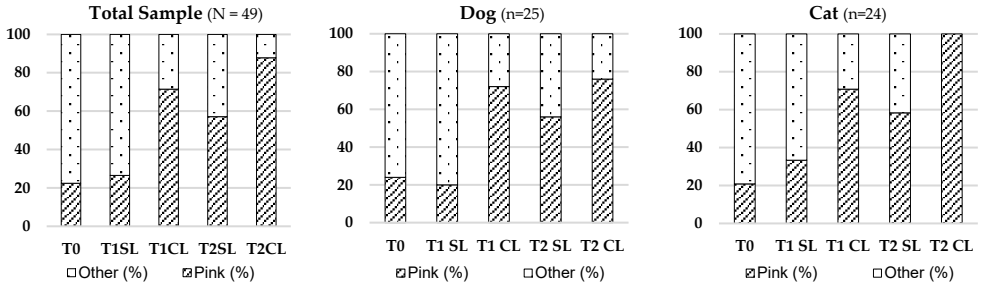

Figure 2. Evolution of Skin Colour in the Total Sample (N = 49), Dogs (n = 25), and Cats (n = 24) across Different Evaluation Timepoints (T0, T1, T2), Considering LASER-Treated (CL) and Untreated (SL) Regions.

3.2.3. Presence of Haematoma

There was a significant reduction in hematoma presence across timepoints in the Total Sample (χ^2 (4) = 77.353; p < 0.001), dogs (χ^2 (4) = 41.941; p < 0.001), and cats (χ^2 (4) = 38.038; p < 0.001). The most marked reduction was seen at T2 CL. See Table 4 and Figure 3 for visualization.

Table 4. Characterization of the Total Sample (N = 49) and the two species groups considered in the study — Dog (*Canis familiaris*, n = 25) and Cat (*Felis catus*, n = 24) — regarding Presence of Hematoma (% Discrete or Absent/ % Evident).

Evaluation Moment	Total Sample (N = 49)	Dog (n = 25)	Cat (n = 24)
T0	22.4 / 77.6	24.0 / 76.0	20.8 / 79.2
T1 SL	26.5 / 73.5	20.0 / 80.0	33.3 / 66.7

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T1 CL	69.4 / 30.6	76.0 / 24.0	62.5 / 37.5
T2 SL	79.6 / 20.4	72.0 / 28.0	87.5 / 12.5
T2 CL	92.9 / 4.1	96.0 / 4.0	95.8 / 4.2

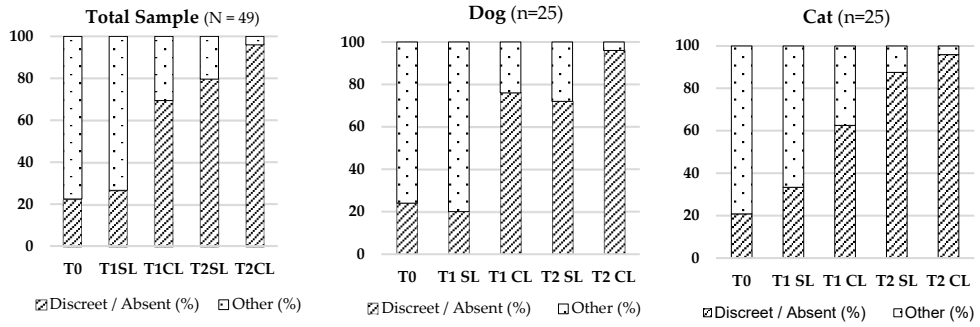

Figure 3. Evolution of Presence of Hematoma in the Total Sample (N = 49), Dogs (n = 25), and Cats (n = 24) across Different Evaluation Timepoints (T0, T1, T2), Considering LASER-Treated (CL) and Untreated (SL) Regions.

3.2.4. Regional Temperature

Significant variations in regional temperature were observed across timepoints in the Total Sample (χ^2 (4) = 86.188; $p < 0.001$), dogs (χ^2 (4) = 53.438; $p < 0.001$), and cats (χ^2 (4) = 36.750; $p < 0.001$), with normalization more prominent in the LASER-treated area at T2. Refer to Table 5 and Figure 4.

Table 5. Characterization of the Total Sample (N = 49) and the two species groups considered in the study — Dog (*Canis familiaris*, n = 25) and Cat (*Felis catus*, n = 24) — regarding Regional Temperature (% Normal/ % Other).

Evaluation Moment	Total Sample (N = 49)	Dog (n = 25)	Cat (n = 24)
T0	22.5 / 77.6	24.0 / 76.0	20.8 / 79.2
T1 SL	30.6 / 69.4	24.0 / 76.0	37.5 / 62.5
T1 CL	71.4 / 28.6	84.0 / 16.0	58.3 / 41.8
T2 SL	87.8 / 12.2	88.0 / 12.0	87.5 / 12.5
T2 CL	98.0 / 2.0	100.0 / 0.0	95.8 / 4.2

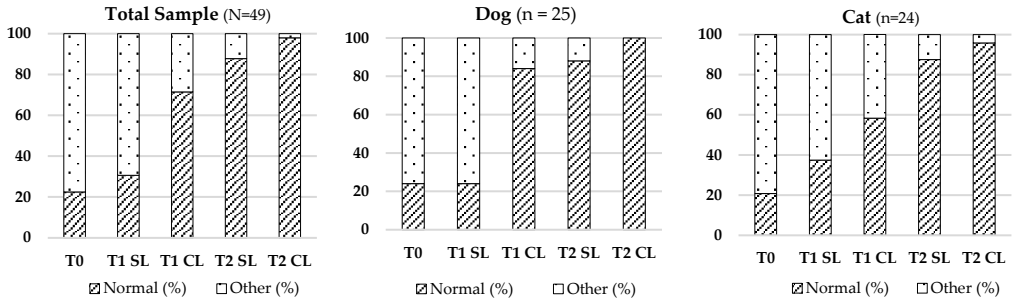

Figure 4. Evolution of Regional Temperature in the Total Sample (N = 49), Dogs (n = 25), and Cats (n = 24) across Different Evaluation Timepoints (T0, T1, T2), Considering LASER-Treated (CL) and Untreated (SL) Regions.

3.2.5. Skin Elasticity

Skin elasticity improved significantly over time in all groups, particularly after LASER application. These changes were confirmed by Cochran's Q test (Total Sample: χ^2 (4) = 32.046; $p < 0.001$). Complete results are shown in Table 6 and Figure 5.

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Table 6. Characterization of the Total Sample (N = 49) and the two species groups considered in the study — Dog (*Canis familiaris*, n = 25) and Cat (*Felis catus*, n = 24) — regarding Skin Elasticity (% Normal/ % Other).

Evaluation Moment	Total Sample (N = 49)	Dog (n = 25)	Cat (n = 24)
T0	22.5 / 77.6	24.0 / 76.0	20.8 / 79.2
T1 SL	26.5 / 73.5	20.0 / 80.0	33.3 / 66.7
T1 CL	67.4 / 32.7	76.0 / 24.0	58.3 / 41.7
T2 SL	67.4 / 32.7	64.0 / 36.0	70.8 / 29.2
T2 CL	89.8 / 10.2	84.0 / 16.0	95.8 / 4.2

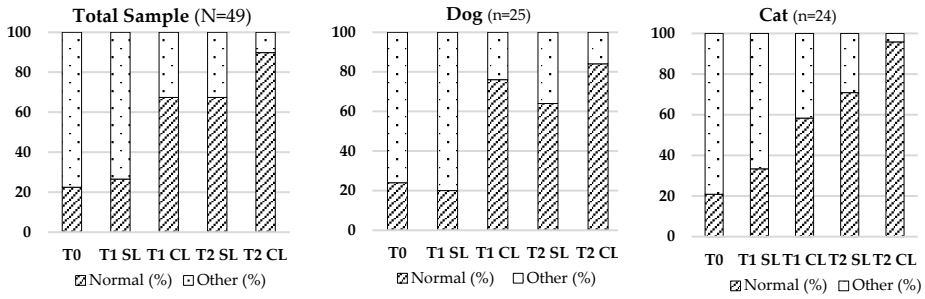

Figure 5. Evolution of Skin Elasticity in the Total Sample (N = 49), Dogs (n = 25), and Cats (n = 24) across Different Evaluation Timepoints (T0, T1, T2), Considering LASER-Treated (CL) and Untreated (SL) Regions.

3.2.6. Presence of Fluids

There was a statistically significant reduction in fluid presence in all groups over time (Total Sample: χ^2 (4) = 73.508; $p < 0.001$). Near-complete resolution was observed at T2 CL. See Table 7 and Figure 6 for graphical data.

Table 7. Characterization of the Total Sample (N = 49) and the two species groups considered in the study — Dog (*Canis familiaris*, n = 25) and Cat (*Felis catus*, n = 24) — regarding Presence of Fluids (% Normal/ % Other).

Evaluation Moment	Total Sample (N = 49)	Dog (n = 25)	Cat (n = 24)
T0	22.5 / 77.6	28.0 / 72.0	16.7 / 83.3
T1 SL	28.6 / 71.4	20.0 / 80.0	37.5 / 62.5
T1 CL	75.5 / 24.5	88.0 / 12.0	62.5 / 37.5
T2 SL	44.9 / 55.1	48.0 / 52.0	41.7 / 58.3
T2 CL	98.0 / 2.0	100.0 / 0.0	95.8 / 4.2

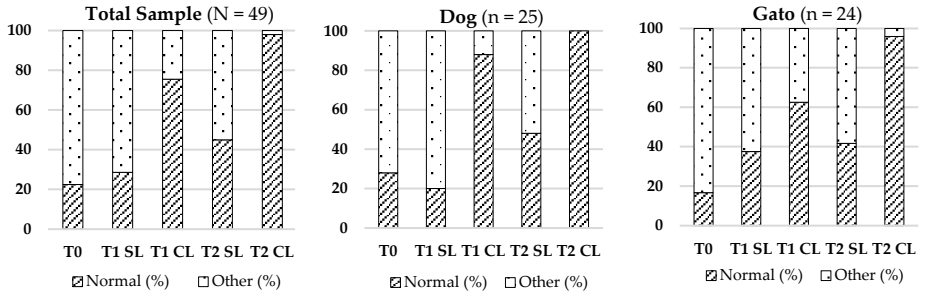

Figure 6. Evolution of Presence of Fluids in the Total Sample (N = 49), Dogs (n = 25), and Cats (n = 24) across Different Evaluation Timepoints (T0, T1, T2), Considering LASER-Treated (CL) and Untreated (SL) Regions.

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3.3. Inter-Species Comparison (Dogs VS Cats)

No significant differences in most parameters between dogs and cats, except for higher pink coloration in cats at T2 CL (p-value = 0.022) [19]. Results are presented in Figure 7.

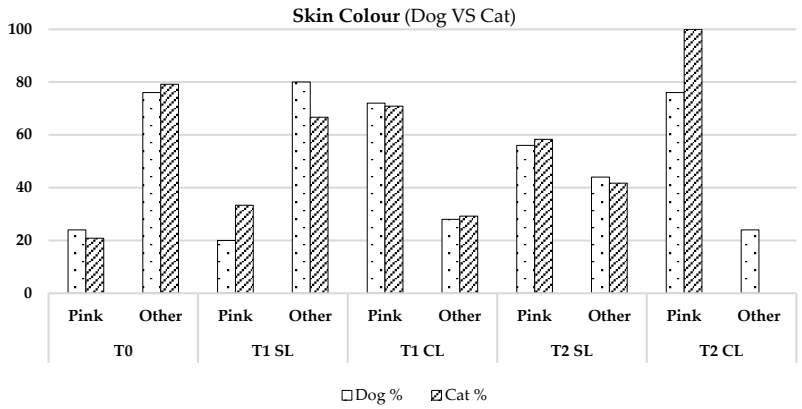

Figure 7. Comparison between Dogs (*Canis familiaris*, n = 25) and Cats (*Felis catus*, n = 24)—regarding the Parameter: Skin Colour.

3.4. Influence of Age, Sex, and Body Condition

No age or sex differences were found for skin thickness or elasticity.

Logistic regression indicated that sex was a significant predictor for pinkish skin colour in the untreated area (SL) (p = 0.014), with females more likely to present normal colour. No significant predictors were identified for the treated area (CL) (Table 8) [7,20].

Table 8. Logistic regression statistics illustrating the influence of age, gender and body condition on skin colouring.

Parameters / Coefficient		B	SD	Wald	df	p-value
SL	Gender	-1,421	0,881	2,601	1	0,107
	Body Condition	0,570	0,254	5,053	1	**0,025**
	Age	0,001	0,106	0,000	1	0,993
CL	Gender	-18,911	876,77	0,000	1	0,998
	Body Condition	-0,040	0,477	0,007	1	0,933
	Age	0,032	0,210	0,023	1	0,880

B - Unstandardised Coefficient; **SD** - Standard Deviation; **Wald** – Statistical test B^2/SD^2; **df** – degrees of freedom; **p-value** - Statistical Significance.

In the SL area, body condition score was a significant predictor (p = 0.025), with worse condition associated with fewer visible hematomas. No significant effects were found in the CL area. Results are detailed in Table 9 [14,20].

Table 9. Logistic regression statistics illustrating the influence of age, gender and body condition on the parameter: Presence of Haematomas.

Parameters / Coefficient		B	SD	Wald	df	p-value
SL	Gender	1,764	0,719	6,024	1	**0,014**
	Body Condition	0,367	0,222	2,864	1	0,091
	Age	0,016	0,089	0,031	1	0,859
CL	Gender	0,600	0,985	0,371	1	0,543
	Body Condition	-0,114	0,298	0,147	1	0,701
	Age	-0,091	0,124	0,541	1	0,462

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B - Unstandardised Coefficient; **SD** - Standard Deviation; **Wald** – Statistical test B^2/SD^2; **df** – degrees of freedom; **p-value** - Statistical Significance.

4. Discussion

This study aimed to evaluate the therapeutic effects of Class IV LASER therapy on the healing process of post-surgical wounds in dogs and cats. The sample included 49 animals of varying ages, sex, weight, and body condition, enabling a comprehensive analysis of the biological effects of photobiomodulation using Class IV laser. The experimental design considered each animal as its own control. Each surgical incision was divided into two distinct anatomical zones: one treated with Class IV laser therapy (CL) and one left untreated (SL). This intraindividual approach isolated the independent variable (laser treatment), ensuring that all other intrinsic and extrinsic variables remained constant. This design substantially increased control over confounding factors, reducing bias and providing higher internal validity and statistical robustness to the findings. This model is aligned with other studies that advocate for intraindividual experimental designs, such as the "split wound" model, which is recognized as a gold standard for evaluating the effects of specific parameters in clinical trials [1–4,8,15,16,18].

Skin thickness significantly decreased in the laser-treated areas (CL) across all time comparisons, indicating not only a lower local inflammatory response but also reduced extracellular matrix (ECM) density. During the inflammatory phase, vasodilation and increased vascular permeability facilitate immune cell infiltration and protein extravasation, leading to localized swelling. The progression into the proliferative phase is characterized by reduced inflammation, granulation tissue formation, and fibroblast proliferation. Class IV laser therapy accelerates this transition by modulating pro-inflammatory cytokines (e.g., TNF-α, IL-1β) and increasing anti-inflammatory mediators like IL-10 [5,25]. Furthermore, laser stimulation activates cytochrome C oxidase in the mitochondrial respiratory chain, enhancing ATP production and cellular metabolism. This bioenergetic effect promotes fibroblast proliferation, collagen type III synthesis, and differentiation into myofibroblasts, which support wound contraction and tissue remodelling [5,6]. The action of growth factors such as TGF-β, FGF, and IGF is enhanced following laser exposure, contributing to the observed reduction in skin thickness as a marker of rapid and efficient healing.

No significant differences in skin thickness were observed between dogs and cats, or across different ages and sexes, indicating that the laser consistently modulated healing mechanisms regardless of physiological profile. While estrogens typically promote tissue regeneration by enhancing epidermal thickness, vascularization, and collagen synthesis, and androgens suppress these processes [7,8,11], the uniform effect observed here suggests that laser therapy can override baseline hormonal differences.

Skin coloration, an indicator of vascular perfusion and oxygenation, was visibly improved in the CL areas, especially at T2, which corresponds with the proliferative phase peak. This phase involves angiogenesis mediated by VEGF, FGF, and TGF-β [9,27]. Class IV laser therapy enhances angiogenesis by increasing ATP and VEGF expression in endothelial and fibroblast cells, leading to improved vascularization and more vibrant skin coloration. This angiogenic effect was more prominent in cats, possibly due to their thinner epidermis, lower subcutaneous fat, and more superficial vascular networks, facilitating better light absorption [10].

In untreated zones (SL), females showed more pronounced pinkish coloration than males, likely due to estrogenic-mediated vasodilation and angiogenesis. However, in laser-treated areas (CL), this gender difference disappeared, suggesting that the laser-induced vascular response compensated for hormonal variability. The involvement of ROS and mitochondrial pathways (especially cytochrome C oxidase) likely contributed to the increased VEGF expression and vascular homogeneity.

Hematoma resolution was notably faster in CL areas, reflecting more effective inflammation control and vascular repair. Laser therapy is thought to stimulate lymphatic drainage, nitric oxide release, and endothelial stabilization. These effects facilitate macrophage recruitment, erythrocyte phagocytosis, and degradation of extravascular haemoglobin [11,12,24,27]. ROS-mediated signalling

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and cytochrome C oxidase activation also reduce capillary permeability and enhance macrophage activity [4,6,12,24]. Although sex hormones affect vascular fragility, the laser's modulatory effects likely overrode these influences, promoting consistent hematoma resolution across groups. Interestingly, animals with higher body condition scores exhibited prolonged hematoma presence in SL areas, possibly due to greater subcutaneous fat and associated vascular fragility. Yet, this difference was mitigated in CL areas, where the laser's anti-inflammatory and lymphatic activation effects were effective.

Skin temperature increased significantly over time in CL regions, consistent with the metabolic activation induced by laser therapy. Enhanced mitochondrial function leads to ATP production, vasodilation, and improved perfusion—key indicators of active tissue repair [4,6,13]. These effects, absent in SL areas and unaffected by sex, age, or species, further support the uniform physiological response elicited by Class IV laser.

Elasticity, a biomechanical marker of ECM quality, improved significantly in laser-treated areas. The synthesis of collagen types I and III, elastin, and fibronectin was stimulated by ATP and fibroblast activation, improving tissue resilience [14,23]. Laser therapy also regulates MMPs and TIMPs, ensuring balanced ECM remodelling [13,15]. While estrogens enhance elasticity and androgens impair it, these hormonal effects were neutralized by laser-induced fibroblast stimulation and ECM regulation, resulting in homogeneous outcomes.

Finally, the presence of regional fluids, including lymphatic and serosanguinous exudate, decreased more rapidly in CL areas. This improvement was attributed to enhanced endothelial and lymphatic recovery via cytochrome C oxidase activation and ATP-driven cellular function [4,6,16,26]. Ion channel activity and aquaporin regulation facilitated interstitial fluid reabsorption, supported by decreased histamine release due to reduced inflammatory cytokine expression [14,17,23,27]. The resulting vascular stabilization and reactivation of lymphatic flow created a more favourable wound environment. The laser's robust effect, unaffected by species, sex, or age, highlights its broad therapeutic applicability in surgical wound management.

5. Conclusions

This preliminary study demonstrated that Class IV laser therapy significantly enhances the post-surgical wound healing process in dogs and cats. The intraindividual design enabled a controlled evaluation of the photobiomodulation effects, showing consistent benefits across various physiological profiles regardless of species, age, or sex. Laser-treated regions (CL) presented faster resolution of inflammation, reduced skin thickness, improved elasticity, enhanced vascularization, and more efficient fluid drainage compared to untreated regions (SL). These findings suggest that Class IV laser therapy is a promising, non-invasive adjunct for improving wound healing outcomes in veterinary practice.

Further studies with larger sample sizes and long-term follow-up are recommended to confirm these results and to better elucidate the molecular mechanisms underlying the observed clinical effects.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization: AL, PA and LMC; Methodology: AL, PA and LMC.; Software, AL.; Validation: AL, PA and LMC; Formal Analysis: AL.; Investigation: AL, PA and LMC; Resources: AL, PA and LMC; Data Curation: AL, PA and LMC.; Writing—original draft preparation: AL; Writing—review and editing: AL, PA and LMC, Visualization: AL, PA and LMC; Supervision: LMC; Project Administration, AL and LMC;

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Abbreviations

The following abbreviations are used in this manuscript:

ECM	Extracellular Matrix
CL	Cranial LASER-treated area
SL	Caudal non-treated area
ATP	Adenosine Triphosphate
ROS	Reactive Oxygen Species
NO	Nitric Oxide
VEGF	Vascular Endothelial Growth Factor
FGF	Fibroblast Growth Factor
TGF-β	Transforming Growth Factor Beta
IL-1β	Interleukin 1 Beta
IL-10	Interleukin 10
TNF-α	Tumor Necrosis Factor Alpha
MMP	Matrix Metalloproteinase
TIMP	Tissue Inhibitor of Metalloproteinase
T0, T1, T2	Timepoints: immediately post-op, 48h, day 8
PBMT	Photobiomodulation Therapy
IGF	Insulin-like Growth Factor

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