SUMMARY

of the doctoral thesis entitled:

INNOVATIVE APPROACHES IN THE THERAPEUTIC MANAGEMENT OF WOUNDS IN VETERINARY MEDICINE

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Veterinary medicine is a continuously evolving field, with constant improvements in diagnostic methods, therapies, and consequently superior clinical outcomes. The foundation of clinical success lies in a vast amount of research and analysis that allows for an advanced understanding of physiological and pathophysiological processes, enabling clinicians to anticipate outcomes. Knowing a patient's future needs is crucial in choosing therapies and developing new products, technologies, and therapeutic protocols. Patients with skin wounds represent a significant percentage of trauma cases. This significant proportion has led to the development of a rich industry of wound care products for human medicine, which has also allowed the development of similar products for veterinary use, especially for companion animals and equines.

This advancement has facilitated the transition from rudimentary wound care, based on knowledge lacking a strong scientific foundation and limited products, to the adoption of the latest wound healing guidelines in Romanian veterinary medicine. The aim of this work was to contribute to optimizing the management of companion animals with wounds by analyzing the multiple therapeutic directions needed to achieve satisfactory results and to highlight the complexity of wound patients and the frequently overlooked changes in therapy.

The thesis titled **"Innovative approaches in the therapeutic management of wounds in veterinary medicine"** is structured according to current standards into two parts: the first part is associated with the bibliographic study and consists of four chapters, while the second part represents the author's own research and contains seven chapters. The doctoral thesis includes 41 tables, 64 figures, and 161 bibliographic references.

Chapter I, titled "*Bibliographic data on the anatomy and physiology of the integumentary system in dogs and cats*," comprises three subchapters presenting concepts on skin structure, vascularization, and innervation.

Chapter II, titled "*The cutaneous healing process*," contains four subchapters detailing the phases of the healing process, individually described, and the four main types of healing related to the presence or absence of surgical intervention.

Chapter III, titled "*Dressings, bandages, and alternative therapies in wound management,"* contains two subchapters dividing the products used in wound therapy into dressings for moist healing and alternative therapies such as the use of ozone therapy or negative pressure therapy.

Chapter IV, titled *"Surgical techniques for skin plasty in complex wounds in domestic carnivores,"* describes the main methods of skin reconstruction in dogs and cats, namely skin flaps and skin grafts.

The second part of the doctoral thesis includes the "Aym and objectives of the research," which presents the motivation for the studies conducted and the proposed goals, namely the approach to patients with wounds as a global system, including the remediation of both systemic and localized impairments.

Chapter V, titled "*Global context of the research: animals included in studies; research methodology,*" includes a subchapter detailing the population included in the doctoral studies, namely 223 animals, of which 118 were canines and 105 felines. This subchapter includes the case selection criteria and descriptive

characteristics such as species, breed, age, and sex. The second subchapter refers to the research methodology, detailing the specially designed work sheet for patients with wounds, and continues with the third subchapter on planimetry concepts.

Chapter VI, titled "*Research on the optimization of the use of bacteriological examination and the use of antibiotic therapy in wound management in domestic carnivores,"* aimed to retrospectively analyze cases of cutaneous wounds in terms of infection management, antibiotic therapy, and bacteriological examination. This chapter included 74 patients (46 dogs; 28 cats) who underwent bacteriological examination with an associated antibiogram. Samples were taken from the wounds using sterile swabs or biopsy samples and sent to a specialized laboratory for bacteriological examination and antibiograms as needed.

Additionally, an analysis of the antibiotic therapy implemented for each case where a bacteriological examination was requested was conducted. Aspects related to "prophylactic" antibiotic therapy, antibiotics used in accordance with antibiogram results, and compatibility between them were monitored.

To analyze the impact, efficacy, and frequency of antibiotic therapy in patients with cutaneous wounds, an analysis was conducted for all 223 patients included in the doctoral thesis, beyond the studies conducted on the 74 cases that underwent bacteriological examination with an associated antibiogram.

This chapter includes case studies that exemplify the complexity of identifying the bacterial agent responsible for the infection and the necessity of individualized therapy. Positive results for aerobic bacterial species were identified in 69 cases. The most frequently encountered were Staphylococcus spp. strains with a percentage of 39.13%, followed by Streptococcus spp. strains (28.99%).

The antibiotic with the broadest spectrum, to which almost half of the identified bacterial species (45.78%) are sensitive, is doxycycline (tetracyclines), followed by antibiotics from the fluoroquinolone class (enrofloxacin-42.16%, marbofloxacin-34.93%, pradofloxacin-36.14%), and aminoglycosides (gentamicin-42.16%).

A distinct analysis of antibiotic use in patients with wounds included all cases used in the doctoral thesis (a total of 223) with the following results: in 130 cases, systemic antibiotics were used, while in 93 cases, wound management was based exclusively on local treatments, without the need for antibiotics.

A case study of cutaneous lesions caused by Nocardia spp. infection was detailed to emphasize the importance of identifying the etiologic agent.

Chapter VII, titled "*Research on hematological and biochemical changes in domestic carnivores with wounds,*" aimed to analyze the complete blood count and biochemical parameters in the context of domestic carnivores with complex cutaneous wounds. The goal of this study was to identify changes in these parameters. In the present study, 124 domestic carnivores were included as follows: 58 dogs and 66 cats.

To evaluate the hematological and biochemical profile of dogs and cats with severe wounds, a quantification and analysis of 97 hemograms for dogs and 87 for cats, and 65 blood biochemical examinations for canines and 69 for felines, were performed.

For biochemical examination, blood was collected on lithium heparin anticoagulant, and for hematological examination on EDTA. Samples were analyzed within 10 to 30 minutes after collection, using analyzers from the IDEXX series.

To facilitate understanding of hematological and biochemical changes, a three-division classification was used: values below reference, values within the reference range, and values exceeding the reference range. This counting was performed for each analyzed parameter, differentiated for dogs and cats. One aspect studied was the interval from the time of trauma to blood testing. For this, the number of analysis sets showing changes related to the following time intervals was counted: 0-24 hours, 24-72 hours, 4-7 days, 8-14 days, 15-21 days, 22-30 days, over 30 days. The results are presented in detail by species and hematological and biochemical parameters.

To illustrate the trends of hematological parameters, a case study of a feline patient with severe thermal burns is presented. The results obtained from the analysis of the hematological and biochemical profile of dogs and cats with wounds led to the observation of repetitive patterns, especially in patients with severe lesions.

Anemia in patients with wounds is associated with the inflammatory process and correlates with the literature as a stable, moderate anemia. Although this type of anemia is described as non-regenerative, mild and severe forms show reticulocytosis and gradual return to physiological values. Anemia remission correlates with wound healing or the advancement of the healing process. The inflammatory process secondary to large wounds is associated with neutrophilia and monocytosis.

Hematological and biochemical evaluation facilitates creating a detailed profile of patients with complex cutaneous wounds.

Chapter VIII, titled "*The role of nutrition in domestic carnivores with wounds*," aimed to highlight the importance of nutrition in wound management, detailing the results obtained from analyzing cases included in the doctoral research regarding body weight variation and the need to develop a nutritional plan.

This study included 110 dogs and 97 cats with complex wounds, with a healing period longer than 21 days. For each patient, the body condition score (BCS) and muscle condition score (MCS) were estimated. In addition to estimating these scores, body weight was monitored.

A higher number of cats (26.80%) compared to dogs (13.64%) initially had a body condition score below the ideal, and this number increased at the 21-day evaluation (16.36% dogs and 30.93% cats).

The objective clinical component that draws attention to the degradation of the general condition and the need to revise the nutritional approach refers to body weight variations. Cats are significantly affected by weight loss. They recorded body weight losses of up to 25-30% of their initial weight. Almost half of the cats with wounds (44.32%) were affected by weight loss when evaluated three weeks after the wound induction. Only 15.45% of dogs had weight loss at the 21-day reevaluation.

Case studies were used to explain and demonstrate the patterns observed in weight variations and the type of nutritional management instituted.

Chapter IX, titled "Clinical, microbiological, and experimental studies on the efficacy of white and kale cabbage extracts in wound management in companion animals" aimed to investigate the properties of white cabbage and kale useful in wound healing, both in preclinical and clinical contexts. The study on the efficacy of white and kale cabbage extracts followed three distinct directions: antibacterial effect, anti-inflammatory effect in a paraclinical context, and healing effect in a clinical context.

To test the properties of Brassicaceae, a gel product incorporating hydro-alcoholic extracts of white cabbage and kale was created in the Hofigal S.A. laboratories. For the microbiological study, both bacterial species isolated from wound samples and laboratory strains used as a control group were utilized. The adapted antibiogram method standardized in CLSI 2020 was employed. For determining the minimum inhibitory concentration, a quantitative method based on serial microdilutions in nutrient broth distributed in 96-well sterile plates was used. Evaluation of bacterial growth inhibition revealed that among the tested compounds, the cinnamon and thyme spray exhibited antimicrobial effects on both Gram-positive and Gram-negative strains.

To highlight the efficacy of the samples (white cabbage extract and kale extract), the carrageenan-induced acute rat paw edema model was used experimentally. The anti-inflammatory effect was determined in vivo by computerized plethysmometry. For testing, animals were grouped into equal batches of 6 individuals each, as follows: 1 test batch with White Cabbage Extract; 1 test batch with Kale Extract; 1 reference batch with Diclofenac – commercial product "Diclofenac Cream 10 mg/g, 35 g, Fiterman"; 1 untreated irritated control batch.

The clinical study was conducted on cats and dogs, based on spontaneous cases presented at the clinic. Each group included 10 cases with surgical wounds and 10 cases with traumatic skin lesions. For sutured wounds, a post-surgical protocol without the use of bandages was applied. For traumatic wounds treated for secondary healing, a covering bandage was used. A decrease in the total score was observed in all wounds, indicating the initiation of the healing process and reduction of the inflammatory phase. Overall, the gel was well tolerated by both dogs and cats. Healing periods were longer for traumatic wounds regardless of the product used, with a 10% healing rate between 25 and 30 days.

By conducting research in this field, a deeper understanding of the efficacy of phytopharmaceuticals as alternatives to antibiotics and other topical products was obtained.

Chapter X: "The use of modern therapies in wound healing in companion animals" contains two major subchapters. The first is a study that refers to the creation of systems for classifying and ranking wounds and the results of the healing process, and the second refers to the long-term complications of secondary intention healing.

For the study on the efficacy of therapies used for wound healing, 183 cases from the total cases mentioned in the population description in Chapter V were included. Of these patients, 99 were dogs and 84 were cats.

To better group the cases, considering the difficulty in creating a homogeneous batch in terms of breeds, ages, etiology, wound location, number of tissues involved, and wound size, a series of 8 scores were created to characterize each case as accurately as possible: severity score, healing time, etiology score, wound location score, complication score, hematological variations score, bacteriological examination influence score, and a global case approach score. The scores obtained were divided into seven categories to create patient batches based on wound severity and healing process complexity. The highest proportion was associated with a score of 16-20 points with a number of 56 patients (30.60%).

To facilitate data presentation, wound etiology was divided into two main categories, traumatic and non-traumatic, and then into subcategories.126 wound cases (69%) had traumatic etiology, while 57 patients (31%) had cutaneous wounds secondary to non-traumatic processes.The two main categories account for half of the traumatic etiology cases and refer to bite wounds (28%) and auto-traumas (21%).

Depending on the type of healing, the 183 cases included in the study were divided as follows: primary healing by direct suture – 15 cases; secondary healing – application of active dressings, advanced local therapies – 136 cases; tertiary healing – 32 cases. Several healing times were recorded to more accurately reflect the healing evolution: granulation tissue formation, 50% wound reduction (1/2), 75% wound size reduction (3/4), and approximately 90% wound size reduction (7/8).

Case studies were used to illustrate the complexity of wounds included in the study and therapeutic management, as well as the need to adapt the protocol according to each individual case.

Long-term complications of secondary intention healing in domestic carnivores with extensive wounds were analyzed in a study conducted on 157 companion animals (92 dogs and 65 cats). The study aimed to divide the cases by location into two main categories: limb region (distal extremities) and other body regions. To facilitate data presentation and documentation, complications were classified into three grades: 0, I, and II. The initial hypothesis was confirmed; limb wounds healed by secondary intention with abundant scar tissue (score 2-3) present a higher probability of long-term complications. In 91 cases, epithelialization had the highest prevalence compared to 62 cases for contraction. To evaluate healing quality and resulting scar tissue, a 3-grade score was applied.

Chapter XI: "General conclusions and recommendations"summarizes the conclusions of the individual studies and provides an overview of the results of the doctoral thesis. As a result of the analyzed data and the experience gained in this field, it was possible to conceive a decision tree that offers guidelines regarding wound therapy in companion animals.