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Time-Course Analysis of Pain and Biochemical Stress Responses After Canine Gastrotomy

Kanissarinn Sakundech[1], Wareeruk Permpoon[1], Thanatchaporn Puddee[1], Kampeebhorn Boonloh[2] and Worapol Aengwanich[1,3]*

[1]Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham 44000, Thailand
[2]Faculty of Medicine, Khon Kaen University, Khon Kaen 40000, Thailand
[3]Stress and Oxidative Stress in Animal Research Unit of Mahasarakham University, Maha Sarakham 44000, Thailand
***Corresponding author:** worapol.a@msu.ac.th

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ABSTRACT

Gastric obstruction in dogs is a common condition that requires surgical removal of the obstruction. Gastrotomy is painful, stressful, and requires appropriate postoperative care. The aim of this study was to examine the physiological changes, including pain, stress, oxidative stress, and nitric oxide levels, after gastrotomy in dogs. Data were collected at five time points: before surgery, immediately after extubation, and on days 1, 3, and 7 postoperatively. Pain scores, stress, and oxidative stress markers (malondialdehyde and hydrogen peroxide), total antioxidant capacity, and nitric oxide levels were measured. On day 1 postoperatively, the dogs showed increased pain scores, neutrophil-to-lymphocyte ratio, and malondialdehyde levels ($P<0.05$), while total antioxidant capacity and hydrogen peroxide levels decreased ($P<0.05$). After extubation, body temperature and respiratory rate decreased ($P<0.05$), whereas heart rate increased ($P<0.05$). Additionally, nitric oxide levels after extubation and on day 1 postoperatively were significantly lower than those measured before surgery and on days 3 and 7 postoperatively ($P<0.05$). These phenomena indicate that gastrotomy has an impact on the physiological responses of dogs, especially during the 24 hours following surgery. Therefore, it is important to manage pain, oxidative stress, and inflammation during this time. Indicators such as stress, oxidative stress, and nitric oxide levels can help veterinarians determine an effective treatment plan for dogs undergoing gastrotomy.

Key words: Stress, Neutrophil-to-lymphocyte ratio, Malondialdehyde, Nitric oxide, Total antioxidant capacity, Hydrogen peroxide.

INTRODUCTION

The stomach acts as a reservoir that regulates the flow and size of ingesta entering the small intestine. It initiates the breakdown of proteins and fats, and assists in the absorption of vitamins and minerals to support the body (Abdelkader et al. 2023). However, gastrointestinal obstructions from foreign bodies (rubber, metal, plastic, or stones) can cause complications, including acid-base and electrolyte imbalances due to fluid loss, hypotension, or toxemia (Sharma et al. 2017; Di Palma et al. 2022; Sharma et al. 2022). Gastrotomy is the surgical procedure used to remove such obstructions. It is also used to manage gastritis, gastric ulcers, or to collect biopsy samples (Abdelkader et al. 2023). Nevertheless, gastrotomy may impair gastric motility and cause postoperative intestinal obstruction (Mazzotta et al. 2020). It can also alter acid secretion, disrupting digestion, mucosal protection, and drug absorption (Hua and Lye 2022).

Pain involves both sensory and emotional components and is categorized as acute or chronic pain (Monteiro et al. 2023). Pain is considered chronic if it persists or recurs for more than three months after the initial injury or triggering event has resolved (Waldron et al. 2025). Acute pain resulting from injury or inflammation may progress to chronic pain through mechanisms such as maladaptive neuroplasticity and behavioral reinforcement (Fang et al. 2023). Notably, postoperative pain and stress responses differ depending on the nature, complexity, and duration of the surgical procedure, as indicated by variations in hematological and

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biochemical parameters observed in dogs subjected to different types of surgery (Kisani et al. 2021). When pain is not adequately managed, it may trigger the sympathetic nervous system, which can lead to immune suppression, metabolic imbalances, and delayed tissue repair (Menéndez et al. 2023). The Glasgow Composite Measure Pain Scale, created through psychometric methodologies, is a reliable tool for identifying acute pain in dogs and is commonly applied in both clinical practice and research (Reid et al. 2007). The criteria used for assessment include vocal expressions, attention directed toward wounds, physical mobility, reactions to tactile stimuli, and behavioral or postural changes (Testa et al. 2021). The use of this scale can help to manage appropriate pain in animals (Marco-Martorell et al. 2024).

Surgical procedures induce oxidative stress in dogs (Salavati et al. 2021; Gültiken et al. 2022). Excess reactive oxygen species (ROS) damage proteins, lipids, and DNA, hindering cell function and tissue repair (Wang et al. 2023). ROS disrupts antioxidant defenses, causes mitochondrial injury, and impairs energy supply (Hunt et al. 2024). Under oxidative stress, fibroblasts diminished functionality, resulting in lower production of collagen and other structural elements of the extracellular matrix (ECM) (Lopes et al. 2024). This stress condition also induces matrix metalloproteinases (MMPs), which degrade ECM integrity and impede the regenerative process (Zivkovic et al. 2024). Moreover, it stimulates the release of inflammatory mediators such as IL-1, IL-6, and TNF-α, intensifying nociception and inflammatory signaling (Savic Vujovic et al. 2023). Increased levels of malondialdehyde and superoxide dismutase have been linked to a greater sensitivity to pain (Zivkovic et al. 2024). Sustained oxidative stress disrupts the function of immune cells, particularly by reducing macrophage effectiveness, thereby raising infection susceptibility (Tepebaşı et al. 2023) and prolonging tissue repair due to persistent inflammation (Hunt et al. 2024).

Nitric oxide (NO) plays a crucial part in tissue recovery by causing vascular dilation, aiding in antimicrobial defense, and regulating inflammatory processes. It improves blood circulation through the enzymatic action of endothelial nitric oxide synthase (eNOS) and helps limit the overaccumulation of leukocytes at the site of injury (Duchesne et al. 2025). NO also interferes with microbial respiration and helps lower infection risk through the application of NO-releasing nanoparticles (Mihu et al. 2024). Additionally, it influences cytokine signaling, strengthens immune defense, and facilitates the transition of macrophages from the pro-inflammatory M1 phenotype to the reparative M2 state (Xia et al. 2025). NO further promotes fibroblast activation, enhances collagen formation (Huang et al. 2023), and regulates vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1-alpha (SDF-1α), both of which are critical for new blood vessel formation (Duchesne et al. 2025).

Although gastrotomy is a commonly performed procedure, information regarding the associated physiological and biochemical alterations in dogs remains limited. This research proposed that the surgery may induce pain and stress, disrupt oxidative balance, reduce total antioxidant, and influence nitric oxide dynamics. Accordingly, the study aimed to evaluate indicators such as pain levels, physiological responses, oxidative stress, total antioxidant capacity, and nitric oxide production at various stages-before, during, and after gastrotomy—to improve insight into postoperative changes and guide effective clinical management.

MATERIALS AND METHODS

Animals

Nine neutered dogs, ranging in age from 1 to 5 years and weighing between 10 and 25kg, were enrolled in this study. The group consisted of both male and female dogs, all of which had completed their standard vaccination schedules. Each dog was subjected to a comprehensive veterinary health check to verify that no clinical issues were present before inclusion in the study. The surgical procedures were carried out at the surgery unit of the Faculty of Veterinary Sciences, Mahasarakham University, Thailand, over the course of January to February 2024.

Experimental design

The study employed a completely randomized design, with the pre- and post-operative periods considered as treatments. Measurements were taken at several time points: before surgery, after extubation, and on days 1, 3, and 7 postoperatively. Each dog served as a replicate for each period. The parameters assessed at each time point were pain scores, body temperature, heart rate, respiratory rate, neutrophil-to-lymphocyte ratio, malondialdehyde levels, hydrogen peroxide levels, nitric oxide levels, and total antioxidant capacity.

Surgical procedure

Pre-surgery preparation

Dogs were fasted for 8 hours prior to anesthesia. Each of the nine dogs underwent a thorough pre-surgery examination. Pain scores, body temperature, heart rate, and respiratory rate were documented. Blood samples were drawn from the saphenous vein.

Gastrotomy procedure

Each dog was sedated with an intramuscular injection of Acepromazine (Combistress®, Belgium) at a dosage of 0.2mg/kg to induce calmness and reduce stress. Following sedation, Lactate Ringer's Solution (LRI®, Thailand) was administered intravenously via the cephalic vein to maintain fluid balance. Cefazolin (Cefaben®, Thailand) at a dose of 20–30mg/kg was delivered through the IV fluid line, and Tolfenamic acid (Tolfedine® CS, France) at 4mg/kg was administered intramuscularly. For induction of anesthesia, Propofol (Troypofol®, India) was administered intravenously at 3-6mg/kg to facilitate the insertion of a breathing tube. Anesthesia was maintained with Isoflurane (Aerrane®, USA) at 1-3%. Once the animal was fully anesthetized, it was positioned in dorsal recumbency. The body was stabilized with ropes, and the limbs were secured to the edges of the surgical table to prevent involuntary movements. The abdominal area was then aseptically prepared for the operation.

The gastrotomy procedure was performed according to the surgical principles outlined by Radlinsky and

Fossum (Radlinsky and Fossum 2019). A cranial midline laparotomy incision was made, starting from the xiphoid process and extending to the umbilicus. The stomach was gently exteriorized and wrapped in sterile gauze soaked in warm normal saline solution (NSS) to keep the tissues hydrated. An incision was then made between the lesser and greater curvature of the stomach, carefully avoiding highly vascularized areas and the pylorus to prevent postoperative stenosis (Fig.1). Stay sutures were placed at the cranial and caudal ends of the incision to lift the stomach wall and reduce the risk of gastric contents leaking into the abdominal cavity. A scalpel blade was used to create an initial incision of approximately 1cm, which was subsequently enlarged using surgical scissors. Gastric contents were aspirated using a suction pump to minimize contamination. The interior of the stomach was carefully examined (Fig.2).

Fig. 1: The locations of the greater curvature and lesser curvature of the stomach.

Fig. 2: Examination of foreign objects in the stomach.

Closure of the gastric incision was performed in two layers using 2-0 polyglyconate (Maxon®). The first layer included the serosa, muscularis, and submucosa, closed using a simple continuous suture pattern. The second layer involved only the serosa and muscularis and was sutured using a Cushing pattern (Fig.3). Although mucosal suturing with a simple continuous pattern can be beneficial in reducing postoperative gastric bleeding, it was not required in this case, as bleeding had ceased by the time the stomach was closed. No evidence of foreign body perforation through the gastric wall was observed in any of the dogs, and therefore bacterial culture and antibiotic sensitivity testing were deemed unnecessary. All contaminated instruments and gloves were replaced before proceeding with abdominal closure. The abdominal wall was closed in layers. The rectus sheath was sutured with 2-0 polyglycolic acid (Max®, Belgium), followed by closure of the subcutaneous tissue and fat using the same suture material. Finally, the skin was closed using 2-0 polyamide (Seralon®, Germany).

Fig. 3: Two-layer stomach suturing. The first layer is sutured using a simple continuous pattern, while the second layer is sutured using a Cushing pattern.

Post-operative care

Following surgery, none of the dogs showed signs of hematemesis. Water was first offered six hours after the procedure. Only one dog experienced a mild, blood-tinged episode of vomiting within two hours of its initial water intake. This isolated event resolved on its own without the need for medical intervention. After the first meal was introduced 12 hours post-surgery, there were no further incidents of vomiting or diarrhea observed in any of the dogs.

To support hydration and recovery, Lactated Ringer's Solution (LRI®, Thailand) was administered once daily at a dosage of 40 to 60mg/kg of body weight. This continued until the animals resumed normal eating behavior. For analgesia, Tolfenamic acid (Tolfedine®, France) was given orally at 4mg/kg every 24 hours for three consecutive days to manage postoperative discomfort.

Skin sutures were removed on the tenth postoperative day. Throughout the recovery period, which lasted ten days, none of the animals required additional medical treatment. The absence of complications suggested effective surgical and postoperative care.

However, in cases where dogs exhibit signs such as fever or abdominal tenderness after surgery, there is a possibility of secondary infection due to bacterial contamination introduced during the operation. Under these circumstances, radiographic imaging becomes an important diagnostic tool. If needed, a second surgical intervention may be carried out—to assess tissue integrity, obtain samples for bacterial culture and sensitivity testing, and conduct peritoneal lavage to help resolve infection. Based on the results of antimicrobial sensitivity testing, a targeted antibiotic regimen should be selected to optimize therapeutic success.

Blood sample collection

Blood samples of 3mL were collected from the saphenous vein at 5 pre-operative and post-operative time points: Before surgery, after extubation, and on days 1, 3, and 7 postoperatively. The blood 0.5mL was transferred to EDTA tubes for hematological analysis, and the remaining 2.5mL was placed in heparin-coated tubes for biochemical measurements. These samples were centrifuged at 2500rpm (769 × g) for 5min, and the plasma fraction was transferred to microtubes and stored at −20°C until the time of biochemical determination.

Determination of indicators

Physiological parameters

This study measured pain scores and physiological parameters, including body temperature, heart rate, respiratory rate, and neutrophil-to-lymphocyte ratio. The methods for measuring each parameter are detailed as follows:

Pain scores

Pain scores were evaluated using the Glasgow Composite Measure Pain Scale, which assesses both spontaneous and elicited behaviors, as well as interactions and clinical observations (Reid et al. 2007).

Physical Parameters

Body temperature was measured rectally using a rectal thermometer (Yuwell® YT308, Chaina). Heart rate was determined by auscultating the left chest with a stethoscope (3M™ Littmann® Cardiology III™, USA) and counting the beats per minute (Sakundech and Aengwanich 2021). Respiratory rate was counted as the number of breaths per minute (Duguma 2016).

Neutrophil-to-lymphocyte ratio

Blood films were prepared, fixed with methanol, stained with Giemsa-Wright solution, and a differential white blood cell count was performed to calculate the neutrophil-to-lymphocyte ratio (Aengwanich et al. 2019).

Biological parameters

Total antioxidant capacity

The total antioxidant capacity in plasma was measured using the Ferric Reducing Ability of Plasma (FRAP) method. The FRAP solution was freshly made by combining 300mM acetate (Ajax Finechem Pty. Ltd., Australia) buffer (pH 3.6), 10mM TPTZ (Aldrich, U.S.A.) in 40mM hydrochloric acid (QRëC™, New Zealand), and 20mM Iron (III) chloride hexahydrate (PanReac AppliChem, Germany). Plasma (20µL) was mixed with 180µL of the FRAP solution in a 96-well plate and left at room temperature for 5 min. Absorbance was measured at 595nm using ferrous sulfate heptahydrate (Ajax Finechem Pty. Ltd., Australia) as the standard (Sakundech et al. 2021).

Hydrogen peroxide

Plasma hydrogen peroxide was measured by mixing 1mL of 2.25mM ferrous sulfate heptahydrate (Ajax Finechem Pty. Ltd., Australia) with 1mL of plasma for 5min. Then, 1mL of 4mM norfloxacin (Sigma-Aldrich, U.S.A) was added and reacted for min. Absorbance at 440nm was recorded using hydrogen peroxide (Vidhyasom Co. Ltd., Thailand) as the standard (Wandee et al. 2022).

Malondialdehyde

Plasma (0.01mL) was mixed with 3mL of 0.05M/L hydrochloric acid (QRëC™, New Zealand) and 1mL of 0.67% thiobarbituric acid (Fluka, Germany). The mixture was heated at 100°C for 30 min, cooled, and 4mL of n-butyl alcohol (QRëC™, New Zealand) was added. After vortexing and centrifuging at 3000rpm (1008 × *g*) for 10 min, absorbance at 450nm was measured. The standard used was 1,1,3,3-tetramethoxypropane (Aldrich, Germany) (Srinontong et al. 2023).

Nitric Oxide

Plasma nitric oxide levels were measured using Griess's reagent, made from 1% sulfanilamide (DC Fine Chemicals, Spain), 0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride (PanReac AppliChem, Germany), and 1.25% phosphoric acid (QRëC™, New Zealand). The reagent was diluted to 100mL with distilled water. Equal parts of plasma and reagent were mixed, left for 15min, and the absorbance at 540nm was recorded. Sodium nitrate (APS Finechem, Australia) was used as the standard (Giustarini et al. 2008).

Statistical analysis and power analysis

Statistical analysis was performed using PROC GLM. Duncan's multiple range tests (SAS® Studio) were used to separate means, with significance set at $P<0.05$.

A post hoc power analysis was conducted based on the observed mean differences and standard errors from primary outcome variables. The study employed a completely randomized design with repeated measures, in which nine dogs were assessed across five distinct timepoints (before surgery, after extubation, and days 1, 3, and 7 postoperatively). The analysis demonstrated that this sample size (n=9) provided sufficient statistical power (>95%, $\alpha=0.05$) to detect significant changes in key variables such as pain scores and nitric oxide levels. This indicates that the sample size was adequate for detecting biologically meaningful differences within the CRD with a repeated measures framework.

RESULTS

Pain scores, physiological parameters, total antioxidant capacity, hydrogen peroxide, malondial-dehyde, and nitric oxide levels in dogs were assessed at multiple time points: before surgery, after extubation, and on days 1, 3, and 7 postoperatively. The findings are summarized as follows:

Pain scores

Pain scores were significantly elevated on day 1 compared to all other time points ($P<0.05$; Fig.4).

Physical Parameters

Body temperature was significantly decreased after extubation compared to other experimental periods ($P<0.05$) (Fig. 5). The respiratory rate after extubation was significantly lower than during other measurement periods ($P<0.05$) (Fig. 6).

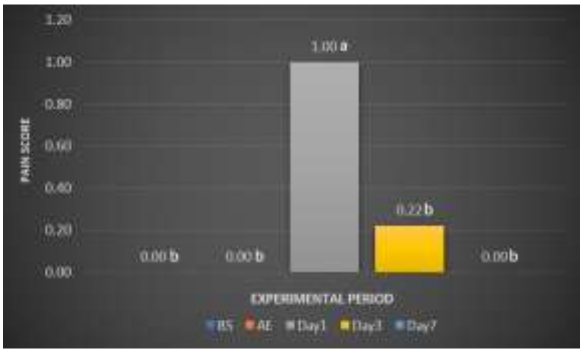

Fig. 4: Pain scores in dogs before surgery (BS), after extubation (AE), and on days 1, 3, and 7 postoperatively. Different letters indicate statistically significant differences (P<0.05); P= 0.0052; SEM=0.263.

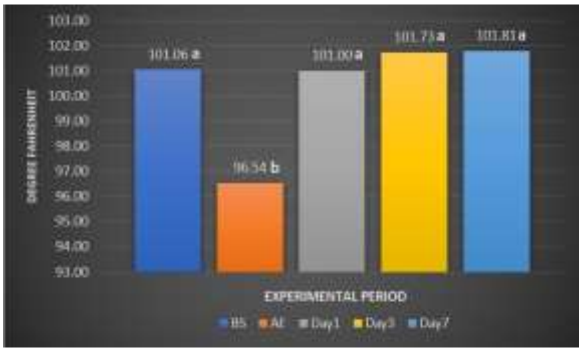

Fig. 5: Body temperature in dogs before surgery (BS), after extubation (AE), and on days 1, 3, and 7 postoperatively. Different letters indicate statistically significant differences (P<0.05); P<0.0001; SEM=0.313.

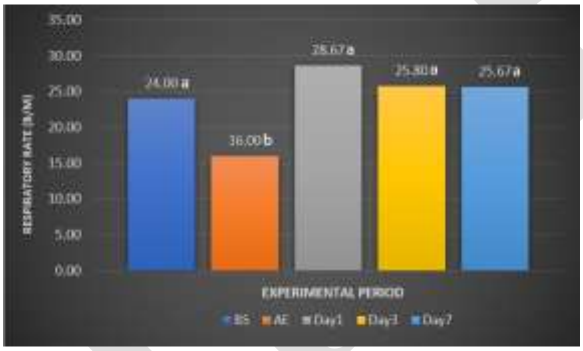

Fig. 6: Respiratory rate in dogs before surgery (BS), after extubation (AE), and on days 1, 3, and 7 postoperatively. Different letters indicate statistically significant differences (P= 0.0082; SEM=0.990.

Heart rate after extubation was significantly higher than before surgery, as well as on days 3and 7 postoperatively (P<0.05) (Fig. 7).

Neutrophil-to-lymphocyte ratio

On day 1, the neutrophil-to-lymphocyte ratio was significantly higher than before surgery, after extubation, and on days 3 and 7 postoperatively (P<0.05) (Fig. 8).

Total antioxidant capacity

Total antioxidant capacity on day 1 was significantly lower than other experimental periods (P<0.05). Total antioxidant capacity on day 3 was significantly lower than before surgery (P<0.05). Total antioxidant capacity on day 7 was significantly higher than at other times (P<0.05) (Fig. 9).

Hydrogen peroxides

Hydrogen peroxide levels were significantly lower on day 1 compared to days 3 and 7 postoperatively, before surgery, and after extubation (P<0.05). Hydrogen peroxide on day 3 was significantly lower than before surgery, after extubation, and on day 7 (Fig. 10).

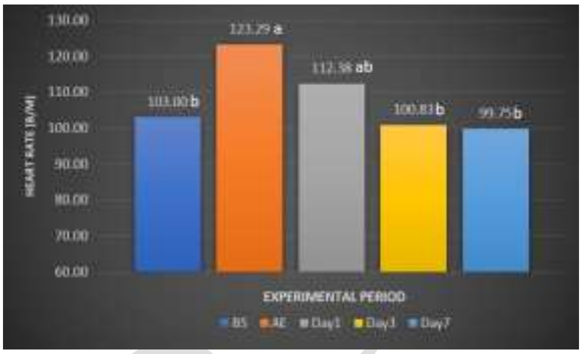

Fig. 7: Heart rate in dogs before surgery (BS), after extubation (AE), and on days 1, 3, and 7 postoperatively. Different letters indicate statistically significant differences (P= 0.0021; SEM=1.257.

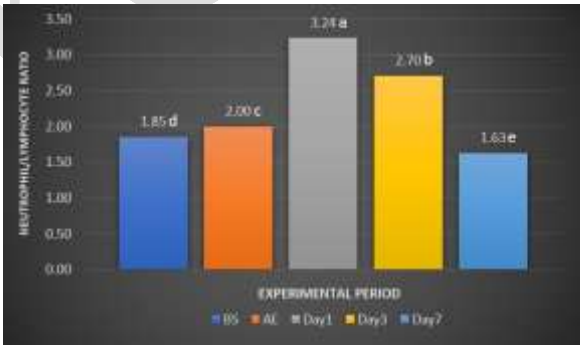

Fig. 8: Neutrophil/ Lymphocyte ratio in dogs before surgery (BS), after extubation (AE), and on days 1, 3, and 7 postoperatively. Different letters indicate statistically significant differences (P<0.0001; SEM=0.138.

Fig. 9: Total antioxidant capacity in dogs before surgery (BS), after extubation (AE), and on days 1, 3, and 7 postoperatively. Different letters indicate statistically significant differences (P<0.0001; SEM=0.970.

Malondialdehyde

Levels of malondialdehyde were significantly elevated on day 1 compared to days 3 and 7, before surgery, and after extubation (P<0.05). Malondialdehyde on day 3 was significantly higher than before surgery, after extubation, and on day 7 (P<0.05) (Fig. 11).

Nitric oxide

Nitric oxide levels on days 3 and 7 were significantly higher than after extubation and day 1 (P<0.05). Nitric oxide on day 7 was significantly higher than before surgery (P<0.05) (Fig. 12).

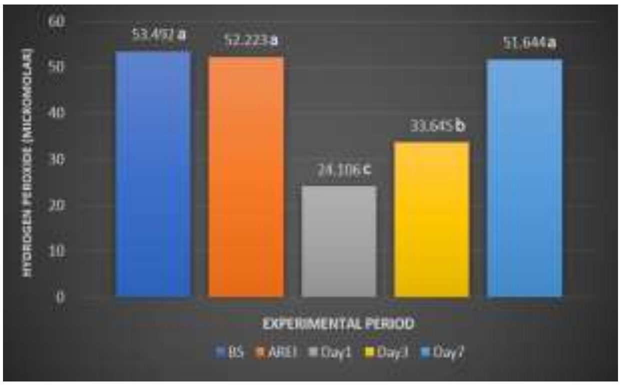

Fig. 10: Hydrogen peroxide in dogs before surgery (BS), after extubation (AE), and on days 1, 3, and 7 postoperatively. Different letters indicate statistically significant differences (P<0.0001; SEM=0.988.

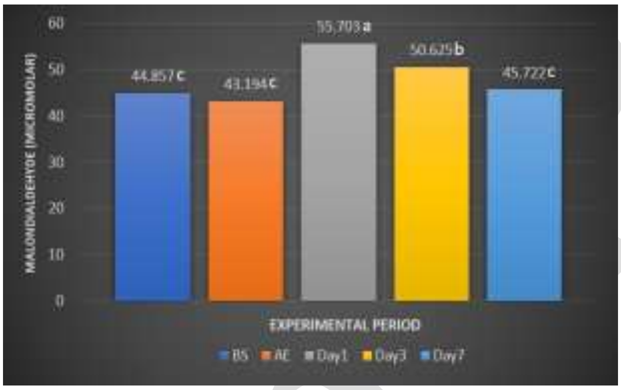

Fig. 11: Malondialdehyde in dogs before surgery (BS), after extubation (AE), and on days 1, 3, and 7 postoperatively. Different letters indicate statistically significant differences (P<0.0001; SEM=0.654.

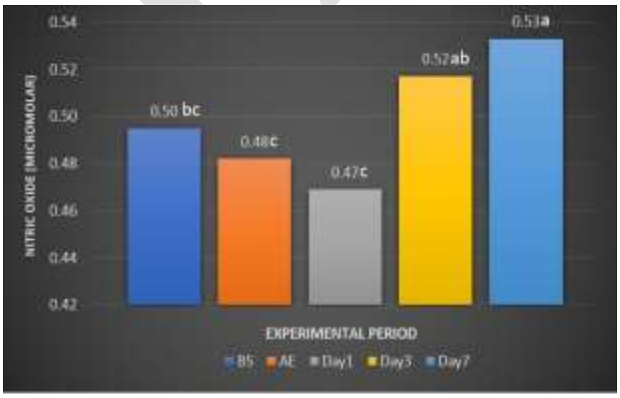

Fig. 12: Nitric oxide in dogs before surgery (BS), after extubation (AE), and on days 1, 3, and 7 postoperatively. Different letters indicate statistically significant differences (P=<0.0001; SEM=0.055.

DISCUSSION

Gastrotomy is a surgical procedure that damages multiple tissue layers, including the skin, abdominal muscles (Radlinsky and Fossum 2019), and the four stomach wall layers: serosa, muscularis, submucosa, and mucosa (Abdelkader et al. 2023). This study assessed dogs' pain levels before and after surgery using the Glasgow Composite Measure Pain Scale (Reid et al. 2007; Testa et al. 2021; Marco-Martorell et al. 2024). The peak pain intensity was found to occur within the first 24 hours after gastrotomy, which is consistent with studies in other animal species. For example, sheep undergoing tendon surgery showed increased pain behaviors and elevated respiratory rates, along with reduced locomotion (Zentrich et al. 2023). In guinea pigs that underwent castration, pain increased after surgery but gradually subsided (Ellen et al. 2016), while in dogs, pain was also reported within the first 24hours following hysterectomy (Santana et al. 2020). Ovariectomy and hysterectomy are both painful procedures; however, hysterectomy is generally more painful due to its longer surgical duration (Tallant et al. 2016; Nisson et al 2025).

Orthopedic surgery tends to be more painful than soft tissue procedures such as gastrotomy. Dogs undergoing spinal disc surgery can experience severe postoperative pain, which typically improves over time (Zidan et al. 2020). Therefore, bone-related pain takes longer to resolve (Pickering et al. 2024), whereas gastrotomy-related pain often improves within about a week (Rialland et al. 2012; Bischoff et al. 2020). Postoperative pain may be exacerbated by tissue swelling and nerve injury (Liu and Kelliher 2022), which triggers the release of inflammatory mediators such as prostaglandins, bradykinins, and cytokines (e.g., tumor necrosis factor-alpha) (Mlachkova and Dosseva-Panova 2023). These mediators sensitize peripheral nerves and enhance pain perception (Zidan et al. 2020).

Inflammatory responses at the wound site can also increase pain by recruiting more immune cells to the area (Kanellos et al. 2021). Additionally, the central nervous system, particularly the brain and spinal cord, plays a role in modulating pain. When pain persists, spinal cord excitability increases, making pain sensations more easily triggered (Reid et al. 2017; Karcz et al. 2024). Emotional factors such as stress or anxiety before or after surgery may further intensify pain (Henao Periañez and Castillo-Díaz 2025). This occurs through activation of the hypothalamic-pituitary-adrenal (HPA) axis, which raises cortisol levels and impairs the body's endogenous pain control mechanisms (Kanellos et al. 2021). Moreover, animals that experience pain before surgery-especially those with chronic pain—are more likely to suffer from intensified postoperative pain (Reid et al. 2017; Downing and Della Rocca 2023).

On day 1 postoperatively, the neutrophil to lymphocyte ratio was higher than before and after extubation, and then decreased on days 3 and 7. The increase in neutrophil to lymphocyte ratio in dogs undergoing gastrotomy is consistent with the study of Bojarski et al. (2022), who reported that the neutrophil to lymphocyte ratio in neutered cats peaked on day 2 postoperatively, which is slightly different from that in dogs, where the neutrophil to lymphocyte ratio increased immediately after surgery (An 2008; Ortiz-López et al. 2022). In addition, the type of surgery affected the neutrophil to lymphocyte ratio, with soft tissue surgery showing short-term inflammation, while orthopedic surgery induces long-term inflammation (REx et al. 2024). Laparoscopic techniques generally result in lower neutrophil-to-lymphocyte ratio values than open surgeries (Espadas-González et al. 2023; Haghi et al. 2024). Generally, surgical stress activates the sympathetic nervous system, releasing catecholamines that stimulate neutrophil mobilization while inducing lymphocyte apoptosis (Bojarski et al. 2022). Cortisol release from the hypothalamic-pituitary-adrenal (HPA) axis inhibits lymphocyte proliferation and prolongs the lifespan of neutrophils (Espadas-González et al. 2023). Inflammatory molecules attract neutrophils to the site and inhibit lymphocyte function (Kim and Choi 2012; Zhang et al. 2024). In addition, lymphocytes migrate to lymphocyte organs, resulting in a decrease in circulating lymphocytes after gastric surgery (Haghi et al. 2023). These changes result in an increased neuterophil-to-lymphocyte ratio after gastrotomy.

An increased heart rate in dogs is associated with pain and stress due to stimulation of the sympathetic nervous system, which causes the heart to beat more strongly (Kishi 2012; Suarez-Roca et al. 2021). In this study, dogs exhibited higher heart rates after extubation, which is consistent with the findings of Zhang et al. (2017) and Gunenc et al. (2022), who reported that heart rates were higher after extubation compared to before anesthesia and 24 hours after surgery. After extubation, the dogs' respiratory rates and body temperatures were lower than at other time points. This is consistent with Srithunyarat et al. (2016) and Laporta et al. (2021), who found that respiratory rate decreased during recovery from anesthesia. The use of anesthesia and a cold environment during surgery is a contributing factor to postoperative hypothermia (Luna et al. 2000; Slatter 2003; Hart et al. 2011; Kelleci et al. 2023).

Nitric oxide levels on days 3 and 7 postoperatively were higher than the levels measured after extubation and on day 1. Additionally, levels on day 7 were higher than the preoperative values. Nitric oxide plays an essential role in controlling inflammation and promoting tissue repair (Xia et al. 2025). In rats recovering from wounds, elevated nitric oxide levels promote angiogenesis and contribute to tissue remodeling (Ibrahim et al. 2018). In diabetic wound healing, exogenous nitric oxide enhances vascularization and reduces inflammation (Zheng et al. 2024). Surgical procedures influence nitric oxide elevation. Orthopedic surgeries show prolonged nitric oxide increases, whereas laparoscopic procedures demonstrate transient nitric oxide peaks (Sivaraj et al. 2023). In surgery, nitric oxide promotes angiogenesis and facilitates complete tissue repair (Bussolati et al. 2001; Chelmu et al. 2025). Nitric oxide production is increased after surgery due to the activation of inducible nitric oxide synthase (iNOS) via cytokines such as tumor necrosis factor-alpha and interleukin-1 beta, resulting in increased nitric oxide levels (Ahmed et al. 2021). Endothelium-derived nitric oxide synthase (eNOS) is upregulated due to increased blood flow and shear force, resulting in vascularization and increased tissue perfusion (Gheibi et al. 2020). Macrophage polarization (M1 to M2) promotes angiogenesis and extracellular matrix remodeling, while hypoxia-induced HIF-1α activation increases nitric oxide levels (Xia et al. 2025). L-Arginine metabolism is altered after surgery, resulting in increased nitric oxide synthesis. Through the cofactor tetrahydrobiopterin (BH4) (Lam et al. 2006; Lundberg and Weitzberg 2022), the combination of these processes accelerates wound healing and recovery.

Malondialdehyde levels surged on day 1 postoperatively and decreased on days 3 and 7, indicating a reduction in oxidative stress. Similarly, increased postoperative levels of malondialdehyde have been found in other animals. For example, in buffaloes undergoing hiatal hernia surgery, malondialdehyde levels were increased (Trivedi et al. 2019). Rodent models of oxidative stress also showed malondialdehyde surges within 24 hours post-surgery, indicating acute lipid peroxidation (Tyastono et al. 2021). The extent of malondialdehyde elevation differs based on the surgical procedure. Orthopedic surgeries result in sustained oxidative stress, whereas soft tissue procedures, such as gastrotomy, cause temporary malondialdehyde increases that rapidly decline (Gbu et al. 2024). Laparoscopic surgery reduces oxidative stress compared to open surgery (Tyastono et al. 2021). Elevated malondialdehyde levels are induced by postoperative ischemia and reperfusion, which increases the production of reactive oxygen species (Trivedi et al. 2019; George et al. 2024). Neutrophil activation releases myeloperoxidase (MPO) and nitric oxide synthase (NOS), exacerbating oxidative stress (Christie et al. 2008; Lin et al. 2024). Inflammatory responses (interleukin-6, tumor necrosis factor-alpha, interleukin-1 beta) increase reactive oxygen species and prolong lipid peroxidation (Mogheiseh et al. 2019; Bhol et al. 2024). In addition, reduced antioxidant levels result in persistently elevated malondialdehyde levels (Tyastono et al. 2021).

Total antioxidant capacity on day 1 postoperatively was lower than at other time points, indicating acute oxidative stress. On day 3, total antioxidant capacity was below preoperative levels, this phenomenon showed continued oxidative imbalance. On day 7, total antioxidant capacity increased, indicating recovery of antioxidant defenses. Similar changes were found in dogs undergoing laparoscopic and open ovariectomy, where overall antioxidant capacity was decreased after surgery but returned to normal by day 7 (Lee and Kim 2014). The decrease in overall antioxidant capacity was consistent with the increase in oxygen free radicals induced by surgical injury, resulting in decreased antioxidants such as glutathione and superoxide dismutase (Ghiselli et al. 2000; Zhang et al. 2018).

Hydrogen peroxide levels decreased on day 1 postoperatively. By day 3, levels increased but were lower than before surgery. On day 7, hydrogen peroxide levels increased, indicating a transition from acute inflammation to the tissue repair phase. This pattern of changes also occurs in other animals. For example, in rodents, the initial decrease in hydrogen peroxide indicates immune activation, followed by a sustained increase to stimulate angiogenesis and fibroblast proliferation (Niethammer et al. 2009; Hunt et al. 2024). Surgery influences hydrogen peroxide changes. It has been found that open surgery causes a prolonged decrease in hydrogen peroxide due to severe tissue injury, while less invasive surgical techniques allow for faster recovery (Athre et al. 2007). Hydrogen peroxide levels decrease after surgery because enzymes such as catalase and glutathione peroxidase rapidly degrade to reduce oxidative stress. In addition, wound healing processes and immune modulation also help to reduce the decrease (Loo et al. 2012; Udegbunam et al. 2021).

Conclusion

This study examined physiological and biochemical changes after gastrotomy in dogs. Pain scores and neutrophil-to-lymphocyte ratio peaked on day 1. Malondialdehyde increased, and total antioxidant capacity decreased. On day 3, nitric oxide levels rose. By day 7, oxidative stress decreased, and nitric oxide increased further. The first 24 hours after surgery showed the highest levels of inflammation and stress. Pain medication is necessary during this period. Monitoring oxidative stress markers, such as malondialdehyde and total antioxidant capacity, may guide antioxidant therapy. These findings may improve postoperative care and recovery in dogs following gastrotomy through better pain control and oxidative stress management.

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Author's Contribution: Conceptualization and study design: KS and WA; Data collection and laboratory investigation: KS; Partial data collection and laboratory work: WP, TP, and KB; Data analysis, validation, and methodology: KS and WA; Writing – original draft, review, and editing: WA, KS, and KB; Final approval: All authors read and approved the final version of the manuscript for publication.

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