

Assessment of haptoglobin, ceruloplasmin, albumin, malondialdehyde, and nitric oxide levels and selected vital and hematological parameters in colic affected horses

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Abstract

This study aimed to evaluate selected vital signs, as well as biochemical, hematological, acute phase, and oxidative stress markers, in horses diagnosed with colic. The study population consisted of 50 horses, including 30 colic-affected horses (both male and female, aged 2 to 10 years, various breeds) presented due to dietary changes, and 20 clinically healthy horses of similar age serving as controls. For all horses, rectal temperature, respiratory and heart rates, and capillary refill time were assessed. Blood samples were collected from colic-affected horses immediately upon clinical presentation—approximately 4 to 8 hours after the onset of clinical signs as reported by owners—and before any treatment was initiated. Serum samples were analyzed for albumin (Alb), haptoglobin (Hpt), malondialdehyde (MDA), nitric oxide (NO), and ceruloplasmin (Cp), along with white blood cell (WBC), red blood cell (RBC), hematocrit (Hct), hemoglobin (Hb), and platelet (THR) counts. Hpt ($P<0.001$), MDA, and Cp ($P<0.05$) levels were significantly higher in colic-affected horses compared to controls, whereas Alb was significantly lower ($P<0.05$). WBC, Hct, Hb, and THR were also significantly elevated ($P<0.05$). Among vital signs, respiratory and heart rates, as well as capillary refill time, were significantly increased in the colic group ($P<0.05$). In conclusion, the concurrent assessment of vital signs and selected biochemical, acute phase, and oxidative stress markers may provide valuable diagnostic insights in the evaluation of colic in horses.

Keywords: Acute phase proteins, colic, hematology, horse, oxidative stress

INTRODUCTION

Colic defined as abdominal pain often associated with visceral discomfort or cramping is a common and potentially life threatening condition in horses, particularly when linked to gastrointestinal disorders (Kızıllı, 2006; Karela et al., 2023). Its high incidence is attributed to species-specific anatomical and physiological features such as a small stomach with no vomiting ability, a long intestinal tract, a loosely suspended mesentery, and the cecum's blind sac structure. The equine enteric nervous system's sensitivity to thermal and chemical stimuli further increases susceptibility (Kızıllı, 2006). Severe colic may lead to complications like endotoxemia, impaired perfusion, systemic inflammation, disseminated intravascular coagulation, and multiple organ dysfunction (Dondi et al., 2015). Among the main causes, nutritional mismanagement is significant. Overfeeding grains (e.g., corn, barley, wheat, rye) and concentrates, as well as factors like abrupt dietary changes, excessive salt, moldy feed, and irregular feeding schedules, contribute to risk (Nursoy, 2008).

Acute phase proteins (APPs) are liver-synthesized plasma glycoproteins that play a central role in the immune response to inflammation. Their serum levels may rise (positive APPs) or fall (negative APPs) depending on the

inflammatory stimulus (Deniz and Cıtil 2022; Ludwig et al., 2023). In veterinary medicine, assessing APPs in animals with severe abdominal pain has shown diagnostic and prognostic value (Dondi et al., 2015; Pihl et al. 2016; Peixoto Rabelo et al. 2023). Given the limited data on equine acute phase responses, evaluating these proteins in conditions like colic holds particular clinical relevance (Pihl et al. 2015; Mihelić et al. 2022; Canola et al., 2024).

Haptoglobin (Hpt), an acute phase protein, plays a protective role by binding free hemoglobin (Hb) and myoglobin, thereby mitigating their biochemical toxicity, reducing oxidative damage, and preventing iron loss. By forming stable complexes with Hb, Hpt limits the availability of free iron essential for bacterial growth, thus exerting a bacteriostatic effect (Ludwig et al., 2023). This binding also facilitates the sequestration of Hb and myoglobin within the intravascular compartment, preventing their filtration into the renal tubules and protecting against renal tubular cell injury (Dondi et al., 2015). Ceruloplasmin (Cp) is classified as a positive acute phase protein synthesized by the liver (Ceron et al., 2005). Its primary role is to bind free copper in the serum and to limit oxidative damage associated with iron metabolism during inflammatory responses (Cray, 2012). In addition to its metal-binding properties, Cp functions as a plasma

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antioxidant and exhibits anti-inflammatory activity by reducing neutrophil accumulation in the vascular endothelium (Murata et al., 2004). Albumin (Alb) is a plasma protein synthesized exclusively by the liver and is considered a negative acute phase protein. It plays a critical role in maintaining intravascular oncotic pressure and acts as a reservoir of amino acids for peripheral tissues. Alb constitutes approximately 35–50% of total plasma proteins. During tissue injury and inflammatory states, its synthesis decreases while catabolism increases, resulting in a significant reduction in serum albumin levels (Emek Tuna and Ulutaş, 2015).

Oxidative stress is a pathological condition arising from an imbalance favoring oxidants over antioxidants, leading to lipid peroxidation and reactive oxygen species production (Ercan and Fidancı, 2012). Inadequate antioxidant defenses result in cellular damage and impaired function. In horses, colic-related intestinal ischemia compromises the mucosal barrier, often causing endotoxemia and systemic oxidative stress. Assessing oxidative stress markers offers valuable prognostic insight, particularly in colic. In this study, nitric oxide (NO) and malondialdehyde (MDA) were measured to explore oxidative stress involvement. NO is produced by mononuclear phagocytes during inflammation (Hajimohammadi et al., 2023), while MDA, a byproduct of lipid peroxidation and arachidonic acid metabolism, reflects phospholipid membrane damage and is a recognized marker of oxidative stress (Tesena et al., 2024).

This study aimed to evaluate the levels of Hpt, Cp, Alb, MDA, and NO, along with selected vital signs and hematological parameters, in horses clinically diagnosed with colic. We hypothesized that horses clinically diagnosed with colic would exhibit significant and quantifiable deviations in acute phase proteins, oxidative stress biomarkers, and selected hematological and vital parameters relative to healthy controls, thereby reflecting the systemic inflammatory and pathophysiological perturbations associated with the colic process.

MATERIALS AND METHODS

Ethical approval

This study was conducted following the approval of the Local Animal Experiments Ethics Committee of Kafkas University (KAU-HADYEK/2023-172), Kars, Türkiye.

Animal material

The study population comprised a total of 50 horses. Of these, 30 horses (14 male and 16 female), aged between 2 and 10 years and representing various breeds, were presented to the Department of Internal Medicine at the Animal Health Education, Research, and Application Hospital, Faculty of Veterinary Medicine, Kafkas University, with complaints of colic associated with dietary changes. All colic-affected horses included in the study were classified as medically managed (non-surgical) based on clinical evaluation, and none required surgical intervention. According to owner reports, these horses were brought to the clinic approximately 4 to 8 hours after the initial onset of colic symptoms. Blood samples were collected immediately upon clinical admission and prior to the initiation of any medical treatment. The

remaining 20 horses (8 male and 12 female), clinically healthy and within the same age range, were used as the control group.

Physical examination and evaluation of vital signs

Horses diagnosed with colic were subjected to a thorough physical examination, and their clinical responses during episodes of abdominal pain were closely monitored. The conjunctiva and mucous membranes of all horses were evaluated following standard systemic examination protocols. In addition, rectal temperature, respiratory rate, heart rate (beats per minute), and capillary refill time were measured and documented.

Blood sampling, biochemical and hematological measurements

Blood samples were collected from colic-affected horses prior to treatment and from healthy controls once, using vacuum serum gel tubes and K₃-EDTA tubes. The serum tubes were centrifuged at 3000 rpm for 10 minutes (Hettich Rotina 380R®, Hettich, Germany) to separate the serum, which was then stored at –20°C until analysis.

Acute phase proteins, oxidative stress markers, and selected biochemical parameters were analyzed from the serum samples. Alb concentrations were measured using a fully automated clinical chemistry analyzer (Mindray BS120®, Mindray Medical Technology, İstanbul, Türkiye).

Ceruloplasmin levels were determined spectrophotometrically by measuring the oxidative conversion of p-phenylenediamine to a blue-colored complex at 546 nm, as described by Colombo and Richterich (1964). MDA levels were assessed using the thiobarbituric acid reactive substances (TBARS) assay, in which MDA reacts with thiobarbituric acid under acidic and high-temperature conditions to produce a pink chromogen, measured at 532 nm (Yoshioka et al., 1979).

Nitric oxide levels were quantified using the Griess reaction, involving enzymatic reduction of nitrate to nitrite by nitrate reductase, followed by reaction with Griess reagents to form a chromophore measurable at 540 nm (Miranda et al., 2001). Hpt concentrations were measured colorimetrically using a commercial kit (Tridelta Development Limited, Ireland), based on the formation of a peroxidase-Hpt-Hb complex, with absorbance read at 630 nm on a microplate reader (Epoch, Biotek, USA).

Whole blood samples collected in K₃-EDTA tubes were analyzed using an automated hematology analyzer (VG-MS4e®, Melet Schloesing, France) to determine total leukocyte (WBC, $\times 10^3/\mu\text{L}$), erythrocyte (RBC, $\times 10^6/\mu\text{L}$), hematocrit (Hct, %), Hb, (g/dL), and platelet (THR, $\times 10^3/\mu\text{L}$) counts.

Statistical analysis

Statistical analyses were conducted using SPSS® software (Version 26.0; IBM Corp., Chicago, IL, USA). The distribution of data was assessed with the Shapiro-Wilk test. For variables that were not normally distributed, the Mann-Whitney U test was applied, while normally distributed variables were analyzed using the Independent Samples t-test. A p-value of <0.05 was considered statis-

tically significant in all analyses. Variables that did not show follow a normal distribution included NO, Hct, Hb, capillary refill time, WBC, RBC, rectal body temperature, Hpt, MDA, Cp, and Alb. These were analyzed using the Mann-Whitney U test. Conversely, THR, respiratory rate, and heart rate per minute were normally distributed and analyzed using the Independent Samples t-test. All quantitative data are reported as mean $\pm$ standard deviation (SD).

RESULTS

Physical examination and vital parameters

In horses diagnosed with colic, clinical signs indicative of pain were frequently observed. These included dryness and hyperemia of the conjunctiva and mucous membranes, frequent weight shifting between limbs, sweating, flank-watching behavior, and a tendency to lie down and roll.

Evaluation of vital signs revealed that both respiratory rate and heart rate per minute were significantly increased in the colic group compared to healthy controls ($P<0.001$). Capillary refill time (seconds) was also significantly prolonged in horses with colic ($P<0.001$).

In contrast, rectal body temperature remained within the reference range in both groups and did not show differ significantly ($P>0.05$). A comparison of vital parameters between colic-affected and healthy horses is summarized in Table 1.

Hematological findings

In horses with colic, total leukocyte and platelet (THR) counts were significantly increased compared to the control group ($P<0.05$). Hct (%) and Hb (g/dL) levels were also notably elevated in the colic group ($P<0.05$).

Although red blood cell (RBC) counts in colic-affected horses approached the upper limit of the reference range, no statistically significant difference was observed between the groups ($P>0.05$). Comparative hematological data are presented in Table 2.

Comparative analysis of biochemical parameters between colic-affected and healthy horses is presented in Table 3. Among the acute phase proteins, Hpt levels were significantly elevated in the colic group compared to controls ($P<0.001$). Cp concentrations also demonstrated a significant increase ($P<0.05$). In contrast, Alb a negative acute phase protein, showed a significant decrease in colic-affected horses ($P<0.05$). Regarding oxidative stress markers, MDA levels were markedly higher in the colic group ($P<0.05$), whereas no statistically significant difference was observed in NO levels between the two groups ($P>0.05$).

DISCUSSION

Clinical signs frequently observed in horses with colic include pawing with the forelimbs, sweating, restlessness, frequent attention to the abdominal region, a tendency to lie down, dog-sitting posture, and dryness and

Table 1. Statistical comparison of vital parameter values between colic-affected and healthy horses

Parameters	Colic (n=30)	Control (n=20)	Reference (Alshami et al., 2024)	P value
Rectal Body Temperature (°C)	38 $\pm$ 0.19	38.24 $\pm$ 0.10	37.78-38.44	$P>0.05$
Respiratory rate (Per minute)	35 $\pm$ 2.05	15.30 $\pm$ 0.90	12-19	$P<0.001$
Heart rate (Per minute)	53.90 $\pm$ 1.42	33.20 $\pm$ 1.06	30-39	$P<0.001$
Capillary refill time (Seconds)	2.45 $\pm$ 0.11	1.30 $\pm$ 0.15	1-2	$P<0.001$

Data are presented as mean $\pm$ SD.

Table 2. Comparative analysis of hematological parameters in colic-affected and healthy horses

Parameters	Colic (n=30)	Control (n=20)	Reference (Alshami et al., 2024)	P value
WBC ($\times 10^3/\mu\text{L}$)	11.95 $\pm$ 1.04	7.94 $\pm$ 0.45	7.4-12.4	$P<0.05$
RBC ($\times 10^6/\mu\text{L}$)	10.19 $\pm$ 0.45	8.84 $\pm$ 0.33	7.2-9.2	$P>0.05$
Hct (%)	50.98 $\pm$ 3.38	39.18 $\pm$ 1.08	32.2-41.2	$P<0.05$
Hb (g/dL)	16.81 $\pm$ 0.83	13.63 $\pm$ 0.49	11.2-15.3	$P<0.05$
THR ($\times 10^3/\mu\text{L}$)	315.70 $\pm$ 36.10	149.40 $\pm$ 7.04	180-345	$P<0.05$

WBC: white blood cell; RBC: red blood cell; Hct: hematocrit; Hb: hemoglobin; THR: platelet. Data are expressed as mean $\pm$ SD.

Table 3. Comparison of biochemical parameters between colic-affected and healthy horses

Parameters	Colic (n=30)	Control (n=20)	P value
Hpt (mg/mL)	0.76 $\pm$ 0.04	0.49 $\pm$ 0.04	$P<0.001$
Cp (mg/dL)	11.76 $\pm$ 0.61	8.92 $\pm$ 0.98	$P<0.05$
MDA ($\mu\text{mol/L}$)	2.12 $\pm$ 0.07	1.72 $\pm$ 0.15	$P<0.05$
NO ($\mu\text{mol/L}$)	4.67 $\pm$ 0.10	4.73 $\pm$ 0.13	$P>0.05$
Alb (g/dL)	2.67 $\pm$ 0.06	2.87 $\pm$ 0.05	$P<0.05$

Hpt: haptoglobin, Cp: ceruloplasmin, MDA: malondialdehyde, NO: nitric oxide. Data are expressed as mean $\pm$ SD.

hyperemia of the conjunctiva and mucous membranes (Onmaz and van den Hoven, 2012). In the present study, similar clinical findings were recorded, and these were consistent with the signs previously described in the literature.

Gastrointestinal dysfunction is a common etiological factor in the pathogenesis of colic in horses, with nutritional mismanagement playing a significant contributory role. In particular, excessive intake of grains such as corn, concentrate feed, barley, wheat, and rye has been frequently reported as a trigger for colic episodes (Nursoy, 2008). In the present study, owner-reported history revealed that colic symptoms commonly developed following increased consumption

In colic cases, increased heart rate is commonly attributed to pain, toxemia, and circulatory disturbances, whereas rectal body temperature often remains within the reference range and may not be significantly affected (Clark, 2016). Consistent with these observations, tachycardia was also noted in the present study among colic-affected horses, which may be attributed to stress, impaired circulation, and toxemia. However, no statistically significant difference in rectal body temperature was found between the colic and control groups.

Pain, metabolic acidosis, and gastrointestinal distension associated with colic in horses can result in tachypnea. Alshami et al. (2024) reported that these pathophysiological changes in colic cases lead to elevated respiratory rates and prolonged capillary refill time. Similar findings have also been documented by Khosa et al. (2023) and Sharma et al. (2022). In accordance with these reports, the present study demonstrated a significant increase in respiratory rate and a marked prolongation of capillary refill time in colic-affected horses.

Westerman et al. (2016) reported that the normal WBC count in healthy horses ranges between 4.8 and $9.8 \times 10^3/\mu\text{L}$. In colic cases, leukocyte levels are often elevated as a result of inflammation and endotoxemia (Tripathi et al., 2018; Deniz et al. 2024a). Similarly, Purnama et al. (2021) noted that leukocytosis is commonly observed in horses affected by colic. Consistent with these findings, a significant elevation in WBC levels was observed in the present study, presumably resulting from secondary infection and endotoxemia associated with colic.

In colic cases, elevated Hct levels are primarily attributed to splenic contraction, plasma volume loss due to dehydration, hypovolemic shock, and stress (Alshami et al., 2024). Dehydration, in particular, contributes to venous depletion and peripheral vasodilation, along with a fluid shift from the intravascular compartment to the intestinal lumen (Tripathi et al., 2018). Consistent with these pathophysiological mechanisms, significant increases in Hct percentage and Hb concentration were observed in colic-affected horses in the present study.

Reactive thrombocytosis is a frequently observed hematological response in animals and is commonly associated with acute conditions such as physical trauma, hemorrhage, infection, inflammation, or stress-related stimuli like excitement or intense exertion (Sellon et al., 1997; Kim et al., 2013). In the present study, significantly elevated platelet counts were identified in horses with colic compared to healthy controls. This increase may

be attributed to stress associated with abdominal pain, enhanced physical activity, systemic inflammation, and possible secondary infections.

Colic is a frequently encountered yet potentially life-threatening condition in horses that can induce hemodynamic alterations due to tissue damage and may result in mortality if not promptly addressed. In such cases, accurate prognostic evaluation can be enhanced by the integrated assessment of clinical signs in conjunction with vital, hematological, acute phase, and oxidative stress parameters (Cray and Belgrave, 2014; Merhan and Bozukluhan, 2024).

Haptoglobin, one of the major acute phase proteins, increases in a wide range of inflammatory conditions, including infection, stress, trauma, and allergy (Cray and Belgrave, 2014). During colic, intestinal ischemia frequently occurs in horses, which leads to the systemic release of proinflammatory molecules such as hydrogen peroxide, superoxide radicals, cytokines, and arachidonic acid metabolites. These mediators contribute to tissue damage and are associated with elevated serum levels of oxidative stress markers (Maranon et al., 2009). An increase in MDA levels is considered a reliable indicator of lipid peroxidation secondary to intestinal ischemia and aligns with previous research findings (Hajimohammadi et al., 2023; Maranon et al., 2009). However, some studies have reported a more pronounced elevation in MDA levels in severe colic cases requiring surgical intervention (Smith et al., 2012). In the present study, most cases responded to medical treatment alone, which may account for the relatively moderate increase in MDA concentrations.

Nitric oxide, a marker of oxidative stress, does not always show statistically significant variation in certain pathological conditions, including secondary bacterial infections. This phenomenon has been attributed to the presence of oxygen-free radicals such as superoxide, which can suppress NO activity even though bacterial infections are known to promote lipid peroxidation (El-Bahr and El-Deeb, 2016). While some studies have reported significant increases in NO levels in horses with colic (e.g., Smith et al., 2012), no such increase was observed in the present study. This discrepancy may be related to the relatively mild severity of the colic cases, the timing of blood sample collection, or the inhibitory effect of superoxide radicals on NO synthesis or activity. Nevertheless, colic-affected horses in this study exhibited significantly elevated Hpt and MDA levels, likely reflecting the influence of stress, intestinal ischemia, and endotoxemia. These findings are consistent with previous reports by Hajimohammadi et al. (2023) and Zuluga et al. (2016).

In this study, Cp concentrations were significantly higher in the colic group ($P < 0.05$), a finding consistent with a hepatic acute-phase response triggered by endotoxin and cytokine spillover particularly interleukin-6 following disruption of the intestinal barrier in colic (Murata et al., 2004; Cerón et al., 2005). In horses, Cp is a moderately positive acute-phase protein, and its response typically peaks at 48–72 h; therefore, sampling within 4–8 h of onset is expected to capture the rising phase and yield a modest yet significant increase (Murata et al., 2004; Cerón et al., 2005).

In the literature, experimental obstruction models and clinical series enriched for surgical or severe cases most often inferring Cp indirectly via the α_2 -globulin fraction on serum protein electrophoresis have reported more pronounced increases, generally associated with later sampling windows and greater clinical severity (Di Filippo et al., 2010). By contrast, early-presenting, conservatively managed cohorts have shown limited or equivocal changes (Souto et al., 2019). In our early, non-surgical series, a modest but significant rise detected by direct Cp measurement aligns with the intermediate kinetics of Cp and with this timing- and severity-dependent pattern. Nevertheless, single-time-point sampling and the exclusion of surgical cases warrant cautious interpretation regarding peak magnitude and generalizability to surgical subtypes; even so, the data support the onset of an early acute-phase response in non-surgical colic.

Albumin is a protein synthesized exclusively by the liver, accounting for approximately 35–50% of total plasma proteins, and plays a crucial role in maintaining intravascular oncotic pressure. During inflammation and tissue injury, albumin catabolism increases, typically resulting in a 20–50% decrease in serum Alb concentrations. Therefore, it is classified as a negative acute phase protein (Emek Tuna and Ulutaş, 2015; Deniz et al 2024b). In the present study, a statistically significant decrease in Alb levels was observed in horses with colic, presumably attributable to tissue damage, inflammatory processes, and possible secondary infections. These findings align with the well-documented role of albumin as a negative acute phase reactant in systemic inflammatory conditions.

An increase in Hpt and Cp with a decrease in Alb indicates the onset of an acute-phase response, while a rise in MDA provides additional evidence of oxidative stress. This panel supports triage decisions when the physical examination and routine hematology are borderline, adding weight to the choice between hospital admission and outpatient follow-up. Repeating the measurements within 12–24 hours helps monitor treatment response: decreases in Hpt/Cp and recovery in Alb are consistent with improvement, whereas a continued rise in MDA or persistently high Hpt/Cp suggests the need for closer monitoring, treatment adjustment, or referral. The absence of an early change in NO implies that a single measurement has limited discriminatory value and that this marker may become more informative in serial assessments or at later time points. Given the study design early sampling and exclusion of surgical cases these biomarkers should not be used as definitive diagnostic tools but rather alongside clinical examination and basic laboratory tests, with particular attention to their trends over time.

This study has some limitations. The relatively small sample size, especially in the colic and control group, may restrict the generalizability of the findings. In addition, the study did not show classify colic cases by severity or subtype, which may influence biomarker responses. Only single-time-point sampling was performed, limiting insights into dynamic changes. Future research should include larger populations, classification of colic types, and serial biomarker measurements. Combining clinical, biochemical, and imaging data can enhance diagnostic and prognostic accuracy in equine colic.

CONCLUSION

In conclusion, based on the findings, colic remains a prevalent and potentially life-threatening clinical condition in horses, capable of inducing hemodynamic disturbances due to underlying tissue damage if not managed promptly. In such scenarios, the integrated assessment of clinical signs, vital and hematological parameters, acute phase proteins, and oxidative stress markers may enhance the diagnostic and prognostic precision of clinical evaluations. The findings of this study underscore the relevance of these biomarkers in the comprehensive assessment of equine colic cases and support their use as valuable tools for guiding clinical decision-making.

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Conflict of interest

The authors declare no conflict of interest.

Ethical statement

This study was conducted following the approval of the Local Animal Experiments Ethics Committee of Kafkas University (KAU-HADYEK/2023-172), Kars, Türkiye.

Author contributions

The initial draft, conceptualization, study design, and methodology were conceived and written by YUB and MS. Experimental procedures and clinical data collection were conducted by CŞE, EEE, UA, ET and TG. Laboratory analyses and biochemical evaluations were performed by OM, EA and YUB. Statistical analysis and data interpretation were carried out by GG and AHK. All authors contributed to manuscript editing and revisions. Each author has read and approved the submitted version of the manuscript and agreed to be accountable for the content.

Availability of data and materials

All data generated or analyzed during the current study are included in this article.

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