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Alterations in the lipid profile and oxidative stress associated with anorexia in donkeys with large intestinal impaction

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ABSTRACT

Hyperlipemia is one of the most common and life-threatening conditions, frequently associated with anorexia secondary to many disease conditions. The aim of the present investigation was to assess the lipid profile and oxidative stress associated with different durations of anorexia in donkeys with large intestinal impaction. Twenty-three non-pregnant female donkeys (7–13 years, 270–300 kg) affected by large intestinal impaction and varying durations of anorexia (1, 3, 5, and ≥ 6 days) were investigated. Blood samples were collected and analyzed for lipid profile, oxidative stress markers, hepatic indicators, and antioxidant defenses. Results revealed progressive significant increases ($p < 0.05$) in serum triglycerides, LDL, uric acid, nitric oxide, lipid peroxidase, and bilirubin, indicating enhanced fat mobilization, hepatic strain, and oxidative stress. Conversely, antioxidant defenses such as catalase activity and vitamin C declined sharply, while HDL levels also decreased significantly ($p < 0.05$). Albumin showed a mild reduction, whereas total protein remained stable. The findings highlight that prolonged anorexia, especially beyond 5–6 days, induces severe metabolic dysregulation and oxidative imbalance, predisposing donkeys to hyperlipemia and hepatic dysfunction. Early nutritional and antioxidant intervention is therefore crucial to mitigate these risks and improve survival outcomes in anorexic donkeys suffering from impaction of the large intestine.

Introduction

Donkeys (*Equus asinus*) play a vital role in the livelihoods of rural and agricultural communities, particularly in developing countries. They are widely used for transportation, cart-pulling, and agricultural labor, making them indispensable working animals. Despite their significant socioeconomic value (Rodrigues *et al*., 2021), donkey health remains largely neglected, This has contributed to a lack of research into common diseases affecting donkeys, especially gastrointestinal disorders such as impaction of the large intestine.

Impaction of the large intestine has been identified as a leading cause of morbidity and mortality in donkeys, accounting for up to 54.8% of colic cases in some populations. It is often associated with secondary complications such as anorexia and metabolic disturbances like hyperlipemia, which can be life-threatening if not managed promptly (Cox *et al*., 2007; Hamed *et al*., 2017). Therefore, greater attention to the clinical and metabolic consequences of anorexia associated with impaction of the large intestine is essential for improving donkey welfare and health outcomes.

Hyperlipemia is a dyslipidemia defined as abnormally high blood triglyceride concentrations (hypertriglyceridemia). It is one of the most common diseases in donkeys. Although epidemiologic data are lacking in this species, it is estimated to have a high incidence (10%) (Burden *et al*., 2011). It is caused by a negative energy balance from a primary clinical disorder (e.g., colic, pleuropneumonia, laminitis, parasitism, etc.), stressful situations (e.g., transport, husbandry changes, hospitalization, etc.) or physiological needs (e.g., pregnancy and lactation) (Mendoza *et al*., 2019).

No primary causes of dyslipidemia (as seen e.g., in cats) have been reported in donkeys. Liver disease (19.4%) followed by colic (13.8%) were the most common primary conditions in a study of 449 dyslipemic donkeys (Burden *et al*., 2011).

Regarding the pathogenesis of hyperlipemia is that When donkeys experience anorexia whether due to illness, pain, stress, or medical treatment their energy intake drops below the baseline requirements of essential physiological functions. As a result, they enter a state known as negative energy balance (NEB) (Hassan *et al*., 2022).

Hormone-sensitive lipase starts body fat mobilization, free fatty acids (FFAs) are released to the bloodstream, and the liver begins to re-esterify FFA into triglycerides (Mendoza *et al*., 2018), The donkey liver is very efficient at transforming FFA into triglycerides. When this metabolic pathway is overwhelmed, triglycerides are released into the bloodstream as very low-density lipoproteins (VLDL) (Mendoza *et al*., 2024a). Unlike ruminants and cats, the liver of equids does not produce ketone bodies. If lipolysis persists, plasma triglycerides levels increase (hyperlipemia), resulting in macroscopic lipemia (from turbid to creamy plasma) and ultimately fatty infiltration of multiple organs (most commonly the liver, but also kidneys, intestine, pancreas, heart, and skeletal muscle). Severe liver infiltration, in addition to causing dysfunction may lead to hepatic rupture (Barton, 2010). Cortisol, which increases in stressed animals, may contribute to the development of hyperlipemia by activating hormone-sensitive lipase, fat mobilization (Frank *et al*., 2003), impairing triglyceride clearance by lipoprotein lipase (Watson, 1998), and reducing insulin sensitivity, emphasizing the relevance of stress as a risk factor for this condition in donkeys (Forhead *et al*., 1995).

Lipoprotein lipase is an extracellular enzyme on the endothelial surface (predominantly present in adipose tissue and heart and skeletal muscle) that is important for triglyceride removal from the circulation and subsequent translocation into cells (Mendoza *et al*., 2024a). Transport can be also stressful for donkeys, and some animals may develop hyperlipemia after transportation (Fazio *et al*., 2013). Hormone-sensitive lipase can also be activated by catecholamines, some interleukins, glucocorticoids, adrenocorticotropic hormone (ACTH), and glucagon and is inhibited by insulin (Tarrant *et al*., 1998).

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This metabolic cascade is further exacerbated by hormonal responses: decreased insulin due to low circulating glucose from anorexia increases lipase activity, while elevated cortisol and catecholamines promote lipolysis. The imbalance between fat mobilization and peripheral tissue uptake results in a dangerous accumulation of lipids in the bloodstream and key organs (Morrone *et al*., 2024). Therefore, this study aimed to investigate the impact of anorexia associated with impaction of the large intestine in donkeys over different time intervals specifically after 1, 3, 5, and 6 or more days on various physiological and biochemical changes, including alterations in the lipid profile and other related parameters.

Materials and methods

Animals

A total of 23 donkeys (*Equus asinus*) were included in this investigation. The age of the examined donkeys was 7-13 years old and the weight was ranged from 200-290 kg. All donkeys under investigations were female non pregnant. This study was carried out at Veterinary teaching hospital, Mansoura University, during the period from September 2020 to August 2024.

Clinical examination and selection of cases

Donkeys were clinically examined at the admission according to standard methods (Constable *et al*., 20016). The selection of the clinical cases based on sharing the same clinical signs. All cases were diagnosed to have large intestinal impaction, constipation and anorexia. The duration of anorexia varied from case to another, consequently the classification of animal groups was based on the duration of anorexia associated with impaction of the large intestine.

Samples

Blood samples were collected from each donkey by jugular vein via needle (1.2 mm × 38 mm) to normal tubes then transferred to biochemical lab in ice box and then centrifuged at 3000g for 25 min. Serum samples were retained in Eppendorf tubes in a refrigerator at − 20°C until analysis time (Kısadere *et al*., 2019) for estimation of BUN, creatinine, uric acid, triglycerides, lipid peroxides, GGT, total bilirubin, AST, catalase, LDL, HDL, Vit. C, nitric oxide, total protein, and albumin.

Biochemical analysis

All the serum biochemical parameters were estimated following standard methods of enclosed pamphlet using commercial kits (Spinreact, Girona, Spain). Serum uric acid was determined according to the method of Fossati *et al*. (1980) and Olisekodiaka *et al*. (2017).

Serum lipid profile including serum levels of total cholesterol was determined according described method (El-Kashef, 2022; Stein, 1986), as well as triglycerides (Elgazar *et al*., 2022; Wahlefeld, 1974) and high-density lipoprotein (Burstein *et al*., 1970; Younas, 2025). Meanwhile, low density lipoproteins (LDL) could be calculated according to specific technique (Friedewald *et al*., 1972; Ribeiro *et al*., 2023).

Lipid peroxidation was determined by measuring the formed MDA (an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method (Buege and Aust, 1978). This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and MDA, the product of lipid peroxidation was measured at 532 nm. MDA concentration was calculated using extinction coefficient value(ε) of MDA-thiobarbituric acid complex (1.56×10^5 /M/cm) (Aguilar Diaz De Leon and Borges, 2020). Serum blood urea nitrogen (BUN), creatinine, total bilirubin in addition to serum activities of aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) were measured using commercial kits supplied by Spinreact (Spinreact, GIRONA, Spain), using UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd., Korea) (Abd Ellah *et al*., 2013).

Catalase enzyme was determined by monitoring the decomposition of hydrogen peroxide (Aebi, 1984; Abedi *et al*., 2010). Nitrite concentration (an indirect measurement of NO synthesis) was assayed using Griess reagent (sulfanilamide and N-1-naphthylethylenediamine dihydrochloride) in acidic medium (Bharwani *et al*., 2025; Moshage *et al*., 1995). Vitamin C was estimated using Folin-Ciocalteu reagent. The color developed was read at 760 nm (Jagota and Dani, 1982).

Serum proteins including Spectrophotometric measurements of serum total proteins and albumin were done using commercial kits supplied by Spinreact (Spinreact, GIRONA, Spain) and using UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd., Korea) (Abd Ellah *et al*., 2013).

Statistical analysis

Data analyses were carried out using statistical software program (SPSS for windows Version 15.01, USA). Normal distribution check of the data was applied using Shapiro-Wilk Test. As the data were found normally distributed, the results were presented as means±SD. Analysis of variance (ANOVA) with post hoc Duncan multiple comparison test was used to determine significant differences between values. Differences were considered significant when $P < 0.05$.

Results

Clinical findings in donkeys with large intestinal impaction and associated anorexia varied moderately. Few cases with prolonged anorexia showed collapse and nervous manifestations (Table 1).

Table 1. The characteristic signs that are associated with large intestinal impaction and its outcome in donkeys.

Clinical signs	Frequency	%
Dullness	23	100
Intestinal sound		
Absent	21	91.3
Present (weak)	2	8.69
Defecation		
Constipation	22	95.65
Normal fecal balls	1	4.35
Outcome.		
Collapse	3	13.04
Seizures	1	4.35
Guarded	19	82.6
Respiratory rate (cycle/min)		
Increased (23.0±7.0)	18	78.26
Normal (14.0±4.0)	5	21.73
Mucous membrane		
Pale	17	73.91
Brick red	6	26.08

The results show a progressive increase in serum triglyceride concentrations in donkeys subjected to anorexia associated with impaction of the large intestine. On day 1, the mean triglyceride level was 196.0±23.0 mg/dL, which increased slightly by day 3 to 201.0±43.0 mg/dL. A more noticeable rise was observed by day 5 (220.0±33.0 mg/dL), and the highest levels were recorded after 6 days or more of anorexia, reaching 224.6±36.0 mg/dL (Figures 1).

Lipid peroxides levels, a key marker of oxidative stress, showed a progressive increase in response to prolonged anorexia in donkeys

with impaction of the large intestine. On day 1, the mean level was 5.24±2.10 nmol/mL, which rose to 6.22±1.70 nmol/mL by day 3. This increase continued on day 5 (7.89±2.20 nmol/mL), reaching a peak of 8.64±3.60 nmol/mL after 6 days or more of feed deprivation (Figure 2).

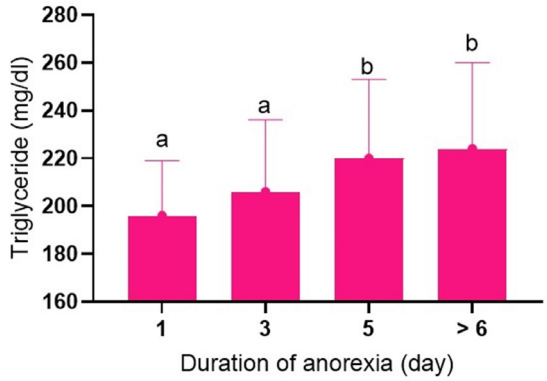

Figure 1. Changes in serum triglyceride concentrations in donkeys with impaction of the large intestine at different durations of anorexia.

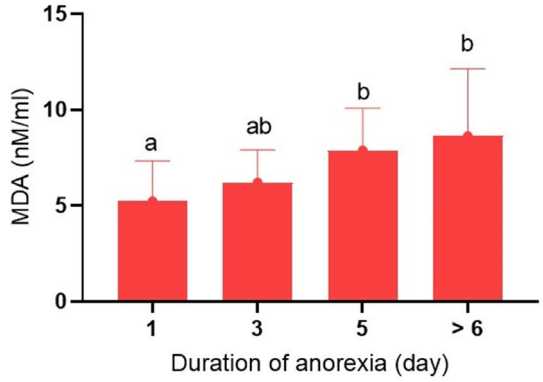

Figure 2. Changes in serum lipid peroxides concentrations in donkeys with impaction of the large intestine at different durations of anorexia.

Low-density lipoprotein (LDL) levels increased progressively with the duration of anorexia in donkeys affected by impaction of the large intestine. On day 1, the mean LDL concentration was 300.0±49.0 mg/dL, which rose slightly by day 3 to 308.0±32.0 mg/dL. A more noticeable increase was observed on day 5 (336.0±51.0 mg/dL), with levels peaking after 6 or more days at 353.0±29.0 mg/dL (Figure 3).

High-density lipoprotein (HDL) concentrations showed a marked progressive decline with increasing duration of anorexia in donkeys suffering from impaction of the large intestine. On day 1, HDL levels were 34.1±9.2 mg/dL, which significantly dropped to 22.7±3.7 mg/dL by day 3. The decline continued on day 5 (20.6±4.5 mg/dL), reaching the lowest recorded level after 6 or more days at 13.8±8.1 mg/dL (Figure 3).

Regarding BUN, as shown in Figure 4; at 1–3 days of anorexia, BUN values are relatively low (~20–22 mg/dl) and not significantly different. After 5 days, BUN increases significantly (~40 mg/dl). As well as at ≥ 6 days, BUN rises further (~55–60 mg/dl), significantly higher than all previous groups.

Concerning creatinine, at 1–3 days, it remains relatively low (~0.9–1.0 mg/dl), with no significant difference. At 5 days, creatinine increases significantly (~1.8–2.0 mg/dl). But at ≥ 6 days, creatinine rises further (~2.5–3.0 mg/dl), still significantly higher than early group (Figure 4).

Serum uric acid levels increased progressively in donkeys undergoing prolonged periods of anorexia due to impaction of the large intestine. On day 1, the mean concentration was 6.88±2.0 mg/dL, rising modestly by day 3 to 7.44±2.30 mg/dL. A more pronounced elevation was observed by day 5 (10.8±2.7 mg/dL), with levels peaking after 6 or more days of anorexia at 14.1±2.8 mg/dL (Figure 4).

Serum total bilirubin concentrations showed also a marked elevation with prolonged periods of anorexia. On day 1, the mean total bilirubin level was within the normal range (0.41±0.12 mg/dL), and it rose slightly by day 3 to 0.49±0.21 mg/dL. However, by day 5, there was a dramatic increase to 50.2±0.6 mg/dL, followed by a further rise to 56.0±0.2 mg/dL after 6 or more days (Figure 5).

Serum AST activity showed a progressive and significant increase with the duration of anorexia in donkeys affected with impaction of the large intestine. On day 1 of anorexia, AST values were relatively low (~20

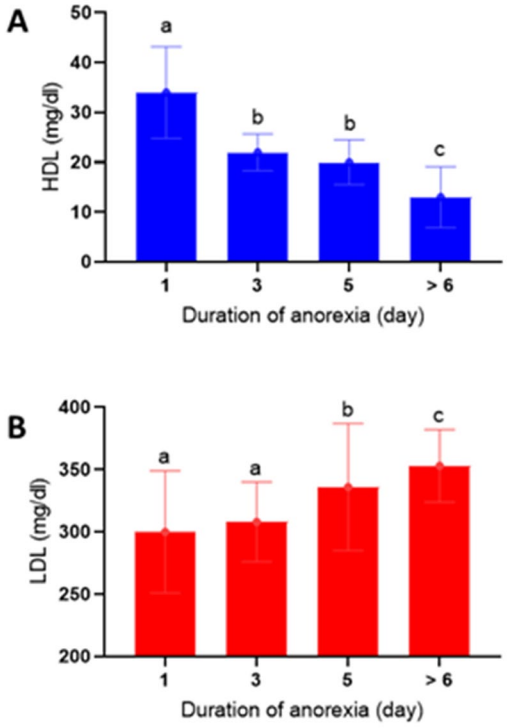

Figure 3. Changes in serum HDL and LDL concentrations in donkeys with impaction of the large intestine at different durations of anorexia.

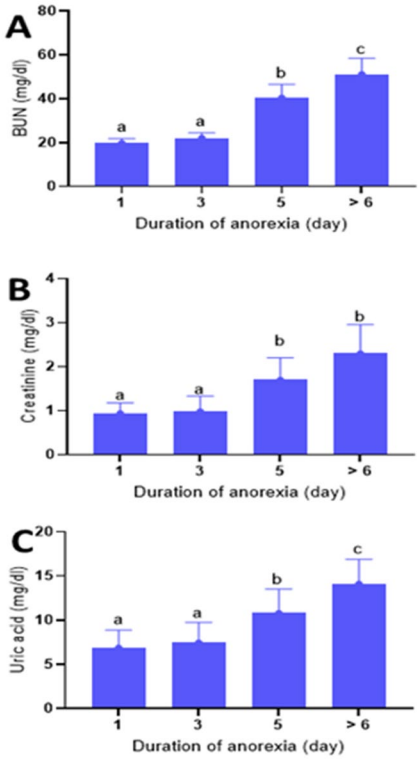

Figure 4. Changes in serum BUN, creatinine and uric acid concentrations in donkeys with impaction of the large intestine at different durations of anorexia.

IU/L). By day 3, levels increased significantly (~30 IU/L). A further marked elevation was observed on day 5 (~50 IU/L). The highest values were recorded after more than 6 days of anorexia (~80 IU/L), which were significantly higher than all previous groups (Figure 5). Concerning GGT; There is a clear progressive increase in GGT levels as the duration of anorexia increases. At 1st day approximately 20 IU/L. At 3rd day approximately 35 IU/L and after 5 days approximately 55 IU/L. in addition to more than 6 days approximately 80 IU/L. This reflects that as the duration of anorexia increases, there is a significant and steady rise in GGT enzyme levels in the blood, with each step showing a statistically meaningful difference from the previous duration group.

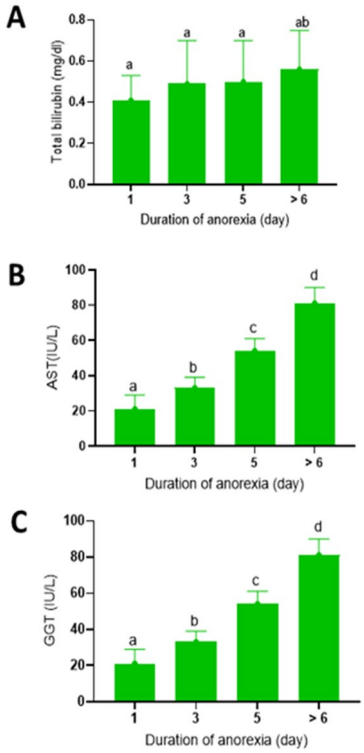

Figure 5. Changes in serum total bilirubin, AST and GGT concentrations in donkeys with impaction of the large intestine at different durations of anorexia.

Catalase activity demonstrated a progressive decline in donkeys suffering from anorexia associated with impaction of the large intestine, with lower values observed as the duration of feed deprivation increased. On day 1 (n = 4), the mean catalase level was 0.088±0.03 U/mL This value decreased by day 3 (n = 7) to 0.070±0.02 U/mL, followed by a sharper drop on day 5 (n = 5) to 0.038±0.01 U/mL, and reached its lowest level after 6 or more days (n = 7) at 0.021±0.01 U/mL (Figure 6).

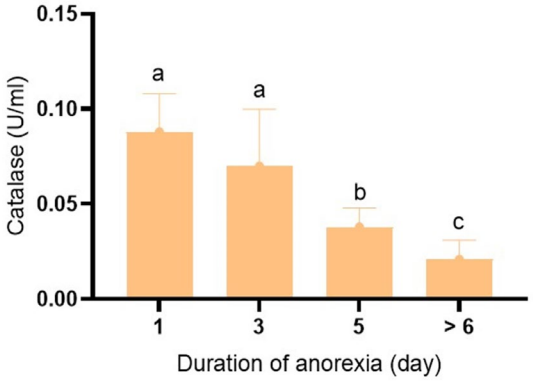

Figure 6. Changes in serum Catalase concentrations in donkeys with impaction of the large intestine at different durations of anorexia.

Vitamin C levels declined significantly with increasing duration of anorexia in donkeys affected by impaction of the large intestine. On day 1, the mean concentration was 6.6±2.4 mg/dL, dropping sharply by day 3 to 3.1±1.6 mg/dL. The decline continued on day 5 (1.8±0.9 mg/dL) and reached its lowest value after 6 or more days at 1.3±0.9 mg/dL (Figure 7).

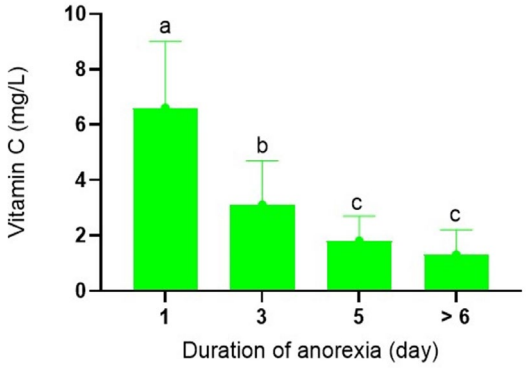

Figure 7. Changes in serum vitamin C concentrations in donkeys with impaction of the large intestine at different durations of anorexia.

Nitric oxide (NO) concentrations increased steadily with prolonged anorexia in donkeys suffering from impaction of the large intestine. On day 1, the mean NO level was 10.6±2.9 µmol/L, rising to 14.8±4.4 µmol/L by day 3. A continued increase was observed on day 5 (15.1±3.6 µmol/L) and peaked after 6 or more days at 16.2±7.1 µmol/L (Figure 8).

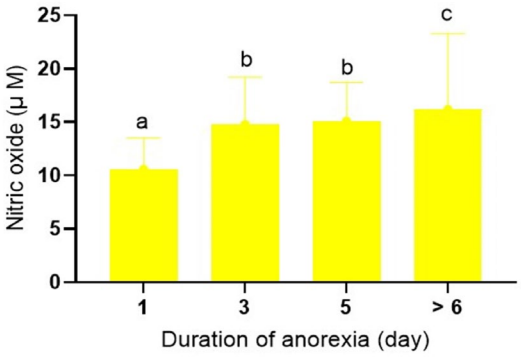

Figure 8. Changes in serum nitric oxide concentrations in donkeys with impaction of the large intestine at different durations of anorexia.

Serum total protein levels remained relatively stable across all durations of anorexia in donkeys affected by impaction of the large intestine. The mean values were 8.1±2.7 g/dL on day 1, 8.2±3.1 g/dL on day 3, 8.0±2.2 g/dL on day 5, and 8.0±3.2 g/dL after 6 or more days.

Serum albumin levels exhibited a mild but progressive decline in donkeys experiencing anorexia due to impaction of the large intestine. On day 1, the mean albumin concentration was 4.1±1.2 g/dL, decreasing slightly to 4.0±2.1 g/dL by day 3. By day 5, levels dropped to 3.9±1.3 g/dL, and further decreased to 3.6±1.8 g/dL after 6 or more days.

Discussion

Donkeys (*Equus asinus*) are indispensable working animals in many rural communities but remain highly neglected in veterinary research, particularly concerning metabolic disorders. Among these, hyperlipemia is one of the most common and life-threatening conditions, frequently associated with anorexia secondary to impaction of the large intestine. The clinical signs of prolonged anorexia associated with large intestinal impaction is not characteristic due to their stoic nature of the donkeys. The animal usually shows less dramatic signs of colic than those seen in horses (Cox *et al*., 2007). The present study demonstrates that anorexia associated with impaction of the large intestine in donkeys initiates a cas-

cade of metabolic and oxidative disturbances, which progressively worsen with time. When compared with findings in the literature, our results align closely with previously described pathophysiological mechanisms in donkeys undergoing negative energy balance (NEB) and hyperlipemia (Mendoza *et al*., 2018; Thiemann and Sullivan, 2019; Meng *et al*., 2024).

The escalation in triglycerides and LDL concentrations over 1, 3, 5, and ≥6 days reflects the intense lipolysis and hepatic lipid re-esterification characteristic of NEB in donkeys. The donkey liver efficiently converts mobilized free fatty acids (FFAs) into triglycerides and very-low-density lipoproteins (VLDL), which eventually spill into the bloodstream when clearance mechanisms are overwhelmed (Mendoza *et al*., 2024b).

In contrast, the progressive decline in HDL levels further indicates impaired lipid transport and reduced antioxidant function, aggravating lipid toxicity–mediated organ stress. HDL's role in reverse cholesterol transport and anti-inflammatory activity is especially critical in counteracting metabolic imbalance (Lewis and Rader, 2005; Chiesa *et al*., 2019; Ouimet *et al*., 2019; Denimal, 2023).

The observed rise in lipid peroxidase (LPO) and nitric oxide (NO), paired with the declines in catalase activity and vitamin C, clearly indicates mounting oxidative stress. Lipid peroxidation degrades cell membranes and generates ROS; the reduced antioxidant reserves (catalase, vitamin C) weaken the donkey's capacity to neutralize this oxidative burden (Mendoza *et al*., 2018; Meng *et al*., 2024; Xu *et al*., 2025).

Marked increase in BUN and creatinine, particularly after 5 days, with the highest levels observed in cases lasting more than 6 days. This pattern strongly suggests evolving prerenal azotemia, most likely due to dehydration, decreased renal perfusion, and catabolism. This finding aligns with the effects of feed deprivation in donkeys and other equids, where prolonged fasting (e.g., 10 days) leads to elevated creatinine, total protein these changes attributed not to primary renal injury, but to reduced glomerular filtration rate (GFR) and prerenal factors (Filippo *et al*., 2021).

In equine medicine, both BUN and creatinine are recognized biomarkers for renal dysfunction but creatinine is generally more specific, while BUN can be influenced by extrarenal variables such as protein intake, dehydration, and catabolism (Frączkowska *et al*., 2021). The rise in both markers in this data likely reflects prerenal mechanisms (e.g., hypovolemia, reduced intake), which impair renal blood flow and filtration, rather than primary renal parenchymal damage.

Although this study focuses on donkeys with impaction of the large intestine, broader equine research supports the notion that colic and associated anorexia can compromise renal indicators. For instance, horses with colic often show significantly higher serum creatinine compared to healthy controls, even when values remain within reference ranges, hinting at early renal stress (Arosalo *et al*., 2007). The progressive nature of these findings and minimal elevation at 1–3 days, followed by stepwise increases at day 5 and beyond strongly mirrors evolving prerenal azotemia under sustained anorexia and colic.

Elevations in uric acid and total bilirubin serve as biomarkers for hepatic strain and oxidative-metabolic dysfunction. Uric acid may function in part as an endogenous antioxidant buffer, whereas bilirubin rises signal hepatocellular impairments due to fat infiltration, as often seen in hyperlipemia–compromised donkeys (Li *et al*., 2018; Mendoza *et al*., 2018; Thiemann and Sullivan, 2019; Nnamdi *et al*., 2020; Akan, 2021; Yánez-Ortiz *et al*., 2021; Li *et al*., 2022; Tripathi *et al*., 2023; Mendoza *et al*., 2024b; Meng *et al*., 2024; Huang *et al*., 2025;Xu *et al*., 2025;).

The progressive increase in serum AST activity observed in donkeys with impaction of the large intestine and prolonged anorexia suggests a gradual development of hepatic and/or muscular stress. AST is a widely recognized enzyme marker in equids, found in both liver and muscle tissues, and its elevation often reflects hepatocellular leakage, muscle injury, or systemic catabolism (Constable *et al*., 2016; Kaneko *et al*., 2008). Comparable findings have been reported in horses, where gastrointestinal disorders and colic are associated with elevated serum hepatic enzymes, including AST, due to both direct hepatocellular damage and systemic metabolic effects (Knottenbelt and Pascoe, 1994). Clinically, the marked increase in AST after 3–5 days of anorexia underscores the importance of early diagnosis and intervention. Monitoring serum AST, alongside other markers such as BUN and creatinine, may help veterinarians detect early signs of organ compromise in donkeys with impaction of the large intestine and guide supportive therapy (Al-Haid *et al*., 2025).

The current study revealed a clear and progressive increase in serum γ-glutamyl transferase (GGT) activity in donkeys with impaction of the large intestine as the duration of anorexia increased, rising from about 20 IU/L on day 1 to nearly 80 IU/L after more than 6 days. GGT is considered one of the most sensitive indicators of hepatobiliary dysfunction in equids, as it is localized in the biliary epithelium and hepatocytes. The gradual elevation observed here likely reflects hepatic stress and cholestasis secondary to prolonged anorexia, dehydration, and intestinal stasis, which impair hepatic perfusion and biliary excretion (Kaneko *et al*., 2008).

The mild downward trend in albumin amidst stable total protein levels suggests that while overall protein balance remains fairly maintained, hepatic albumin synthesis may begin to falter under the stress of NEB, inflammation, or early liver compromise. A relative increase in globulin fractions could mask albumin loss when only total protein is assessed (Shibutani *et al*., 2015).

These findings fit well within the broader understanding of donkey physiology. Donkeys are especially predisposed to hyperlipaemia and oxidative damage due to their efficient fat mobilization coupled with reduced ability to adapt to sustained fasting or physiological stress. In colic cases, this risk is further amplified by stress-induced cortisol release and inflammatory cytokines (e.g. TNF-α, IL-6), which potentiate hormone-sensitive lipase activity and impair lipid clearance by insulin resistance (Mendoza *et al*., 2018).

Conclusion

The results of this study revealed a clear association between the duration of anorexia and progressive biochemical alterations in donkeys suffering from impaction of the large intestine. Triglycerides and LDL levels showed a steady increase over time. indicating enhanced lipolysis and lipid mobilization due to negative energy balance. Conversely, HDL concentrations declined notably, reflecting impaired lipid transport and metabolic stress. Markers of oxidative stress, such as lipid peroxidase and nitric oxide, increase significantly, while antioxidant parameters including catalase activity and vitamin C levels decrease sharply, confirming a shift toward oxidative imbalance. Uric acid and total bilirubin levels also rose markedly, suggesting hepatic strain and metabolic dysregulation. Meanwhile, albumin showed a mild decline, with total protein levels remaining relatively stable throughout the anorexia period. These biochemical patterns collectively highlight the metabolic and oxidative burden imposed by prolonged feed deprivation, particularly after 5 to 6 days of anorexia.

Acknowledgments

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Conflict of interest

Authors declare that there is no conflict of interest.

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