P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

**Research Article** https://doi.org/10.47278/journal.ijvs/2022.145

# The Effects of Acute Blood Loss on Inflammatory and Bone Biomarkers, Acid-base Balance, Blood Gases and Hemato-biochemical Profiles in Sedated Donkeys (*Equus asinus*)

Mohamed Tharwat[1,2,*] and Fahd Al-Sobayil[1]

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah, 51452, Saudi Arabia; [2]Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt
***Corresponding author:** mohamedtharwat129@gmail.com

**Article History:** 22-522 Received: 04-Feb-22 Revised: 18-Feb-22 Accepted: 28-Feb-22

## ABSTRACT

The current experiment was designed to investigate the possible effects of acute blood loss on the inflammatory and bone biomarkers status, acid-base balance, blood gases, and hemato-biochemical profiles in sedated donkeys (*Equus asinus*). For the induction of acute blood loss, collected 900mL of blood from the carotid artery of 10 male donkeys sedated with xylazine HCl (1mg/kg). For analysis, five blood samples were collected; collected the 1st (T0) before induction of blood loss and the other 4 (T1, T2, T3 and T4) collected at 30, 60, 120 and 240min post-blood loss. The serum concentrations of amyloid A (SAA) and haptoglobin (Hp) decreased at all-time points after blood loss compared to T0, but the differences were non-significant. The differences in levels of serum biomarkers osteocalcin (OC), β-alkaline phosphatase (β-ALP) and pyridinoline cross-links (PYD) at all-time points post blood loss compared to T0 values were also non-significant. Except for $PCO_2$, the acid-base status and blood gases, including pH, $PO_2$, $HCO_3$, $TCO_2$, $sO_2$, base excess, anion gap and lactate, increased significantly after blood withdrawal. On the contrary, most of the measured hemato-biochemical parameters decreased significantly after blood loss, except for glucose. In conclusion, acute blood loss in sedated donkeys did not influence serum concentrations of the inflammatory biomarkers SAA and Hp and the markers of bone metabolism OC, β-ALP, and PYD. On the contrary, most acid-base, blood gas parameters, and hemato-biochemical parameters differed significantly after blood withdrawal compared to their values before blood loss.

**Key words:** Acid-base balance, Acute blood loss, Biomarkers, Blood gases, Donkeys.

## INTRODUCTION

Blood is a major component of the mechanism whereby oxygen is transported from the lungs to all organs and tissues of the body. The total blood amount in a horse's body constitutes about 6-10% of its total weight, based on breed (Marcilese et al. 1964). The horse can tolerate up to 15% loss of its blood without requiring blood transfusion. Moreover, it is well known that a conscious horse weighing 500kg may lose around one-third of its whole blood immediately after marked cardiovascular damage (Durham 1996). In horses, the decision of blood transfusion mostly depends on the amount and speed of blood loss, as horses can adapt to chronic anemia compared to sudden blood loss (Williams 2020).

Infection or inflammation biomarkers or acute phase proteins (APPs) are serum proteins, and blood concentrations of these proteins can vary after infection, inflammation, or trauma (Murata et al. 2004; Tharwat et al. 2014a; Tharwat and Al-Sobayil 2015a,b; Tharwat and Al-Sobayil 2018a; Tharwat 2020a). This phenomenon is usually called the acute phase response or reaction (APR) that mostly occurs secondary to various stimuli of tissue damage; however, it may also be physiological during inflammatory episodes (Yazwinsk et al. 2013). In addition, bone biomarkers are mostly used in humans for non-invasive monitoring of bone metabolism and therapy reaction of special musculoskeletal and bone affections (Allen 2003; Sabour et al. 2014). In the veterinary field, these markers are mostly used for evaluating the reaction of bone to either medical or invasive interferences and for the diagnosis of musculoskeletal disorders (Frisbie et al. 2008; Tharwat and Al-Sobayil 2018b; Tharwat 2020b; Al-Sobayil and Tharwat 2021).

**Cite This Article as:** Tharwat M and Al-Sobayil F, 2022. The effects of acute blood loss on inflammatory and bone biomarkers, acid-base balance, blood gases and hemato-biochemical profiles in sedated donkeys (*Equus asinus*). International Journal of Veterinary Science 11(4): 479-485. https://doi.org/10.47278/journal.ijvs/2022.145

Severe bleeding may lead to hypovolemic shock in horses, leading to death if not managed in time. The etiologies of severe bleeding may be internal or external (Mudge 2014). Therefore, it is vital to monitor the signs of this problem, such as harried respiration, elevated heartbeats, depression, hypothermia, and pale mucous membranes in the affected horses. Animals with hemoperitoneum may also show abdominal pain and distension (Pusterla 2005). Whether the acute blood loss in the donkey (*Equus asinus*) affects biomarkers of inflammation and bone, acid-base balance, blood gases, and hemato-biochemical parameters are unknown. Therefore, the present study was carried out to investigate the possible effects of acute blood loss on the inflammatory and bone biomarkers status, acid-base balance, blood gases, and hemato-biochemical profiles in sedated donkeys (*Equus asinus*).

## MATERIALS AND METHODS

### Ethical Approval

Experimental design and procedures were duly approved by the Qassim University animal ethics committee, which basically comply with the Guidelines of Laboratory Animals of the National Institutes of Animal Health (USA, release no. 86-23, reviewed 1996).

### Donkeys

A group of 10 male donkeys, aged 7.6±2.4 years and weighing 116±17kg were used in this study. These animals were assumed to be disease-free. Each donkey was subjected to a full clinical examination, with especial attention to the cardiovascular system. Based on physical and laboratory examinations, echocardiography and electrocardiography, all donkeys were proved clinically healthy.

### Blood Collection

For the induction of acute blood loss, animals were sedated with xylazine HCl (1mg/kg, Bomazine 10%, BOMAC Laboratories Ltd, Auckland, New Zealand). Then 900mL blood was collected in 30 minutes from each donkey in standing position through surgically placing a 14G×45mm cannula (Mais Co, Riyadh, Saudi Arabia) into the carotid artery. Blood was collected into sterile 500mL bags containing 50mL 3.8% sodium citrate, and the collection tube was clamped for a few seconds when the collection bag was changed. For analysis, one blood sample (T0) was collected immediately prior to induction of blood loss and four blood samples (T1, T2, T3 and T4) were collected at 30-, 60-, 120- and 240-minutes post-blood loss. At each sampling, 10mL of arterial blood was collected using aseptic containers. Out of this 10mL of blood, 2mL was collected in heparin tubes for determining acid-base balance and blood gas parameters, 2mL in EDTA tubes for hematology, and the remaining 6mL in plain tubes to harvest serum for the assays of biochemical parameters and biomarkers of inflammation and bone.

### Blood Gases Analyses and Determination of Hemato-biochemical Parameters

Blood samples collected in heparin tubes were used to estimate the acid-base and blood gas parameters using *in situ* Veterinary Analyzer (I-STAT®, Abaxis, California, USA). For this purpose, an immediate analysis of the following parameters was performed: Blood pH, partial pressure of carbon dioxide ($PCO_2$), partial pressure of oxygen ($PO_2$), bicarbonate ($HCO_3$), total carbon dioxide ($TCO_2$), excess of base (BE), oxygen saturation ($So_2$) and lactic acid (LA) (Tharwat et al. 2014b; Tharwat and Al-Sobayil 2014a,b,c; Tharwat 2015). Different blood variables including total and differential white blood cell count, erythrocytic count, hematocrit (HCT), hemoglobin concentration and blood indices were determined using the EDTA blood samples (VetScan HM5, Abaxis, California, USA). An automated Biochemical Analyzer (VetScan VS2, Abaxis, California, USA) was used to determine the serum concentrations of total protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, calcium, glucose, sodium, potassium and chloride. The serum activity of creatine kinase (CK), aspartate aminotransferase (AST), and γ-glutamyl transferase (GGT) were also measured.

### Inflammatory Biomarkers Assay

Serum amyloid A (SAA) was determined in sera using an ELISA kit (Multispecies SAA ELISA kit, Tridelta Ltd., Ireland). A monoclonal antibody specific for SAA has been coated onto the wells of the microliter strips provided. Concentration of the second inflammation biomarker haptoglobin (Hp) in sera was determined using a colorimetric assay (Haptoglobin kit, second generation, Tridelta Ltd., Ireland), as described previously (Tharwat et al. 2014a; Tharwat and Al-Sobayil 2015a,b; Tharwat and Al-Sobayil 2018a; Tharwat 2020a).

### Bone Biomarkers Assay

The bone biomarkers, including osteocalcin (OC), β-alkaline phosphatase (β-ALP) and pyridinoline cross-links (PYD) were measured in sera using an immunoassay kit (Metra Biosystems Inc., a division of Quidel Corp.). Quantification limits of the assay ranged from 2 to 32 ng/mL, 2-140 U/L and 15-750 nmol/L for OC, β-ALP and PYD, respectively. Precision CVs within and between runs were 5-10% for OC, 4-6% and 5-8% for β-ALP, 6-10% and 3-11% for PYD (Tharwat et al. 2014a; Tharwat and Al-Sobayil 2015b; Tharwat and Al-Sobayil 2018a,b; Tharwat 2020b; Al-Sobayil and Tharwat 2021).

### Statistical Analysis

Values (Mean±SD) for different variables were computed and the data were analyzed using a commercially available statistical program (SPSS 2017). A repeated measure analysis of variance (ANOVA) was employed as the statistical model to evaluate the differences over time (T0-T4 time points), followed by Dunnett's multiple comparison. The level of significance was set at $P \leq 0.05$.

## RESULTS

Compared to values at T0, the respiratory and heart rates increased significantly at T1, T2, T3 and T4 ($P<0.05$). Serum concentrations of the APPs, SAA and Hp at different time points are shown in Fig. 1. The serum concentrations of SAA and Hp decreased at all-time points after acute blood loss compared to T0, but the differences were statistically non-significant. Fig. 2 shows serum concentrations of the bone metabolism biomarkers OC, β-ALP and PYD.

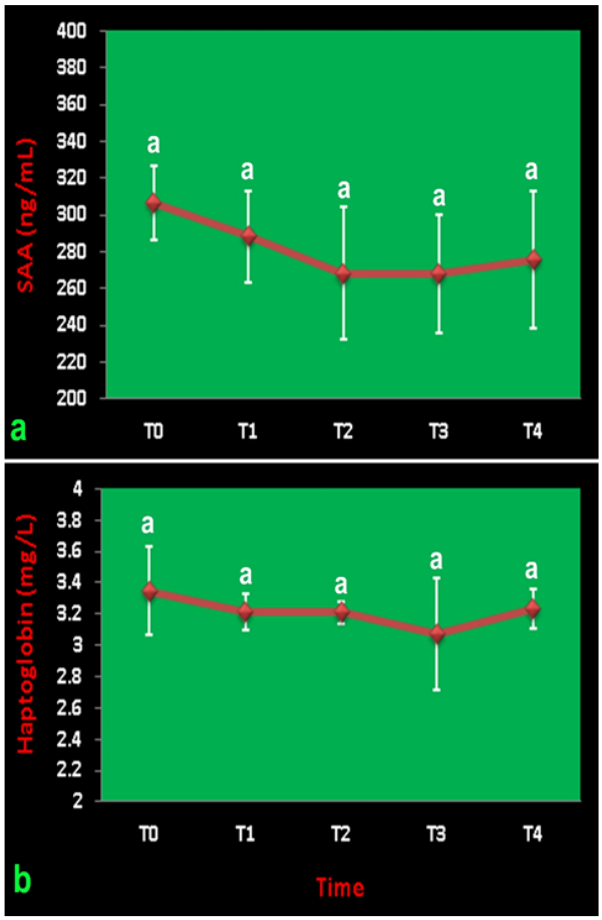


**Fig. 1:** Serum concentrations of amyloid A (SAA) (**a**) and haptoglobin (**b**) in donkeys (*n*=10) with acute blood loss. T0=immediately before blood loss; T1, 30min; T2, 60min; T3, 120min and T4, 240min after blood loss. [a]Values with same letters did not differ significantly.

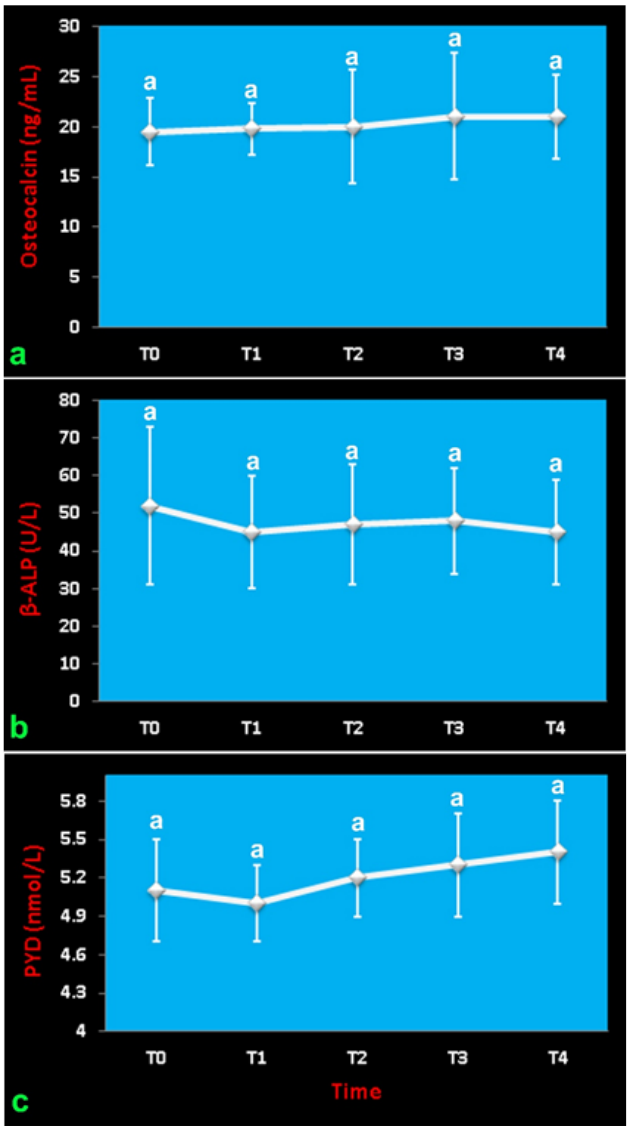


**Fig. 2:** Serum concentrations of the bone metabolism biomarkers osteocalcin (**a**), β-alkaline phosphates (β-ALP) (**b**) and pyridinoline cross-links (PYD) (**c**) in donkeys (*n*=10) with acute blood loss. T0= immediately before blood loss; T1, 30min; T2, 60min; T3, 120min and T4, 240min after blood loss. [a]Values with same letters did not differ significantly.

The differences in serum OC, β-ALP and PYD at all-time points after acute blood loss compared to T0 values were also non-significant.

The mean±SD of acid-base, blood gas parameters and LA in the donkeys before and after the induction of acute blood loss are summarized in Table 1. The blood pH increased significantly at T4 (7.54±0.16) compared to 7.43±0.01 at T0 (P=0.04). Similarly, the $HCO_3$ and $TCO_2$ increased significantly at T4 compared to pre-blood loss values (P=0.03 and P=0.05, respectively). The $PCO_2$ also increased significantly at T4 (P<0.05) and the $PO_2$ concentration increased significantly at T2, T3 and T4 (P=0.01, 0.004 and 0.002, respectively). Moreover, the $sO_2$ increased significantly at T2, T3 and T4 (P=0.01, 0.003 and 0.002, respectively). In all tested times (T1-T4), the anion gap (AnGap) increased significantly compared to T0 (P<0.05). It did not differ significantly at any time point, while the lactate concentration increased significantly at T1, T2 and T3 compared to baseline values (P=0.01, 0.01 and 0.05, respectively).

The hematological alterations detected after acute blood loss are summarized in Table 2. The WBCs, lymphocytes and neutrophils decreased significantly at all-time points (T1-T4) compared to T0 values (P<0.05). The RBCs, hemoglobin concentration and HCT decreased significantly at T1, T2, T3 and T4 compared to pre-blood loss values (P<0.05). On the contrary, the MCV, MCH and MCHC did not differ significantly at any time point compared to T0 values. The PLT count decreased significantly at T3 and T4 compared to T0 values (P=0.01 and 0.003, respectively).

Table 3 summarizes serum biochemical parameters before and at different time points after acute blood loss in donkeys. Compared to T0, the serum concentration of total proteins decreased significantly at T1-T4 (P<0.05), while serum albumin and globulin decreased significantly at T2-T4 compared to T0 (P<0.05). Serum activities of CK, AST and GGT decreased significantly at all-time points after blood loss compared to T0 (P<0.05). Similarly, serum concentrations of calcium and potassium decreased significantly at all-time points after blood withdrawal compared to T0 (P<0.05). On the other hand, serum concentrations of glucose increased significantly at all-time points after blood loss compared to T0 (P<0.0001). However, BUN, creatinine, sodium and chloride concentrations did not differ significantly at any time-point compared to T0 values.

**Table 1:** Values of acid-base balance, blood gases, electrolytes and lactic acid concentrations in donkeys before and after acute blood loss

| Variables | T0 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| pH | 7.43±0.01[a] | 7.43±0.01[a] | 7.43±0.02[a] | 7.43±0.01[a] | 7.54±0.16[b] |
| $PCO_2$ (mmHg) | 43.3±3.2[a] | 43.1±4.4[a] | 41.2±5.8[a] | 44.0±3.8[a] | 40.8±1.7[b] |
| $PO_2$ (mmHg) | 29.8±4.4[a] | 32.1±2.1[a] | 34.6±1.3[b] | 37.0±3.2[b] | 39.2±4.7[b] |
| BE (mmol/L) | 4.3±2.1[a] | 4.0±2.7[a] | 3.2±1.3[a] | 4.8±2.5[a] | 4.7±1.3[a] |
| $HCO_3$ (mmol/L) | 28.8±2.2[a] | 28.4±2.7[a] | 27.3±1.1[a] | 29.1±2.5[a] | 36.3±10.2[b] |
| $TCO_2$ (mmol/L) | 30.2±2.3[a] | 29.8±3.0[a] | 28.5±1.3[b] | 31.0±2.4[a] | 36.8±9.1[b] |
| $sO_2$ (%) | 56.6±9.4[a] | 62.4±4.9[a] | 67.8±2.8[b] | 71.2±4.7[b] | 77.6±10.5[b] |
| Anion Gap (mmol/L) | 5.0±0.0[a] | 7.7±0.8[b] | 7.7±1.2[b] | 7.6±0.7[b] | 7.5±1.0[b] |
| Sodium (mmol/L) | 133±0.8[a] | 133±1.4[a] | 133±0.3[a] | 134±1.4[a] | 132±2.1[a] |
| Potassium (mmol/L) | 4.1±0.3[a] | 3.8±0.3[b] | 3.7±0.2[b] | 3.8±0.3[b] | 3.8±0.3[b] |
| Chloride (mmol/L) | 102±1.7[a] | 102±0.7[a] | 101±1.2[a] | 101±0.8[a] | 101±3.1[a] |
| Lactic acid (mmol/L) | 2.1±0.2[a] | 2.4±0.2[b] | 2.5±0.2[b] | 2.4±0.3[b] | 2.3±0.3[a] |

$PCO_2$, partial pressure of carbon dioxide; $PO_2$, partial pressure of oxygen; BE, base excess; $HCO_3$, bicarbonate; $TCO_2$, total carbon dioxide; sO2, oxygen saturation. T0, immediately before blood collection, and T1, T2, T3 and T4 at 30, 60, 120 and 240min post-blood loss, respectively. [a,b]Values (Mean±SD) with different letters in the same row differ significantly ($P<0.05$).

**Table 2:** Values of hematological parameters in donkeys before and after acute blood loss

| Parameter | T0 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| WBCs ($\times10^9$/L) | 12.8±1.3[a] | 10.9±1.4[b] | 9.4±1.4[b] | 8.6±1.1[b] | 7.9±1.0[b] |
| Lymphocytes ($\times10^9$/L) | 6.2±0.8[a] | 5.3±0.9[b] | 4.5±0.9[b] | 4.1±0.8[b] | 3.7±0.6[b] |
| Neutrophils ($\times10^9$/L) | 5.6±0.6[a] | 4.6±0.5[b] | 4.3±0.5[b] | 3.7±0.2[b] | 3.6±0.4[b] |
| RBCs ($\times10^{12}$/L) | 7.7±0.6[a] | 7.0±0.6[b] | 6.5±0.4[b] | 6.2±0.4[b] | 5.9±0.4[b] |
| Hemoglobin (g/dL) | 12.7±0.7[a] | 11.7±0.7[b] | 10.8±0.6[b] | 10.3±0.5[b] | 9.6±0.5[b] |
| Hematocrit (%) | 38.7±1.0[a] | 35.8±1.6[b] | 33.2±0.8[b] | 31.5±0.5[b] | 30.0±1.4[b] |
| MCV(fL) | 50.8±3.0[a] | 51.2±2.3[a] | 51.4±2.5[a] | 51.1±2.8[a] | 51.4±2.7[a] |
| MCH (pg) | 16.7±0.6[a] | 16.7±0.5[a] | 16.7±0.6[a] | 16.5±0.5[a] | 16.5±0.7[a] |
| MCHC (g/dL) | 33.0±1.0[a] | 32.5±0.7[a] | 32.5±0.9[a] | 32.5±1.4[a] | 32.1±1.2[a] |
| Platelet count ($\times10^9$/L) | 277±25[a] | 221±27[a] | 210±31[a] | 196±24[b] | 196±14[b] |

WBCs, white blood cells; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration. T0, immediately before blood collection, and T1, T2, T3 and T4 at 30, 60, 120 and 240min post-blood loss, respectively. **[a,b]**Values (Mean±SD) with different letters in the same row differ significantly ($P<0.05$).

**Table 3:** Values of serum biochemical parameters in donkeys before and after acute blood loss

| Parameters | T0 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| Total protein (G/L) | 68.6±0.6[a] | 66.4±1.5[b] | 64.6±0.8[b] | 63.3±1.6[b] | 61.3±1.1[b] |
| Albumin (G/L) | 38.1±1.9[a] | 36.9±1.8[a] | 32.7±2.0[b] | 35.7±0.3[b] | 33.8±0.9[b] |
| Globulin (G/L) | 29.8±1.3[a] | 29.5±0.8[a] | 31.9±2.6[b] | 27.6±1.3[b] | 27.5±0.8[b] |
| BUN (mmol/L) | 6.8±0.2[a] | 6.8±0.3[a] | 6.8±0.3[a] | 6.8±0.2[a] | 6.8±0.3[a] |
| Creatinine (µmol/L) | 49.3±5.6[a] | 47.7±7.1[a] | 48.5±2.0[a] | 49.3±2.5[a] | 50.1±7.6[a] |
| AST (U/L) | 389±76[a] | 325±17[b] | 323±12[b] | 312±16[b] | 303±14[b] |
| GGT (U/L) | 33.3±1.1[a] | 31.5±2.5[b] | 31.4±0.3[b] | 30.6±2.6[b] | 28.0±0.9[b] |
| CK (U/L) | 274±11[a] | 259±15[b] | 255±14[b] | 239±20[b] | 234±10[b] |
| Calcium (mmol/L) | 3.2±0.1[a] | 3.1±0.0[b] | 3.0±0.0[b] | 3.0±0.0[b] | 2.9±0.0[b] |
| Glucose (mmol/L) | 5.4±0.3[a] | 6.2±0.5[b] | 7.7±0.2[b] | 8.2±0.1[b] | 8.4±0.2[b] |

BUN, blood urea nitrogen; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; CK, creatine kinase. T0, immediately before blood collection, and T1, T2, T3 and T4 at 30, 60, 120 and 240min post-blood loss, respectively. **[a,b]**Values (Mean±SD) with different letters in the same row differ significantly ($P<0.05$).

## DISCUSSION

In equines, the problem of acute blood loss may lead to severe hypovolemic shock and can be life-threatening if not managed in time. The causes of acute bleeding may be external, such as arterial tears and nasal bleeding, or internal, such as rupture of the spleen or hemorrhage from the uterine artery (Mudge 2014). Principles for handling hypovolemic shock have been described in humans and animals (Rudloff and Kirby 2008; Dutton 2012). Acute bleeding in equines can lead to some special consequences due to the high volume of blood loss and difficult approach to the site of the hemorrhage. To the authors' knowledge, this is the first study investigating the effects of acute blood loss on inflammatory and bone metabolism biomarkers, acid-base balance, blood gases, and hemato-biochemical parameters in sedated donkeys (*Equus asinus*).

In horses, the marker SAA is the master APP that increases due to infectious, non-infectious, and inflammatory disorders such as gastrointestinal, genital, and pulmonary diseases and following surgical interventions. This pattern of marker SAA is useful for diagnosing subclinical conditions that can adversely affect horses' exercise and racing ability. The blood concentrations change dramatically, pointing to the beginning of APR, but the pattern of these changes is specific to different species (Witkowska-Piłaszewicz et al. 2019). Serum concentrations of the inflammatory biomarkers SAA and Hp in this study did not differ significantly at all tested time points following acute blood loss compared to values before blood withdrawal. It has been reported that serum SAA levels increase within 6 h after tissue damage due to infection (Nunokawa et al. 1993; Jacobsen et al. 2006) and jump to 1000 double within 24-

48h of the baseline value (Coutinho-da-Silva et al. 2013) and drop within 12h when inflammation subsides (Coutinho-da-Silva et al. 2013). Therefore, the results of Nunokawa et al. (1993) and Jacobsen et al. (2006) explain why the serum concentrations of SAA in the current study did not differ significantly after blood loss compared with pre-blood loss levels. It appears that the APR failed to emerge due to the short sampling time (240min; 4h) after blood withdrawal.

Haptoglobin is another APP that may be applied as a diagnostic aid in equines experiencing different problems, and its increase may be related to various physiological or pathological conditions (Assuncao et al. 2019). In the current study, parallel to SAA, serum levels of the Hp decreased but did not reach a significant level at all tested time points compared to baseline values. In equines with abdominal pain, serum Hp value was below the reference range in 13/54 of the affected animals (Dondi et al. 2015). In addition, non-survivor horses had significantly lower serum Hp levels than those in survivors. Similarly, horses with intestinal ischemia or strangulation had significantly lower serum haptoglobin concentrations than those in the non-ischemic/non-strangulating animals (Dondi et al. 2015). Likewise, in another study of horses with severe abdominal distress, the serum concentrations of Hp were significantly decreased compared to those in healthy ones (Pihl et al. 2013).

In the present study, relatively short sampling time may also be the reason for non-significant differences in serum OC, β-ALP, and PYD at all test points following acute blood loss compared to T0 values. It is well known that bone metabolism biomarkers are released during the bone remodeling processes by osteoblasts or osteoclasts (Allen 2003). In horses, OC, β-ALP, and PYD are the major bone markers (Mitchell et al. 2019; Silvers et al. 2020). Osteocalcin is synthesized mostly from mature osteoblasts. It is believed that OC is associated with the mineralization of newly formed osteoid. Therefore, OC is an accepted bone formulation and mineralization (Billinghurst et al. 2004). The β-ALP is an isoform of the enzyme ALP and plays an important role in bone formation (McIlwraith 2005). The PYD cross-links are set in the mature collagen of bone and found in mature type I collagen and collagen types II and III (Al-Sobayil and Tharwat 2021). An extreme level of PYD in serum or urine is most commonly considered as an index of bone resorption (Thompson et al. 1992).

The current experiment's pH, $HCO_3$, and $TCO_2$ levels increased significantly at 4h following acute blood loss compared to values before blood withdrawal (T0). The metabolic alkalosis observed 4h after blood withdrawal might have been due to a reduction in $PCO_2$. During another study of severe acute hemorrhage in anesthetized horses, the pH, bicarbonates, and BE did not change significantly compared to controls after collecting a total of 5, 10, 15, and 20kg of blood; only values differed significantly before the horses were euthanized (Wilson et al. 2003). The effect of general anesthesia may be the cause of differences between the results of our study and those of Wilson et al. (2003). At T2, T3, and T4 time points, serum $PO_2$ and $sO_2$ concentrations increased significantly compared to their values before blood loss. An increase in respiratory rates in the donkeys may cause decreased $PCO_2$ and the increased $PO_2$ and $sO_2$ after injection. In our study, the $PCO_2$ did not differ significantly at T1-T4 compared to T0; these results agree with those of Wilson et al. (2003), who induced blood loss by collecting a total of 5, 10, 15, and 20kg of blood. The significantly increased AnGap after blood loss compared to T0 values may be elevated $HCO_3$ after blood withdrawal.

Lactate is produced following high-intensity exercise and plays an important role in producing energy in different body organs (Janssen et al. 2009; Tharwat 2021a,b). Differences in lactate concentration in the blood after acute bleeding appear to be early indicators of decreased blood volume in horses and, therefore, can be used to monitor responses of horses to blood transfusion (Magdesian et al. 2006). In the present study, the lactate level increased markedly at time points T1, T2, and T3 following blood loss compared to baseline values, which the high metabolic signal might have caused during these time points in response to acute bleeding. These results regarding increased serum lactate contents after acute hemorrhage in the donkeys are supported by a previous report in horses with acute blood loss (Magdesian et al. 2006).

In the present study, values of most of the hemato-biochemical parameters decreased significantly after blood loss, and this was expected. According to Wilson et al. (2003), the HCT decreased significantly in anesthetized horses with severe blood loss. The only parameter that increased significantly at all-time points after blood withdrawal compared to values at T0 was glucose. Cannot specify exact cause for this hyperglycemia in all cases. However, previous studies have demonstrated that surgical trauma can increase the stress hormone cortisol, along with catecholamines, which can induce insulin resistance, finally resulting in hyperglycemia (Duggan et al. 2017; Peacock 2019).

## Conclusion

It is concluded from this study that acute blood loss in the sedated donkeys did not influence serum concentrations of the inflammatory and bone biomarkers recorded in this study. On the contrary, most of the acid-base and blood gas parameters differed significantly, where the pH, $PO_2$, $HCO_3$, $TCO_2$, $sO_2$, BE, AnGap, and LA increased significantly as a result of acute blood loss. Except for glucose, most of the measured hemato-biochemical parameters showed a significant decrease after blood loss. As the duration of post blood loss monitoring period in this study was quite short (4h, 240min), another study is warranted to investigate the long-term effects of acute blood loss on the biomarkers of inflammation and bone, acid-base and blood gas parameters, and on the hemato-biochemical variables in donkeys.

## Authors' Contribution

Both authors designed the study and carried out the practical and laboratory work. M. Tharwat wrote the original manuscript draft and prepared figures and tables. F. Al-Sobayil read and revised the manuscript draft. Both authors have read and approved the final manuscript.

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