



Haematological and serum electrolyte responses in goats undergoing tibial fracture reduction

EA Uwagie-Ero^{1*}, RI Udegbumam² & KB Kadima³

- ^{1.} Department of Surgery Faculty of Veterinary Medicine University of Benin, Benin City, Nigeria..
- ^{2.} Department of Veterinary Surgery Faculty of Veterinary Medicine University of Nigeria, Nsukka.
- ^{3.} Veterinary Teaching Hospital, Ahmadu Bello University Zaria

*Correspondence: Tel.: +2348033977590, E-mail: eddyero@yahoo.com

Abstract

Haematologic, biochemical, electrolyte and acid base values were determined in twelve goats prior to (baseline) tibia surgery, during surgery (at 45 min) and subsequently at 24 h and 72 h post operatively. The haematocrit, haemoglobin, blood urea nitrogen, blood glucose, sodium, potassium, chlorine, total carbon dioxide (TCO₂), anion gap, base excess, pH, partial carbon dioxide (PaCO₂) concentration and bicarbonate concentrations in the samples were obtained using the i-STAT EC8+ handheld biosensor analyzer. The osmolarity of the blood was also estimated. To investigate changes in the variables during and after fracture fixation, the control (baseline) values of the data were compared with the mean variables obtained per time point during and after surgery using ANOVA. The haematocrit, haemoglobin, blood glucose, osmolarity, anion gap, chlorine, sodium and potassium concentrations were significantly lower ($p < 0.05$) than control values during surgery and at 24 and 72 h post-surgery. Blood urea nitrogen, base excess, pH, PaCO₂ TCO₂ and bicarbonate concentration (HCO₃⁻) increased significantly ($p < 0.05$) above control values at 24 and 72 h post-surgery. The results obtained from this study showed that tibia surgery led to alteration in fluid, electrolyte and acid base status of goats. Most importantly, metabolic alkalosis ensued post tibia fracture creation and fixation.

Keywords: Haematology, Electrolytes, Acid-base, Tibia, Fracture, Surgery

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Introduction

Trauma induces circulatory, endocrine and metabolic changes referred to as stress response (Desborough, 2000). These changes occur in the body's attempt to maintain homeostasis as well as increase the rate of catabolism (Desborough, 2000; Lobo *et al.*, 2013). In addition to the neuro-endocrine and metabolic changes, acid-base and electrolyte status of animals are also altered peri-operatively (Andersen & Wang, 2000; Rassam & Counsell, 2005; Kwak *et al.*, 2010).

Traumatic injuries such as fractures and their causative agents have been reported in small ruminants (Adeyanju *et al.*, 2004; Udegbumam *et al.*, 2009a). These scholars showed that a high proportion of animals which suffer trauma annually undergoes fracture fixation in veterinary clinics. Despite this, there is a paucity of data on the immediate stress response of this species post fracture. Most empirical data on response to trauma are those drawn from studies conducted in

goats using less traumatic surgical procedures such as enterectomy (Nazifi *et al.*, 2000), rumenotomy (Adamu *et al.*, 1991) and castration (Oyeyemi *et al.*, 2000; Mohammed *et al.*, 2008; Olaifa & Opara, 2011). Thus, since the degree of stress induced changes depends on the intensity of trauma (Desborough, 2000), it is important to investigate the immediate stress response of goats to fracture.

The purpose of this study therefore was to investigate the stress response of goats to tibia surgery. The stress response was evaluated by monitoring the fluid, electrolyte and acid-base status of goats during and post tibia surgery.

Materials and Methods

Twelve savannah brown goats aged between 2-3 years (age determination was done as described by Pasquini & Spurgeon, 1989) and of body weight 13.4 ± 0.3 kg were used for this study. They were

judged clinically healthy by haematologic and biochemical examinations. Prior to the study, they were dewormed using 10 mg/kg Febendazole (Kosher Pharma, India). Blood was collected from the jugular vein of goats into heparinized syringes and mixed repeatedly by rolling between the palms for about 5 seconds to determine the baseline haematologic, biochemical, electrolyte and acid base values which served as the control values. An electronic check was performed on the handheld biosensor analyser using the electronic stimulator before proceeding with the test. To measure the above parameters, 2 drops of blood were discarded after mixing before filling the EC8+ cartridge (Abbott Lab., Illinois, USA) with 2-3 drops of blood. The filled cartridge was closed and inserted into the i-STAT handheld biosensor analyser (Abbott Lab., Illinois, USA) after the prompt from the machine. Test was completed and the results were viewed in the handheld display screen and recorded for each animal. Analysed cartridges were disposed safely.

To prepare the implant for fracture treatment, 0.1 µg/ml of bone morphogenetic protein-2 (BMP-2) [Sigma Aldrich, Germany] was prepared using deionized sterile water. The BMP solution (2ml) was made into a thick paste with hydroxapatite powder (Sigma Aldrich, Germany) and rolled into a cylindrical mold (0.5 cm diameter and 1 cm height). The mold was then cased with absorbable collagen sponges (ACS) [Helistat Integra Life Sciences, Colla-tec Inc, NJ, USA] and allowed to dry into a strip (0.5cm x 1 cm).

All goats were pre-medicated using 0.05 mg/kg xylazine hydrochloride (VMD, Belgium) and general anaesthesia induced using 20 mg/kg ketamine hydrochloride (Pharmax, PVT, India). The right forelimbs were shaved and prepared for aseptic surgery. A 5 cm skin incision was made over the anteriomedial aspect of the tibia of each goat and the tibia bones exposed by blunt dissection. Using an osteotome and bone rongeurs, fracture gaps (1 cm) were created on the

midshaft of each right tibia bone. The fracture sites were flushed with phosphate buffered saline (PBS). The BMP/ACS/hydroxyapatite implants were positioned into the fracture gaps. Subcutaneous tissues were sutured with no. 2 chromic catgut while skin incisions were sutured with no. 2 nylon in horizontal mattress pattern. A plaster of paris cast was applied over the affected limb for external immobilization and stability. The skin incision sites were opened at the cast and sterile compression bandages applied to the limbs. All animals were placed on cephazolin sodium (Lilly Pharma, Isreal) 35mg/kg i.v and acetaminophen (Alpha Laboratories, India) 5 mg/kg p.o for 5 days post-operatively. Diclofenac sodium (Diclonex, Iscon Pharma, India) 2.5 mg/kg i.m was administered for 3 days to improve post-operative analgesia in two animals showing severe pain (data from these animals were excluded from this report).

Blood samples were collected during surgery (at 45 min) and subsequently at 24 h and 72 h post operatively. The haematocrit (Hct), haemoglobin (Hgb), blood urea nitrogen (BUN), blood glucose (BGs), sodium (Na⁺), potassium (K⁺), chlorine (Cl⁻), total carbon dioxide (TCO₂), anion gap, base excess, pH, partial carbon dioxide (PaCO₂) and bicarbonate (HCO₃⁻) concentrations in the samples were obtained using the EC8+cartridge on the i-STAT handheld biosensor analyzer (Abbott Lab, Illinois, USA). The osmolarity of the blood was estimated as described by Pettifer (2003).

To investigate changes in the variables during and after fracture fixation, the control values of the data were compared with the mean variables obtained per time point during and after surgery using ANOVA. Variant means were separated using Turkey post-Hoc test at $p < 0.05$.

Results and Discussion

As shown in Table 1, the haematocrit and haemoglobin of goats decreased significantly ($p < 0.05$) below control value during surgery and

Table 1: Haematocrit, biochemical and electrolyte variables of goats during and after fracture fixation (n=8)

Variables	Control	Intraoperative 45 min	Post-operative	
			24 h	72h
Hct (%)	50.9±1.08 ^a	41.6±2.6 ^b	38.2±0.4 ^b	40.9±0.4 ^b
Hgb (g/dl)	7.54±0.13 ^a	6.16±0.06 ^b	4.79±0.30 ^c	5.3±0.08 ^d
BUN (mg/dl)	16.95±1.92 ^a	19.4±0.80 ^a	25.7±0.26 ^b	23.95±0.30 ^b
Glucose(mg/dl)	117.2±2.80 ^a	91.4±2.70	110.1±0.50	102.4±0.49
Chlorine(mEq/L)	114.5±1.50 ^a	102.9±0.17 ^b	97.1±0.90 ^c	99.3±0.81 ^c
Sodium(mEq/L)	147.7±0.70 ^a	141.5±0.50 ^b	133.1±0.80 ^c	131.7±1.00 ^c
Potassium(mEq/L)	4.6±0.050 ^a	3.7±0.050 ^b	2.7±0.04 ^c	3.02±0.02 ^d
Osmolarity(mOsm/L)	307.6±0.80 ^a	296.3±1.00 ^b	281.5±1.70 ^c	277.7±2.00 ^{ac}

^{a,b,c,d} Different superscripts in a row indicate significant difference at $P < 0.05$

Table 2: Acid-base variables of goats during and after fracture fixation (n=8)

Variables	Control	Intraoperative	Post-operative	
		45 min	24 h	72h
TCO ₂ (mEq/L)	25.2±0.40 ^a	24.7±0.10 ^a	27.4±0.30 ^b	23.9±0.10 ^c
Anion gap(mMol/L)	17.4±0.40 ^a	15.95±0.20 ^b	13.2±0.09 ^c	15.2±0.06 ^b
Base excess(mMol/L)	1.0±0.00 ^a	1.95±0.09 ^b	2.4±0.05 ^c	2.1±0.10 ^{bc}
pH	7.4±0.01 ^a	7.7±0.03 ^b	7.8±0.04 ^{bc}	7.79±0.01 ^c
PaCO ₂ (mEq/L)	41.2±0.84 ^a	56.2±0.80 ^b	48.8±1.01 ^c	43.2±0.40 ^a
HCO ₃ ⁻ (mEq/L)	18.6±0.60 ^a	20.9±0.30 ^b	20.6±0.30 ^b	17.1±0.20 ^c

^{a, b, c} Different superscripts in a row indicate significant difference at P<0.05

post-surgery. Blood glucose concentrations were significantly lower than control value during surgery and at 24 and 72 h post-surgery. Blood urea nitrogen increased significantly ($p<0.05$) above control value at 24 and 72 h post-surgery (Table 1). Serum chlorine, sodium and potassium significantly decreased ($p<0.05$) below normal values during surgery and post-surgery. Blood osmolarity decreased significantly ($p<0.05$) during surgery and post-surgery (Table 1). Blood pH and PaCO₂ increased significantly ($P<0.05$) during surgery and at 24 and 72 h post-surgery (Tables 2). Blood TCO₂ and HCO₃⁻ increased significantly ($p<0.05$) above control value during surgery and 24 h post-surgery (Table 2). Anion gap decreased significantly ($p<0.05$) below control value during surgery and post operatively (Table 2). Base excess increased significantly ($p<0.05$) above control value during surgery and post-surgery (Table 2). The study demonstrated that Hct and Hgb of goat decreased significantly during and after tibia surgery. Similar changes in these parameters have been previously reported in dogs that underwent femorotibial joint surgery (Sibanda *et al.*, 2006). The catabolic phase following trauma is characterized by increased anti-diuretic hormone and aldosterone secretion with consequent water and salt retention (Rassam & Counsell., 2005; Lobo *et al.*, 2013). This causes the expansion of the extracellular fluid leading to haemodilution and drop in haematocrit (Lobo *et al.*, 2013). Also ketamine anaesthesia causes a transient decrease in packed cell volume through splenic sequestration of erythrocytes (Bennett *et al.*, 1992; Ramasamy *et al.*, 2006; Udegbumam *et al.*, 2009b). Thus we infer that decrease in Hct and Hgb noted during this study might be a consequence of haemodilution or anaesthesia. The increase in BUN post-surgery was similar to findings reported in earlier studies post castration (Mohammed *et al.*, 2008; Olaifa & Opara, 2011). After major trauma with zero food intake, there is rapid lysis of skeletal muscles leading to a negative nitrogen balance as amino acids are deaminated (Breznock, 1980). The carbon skeletons are processed into energy while their amine radicals are converted into urea and excreted through the kidneys. The blood urea nitrogen rises slightly if

urea endogenous production from skeletal muscle catabolism outstrips the rate of urinary urea excretion. Also, severe blood loss and decrease in glomerular filtration rate (GFR) in the immediate post-operative period leads to increase in BUN concentration in the immediate post-surgical period (Lobo *et al.*, 2013). Thus, the increase in BUN noted might be due to increased protein catabolism and decreased GFR.

An important response to anaesthesia and surgery in the perioperative period is sodium and water retention in the interstitial space (Rassam & Counsell, 2005; Singh, 2003). The net effect is reduction in plasma concentration of Na⁺ and increase in plasma concentration of K⁺. Thus, the blood sodium and chloride concentrations as well as the plasma osmolarity decreased during and after surgery. However, the significant reduction in plasma K⁺ concentration during this study was not expected since K⁺ often rises as Na⁺ concentration decreases. However, apart from the fall in K⁺, Na⁺, venous gas analysis performed during this study showed that pH, PaCO₂, HCO₃⁻ and base excess significantly increased in the perioperative period. These findings suggested a state of metabolic alkalosis (Pettifer, 2003; Rassam & Counsell, 2005). It has been established that the body tries to control alkalosis through buffering as well as by respiratory compensatory mechanisms. The buffering process leads to a decrease in K⁺ concentration in the ECF since efflux of cellular H⁺ into the ECF leads to influx of K⁺ into the cells. Also increase in HCO₃⁻ stimulates compensatory hypoventilation leading to increase in PaCO₂ (Pettifer, 2003). The body also tries to correct alkalemia by elevating its PaCO₂ level by means of hypoventilation. The finding of a state of metabolic alkalosis in goats during this study is significant since alkalosis induces functional hypocalcaemia by increasing the binding ability of calcium to albumin as pH increases (Pettifer, 2003).

The results obtained showed that anion gap decreased during and after surgery. It has been documented that anion gap decreases in the presence of hypoalbuminaemia (Pettifer, 2003). Therefore, although the total plasma protein (TPP) of goats were not assayed during this study, the finding of reduced anion gap suggests that there

could have been marked reduction in TPP. Decreases in TPP post-surgery have been reported in previous experiments (Oyeyemi *et al.*, 2000; Olaifa & Opara, 2011). The reasons for these findings in the perioperative period include increased leakage of albumin into the interstitial space (Lobo *et al.*, 2013) as well as increased protein catabolism (Pereira, 1996; Desborough, 2000).

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- In conclusion, the study showed that tibia surgery lead to alteration in fluid, electrolyte and acid base status of goats. Most importantly, metabolic alkalosis ensued post tibia fracture creation and fixation. This result highlights the need for administration fluid containing sodium chloride as well as potassium chloride in the perioperative period.
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