Effects of Haemorrhage on Thermoregulation, Heart Rate and Blood Constituents in Goats (Capra hircus)

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Abstract: The effects of two levels of bleeding (15 and 30%) on physiological responses were evaluated in adult goats. The magnitude of haemorrhage was expressed as percentage of initial total blood volume after plasma volume determination by dye dilution. The groups subjected to haemorrhage had higher rectal temperature, respiration rate and heart rate compared to the control, the responses were more marked with the higher level of bleeding. The treated groups had lower Packed Cell Volume (PCV), haemoglobin concentration (Hb) and Total Leucocyte Count (TLC) compared to the control; they were significantly lower with high level of bleeding compared to the control. The ratio of lymphocytes decreased, whereas the neutrophil ratio increased in treated groups compared to the control. The treated groups had lower serum total protein and albumin concentrations compared to the control. The plasma glucose level was higher in treated groups compared to the control and it increased with the increase of bleeding level. The treated groups had lower serum Na, Ca and Mg concentrations compared to the control. The levels of these minerals decreased with increase of bleeding level. The 15% bleeding group returned to normal values within 2 weeks, whereas the 30% bleeding group recovered within 5 weeks.

Key words: Goats, haemorrhage, thermoregulation, heart rate, blood constituents

INTRODUCTION

Goats are considered as multipurpose animals that produce meat, milk, skin and fibre. They are kept as a source of protein and they supplement the family income in rural areas. They are unique in their ability to adapt and maintain themselves in harsh tropical environments. Furthermore, goats are relatively resistant to diseases and dehydration and are endowed with inquisite feeding habits and high digestive efficiency for cellulose (Devendra and McLeroy, 1987). Goats may experience considerable blood loss due to trauma and haemorrhage associated with surgery and gynaecological manipulation. Also internal parasites and blood sucking insects may induce blood loss in certain occasions. Such situations may cause anaemia and influence the productivity of goats, particularly during gestation and lactation.

The goat is extensively used as a mammalian research model in various disciplines, particularly in physiological studies. Haematological investigations into the responses of mammals to haemorrhage provide useful scientific knowledge that could be utilized in medicine, surgery and immunology. Such information is also essential for evaluation of efficiency of haemopoietic and other compensatory mechanisms involved in restoration of homeostasis.

Acute blood loss is a major haemorrhage that occurs within a few minutes to several hours. Severe blood loss threatens homeostasis as it acutely decreases blood volume and can lead to cardiovascular collapse, hypovolaemic shock and death (Hillman, 1995). The physiological responses to haemorrhage in mammals are aimed at the preservation of blood pressure and tissue perfusion. The primary mediator of the blood pressure response in animals is increased activity of the sympathoadrenal system in sheep (Block et al., 1987). Haemorrhage resulted in a fall followed by a partial recovery of arterial blood pressure and significant rise of hepatic artery blood flow in rats (Darlington and Tahirani, 1997).

After moderate haemorrhage, a low PCV is remarkably well tolerated because of compensatory mechanisms such as increase in concentration of 2,3-diphosphoglycerate in red blood cell (Ganong, 2003). Erythropoietin secretion
from the kidneys increases in response to blood loss to stimulate erythropoiesis by the bone marrow (Hillman, 1995) and the iron supply to the red cell production usually reflects the severity of the anaemia (Jain, 1993). The reticulocyte response to acute blood loss is highly variable among species (Tyler and Cowell, 1996). Bleeding in sheep resulted in the appearance of large erythrocytes in peripheral blood (Wintour et al., 1995). The neutrophilia and lymphopenia that are common in anaemias could be attributed to the effects of both haemolysis and endogenous corticosteroids (Duncan et al., 1994). Most of the studies have dealt mainly with the acute haemodynamic and endocrine responses of animals to haemorrhage. Accordingly, the purpose of this study was to investigate the sequential acute and long term changes in thermoregulation, heart rate and blood constituents in response to mild and moderate haemorrhage in goats.

MATERIALS AND METHODS

Animals and diet: Eleven young adult (10 months old), apparently healthy non-gestating and non-lactating desert breed goats weighing an average of 16.5 kg were used in the experiment. The animals were examined clinically and were kept in the animal house for an adaptation period of 14 days, followed by an experimental period of 6 weeks. During these periods, the animals were fed alfalfa hay (CP 18%, ME 7.9 MJ kg⁻¹) and were offered tap water ad libitum. This study was conducted at the Department of physiology during March-April 2006.

Experimental design: For all animals, the initial baseline physiological data were determined. The total blood volume was measured in all animals using Evans blue dye. The animals were randomly assigned to three groups, 3 animals in group A and 4 in each of groups B and C. Group A served as control while the treated groups, B and C were subjected to 15 and 30% bleeding, respectively. Graduated blood collection bags were used to collect the specific volume of blood from the external jugular vein. The acute responses were monitored for 9 days following bleeding and the parameters were determined and blood samples were collected weekly for 6 weeks in order to determine the long-term responses to bleeding.

Collection of the blood samples: Five milliliter of blood samples were collected using plastic disposable syringes. Immediately, 1 mL of blood was transferred to a clean dry test tube containing disodium ethylene diamine tetra acetate (Na₂-EDTA) as an anti-coagulant for blood analysis. One milliliter of blood was also transferred to another test tube containing sodium fluoride to inhibit the enzymatic reaction (Kelly, 1984) and was centrifuged at 3000 rpm for 15 min; the plasma samples were used for glucose determination. The rest of the blood was allowed to stay for 2 h at room temperature and then centrifuged at 3000 rpm for 15 min and haemolysis-free serum samples were pipetted into clean vials and immediately frozen at -20°C for subsequent analysis.

**Measurement of blood volume:** The blood volume was determined using Evans blue dye (T-1824) according to the method described by Pirkle and Gann (1976).

**Blood analysis:** The Haemoglobin concentration (Hb), Packed Cell Volume (PCV), total leukocyte count (TLC) and differential leukocyte count (DLC) were determined according to the standard methods described by Kelly (1984) and Jain (1993).

**Serum and plasma analysis:** The serum total protein concentration was determined using Biuret reagent as described by King and Wooton (1965). Serum albumin concentration was determined by the colorimetric method of Bartholmew and Delaney (1966). Plasma glucose concentration was determined by the enzymatic colorimetric method using a kit (Spinreact, S.A., Spain). Serum Na concentration was determined by flame photometer technique as described by Wooton (1974). Serum Ca concentration was determined by the colorimetric method using chloramidine acid and ferric nitrate (Trinder, 1960). The concentration of Mg in serum was determined as described by Neil and Neely (1956).

**Statistical analysis:** The experiment was performed according to the complete randomized design (Factorial arrangement). The data collected were subjected to appropriate General Linear Model (GLM) procedure of statistical analysis using the SAS (1988). The SAS was used to perform analysis of variance (ANOVA) to evaluate the effects of bleeding on the responses of goats. The values of parameters measured are expressed as Means±Standard Deviation (SD). The separation of means was done by Duncan multiple range test.

RESULTS

**Rectal temperature (T_r):** Figure 1 shows that the initial values of T_r of the goats ranged between 38.2 and 39.2°C. The treated groups had higher mean initial values of T_r compared to the mean value measured for the control group. Generally, for the treated groups of goats, there was an initial gradual increase in T_r following bleeding for 2 h and the normal values of T_r were recovered after about 5 h.
Respiration rate (RR): Figure 2 shows that the initial mean values of RR of the experimental groups were almost similar (= 25 breaths min⁻¹). The control group maintained this value until the end of experimental period. Generally, the haemorrhaged groups of goats maintained higher values of RR following bleeding until day 4 and there was an increase in RR with increase of bleeding level. Immediately after bleeding, compared to the values obtained for the control group, RR was higher (p<0.05) with 30% bleeding. The 30% bleeding group had higher (p<0.01) RR values compared with control and 15% bleeding group at 1, 2, 3, 4, 5 and 6 h after bleeding.

Heart rate (HR): Figure 3 shows that the initial mean value of HR was higher in the control group. Following bleeding, treated groups had higher mean values of HR compared with the control until day 2. The values of treated groups returned to normal after 4 days. There was increase in HR with the increase of bleeding level. Immediately after bleeding, the 30% bleeding group had higher (p<0.05) HR compared with control group value. At 5 and 6 h after bleeding, the 30% bleeding group had higher (p<0.01) HR values compared with control. The 30% bleeding group also had significantly higher HR values compared with control at 6 h (p<0.05) and at 24 h (p<0.01) following bleeding.

Packed cell volume (PCV): Immediately post-bleeding, the PCV was steady with 15% bleeding and it showed a slight decrease with 30% bleeding (Fig. 4). There was a sharp decrease in PCV of treated groups at 6 h post-bleeding. The groups subjected to bleeding had significantly (p<0.01) lower values of PCV at 6, 24, 48 and 96 h post-bleeding compared with the control. The 30% bleeding group had lower (p<0.05) PCV mean values compared with the control at days 6 and 9 after bleeding. The PCV recovered the initial normal value at day 6 with 15% bleeding and after 2 weeks with 30% bleeding.

Haemoglobin concentration (Hb): Figure 5 shows that the initial values of Hb ranged between 9.8 and 11.0 g dL⁻¹. There was no marked change in Hb concentration.
immediately post-bleeding. However, the treated groups showed a sharp decrease in Hb level at 6 h post-bleeding and the decrease was more pronounced with 30% bleeding. Thereafter, the control group showed an almost steady level and the treated groups showed gradual increase in Hb level. The 15% bleeding group recovered the normal Hb level at day 6. The 30% bleeding group showed a gradual increase in Hb level until day 9, maintained an almost steady level at weeks 2, 3 and 4 and then the level increased to assume levels similar to the control and 15% bleeding group in weeks 5 and 6. At 6, 24 and 48 h, the treated groups had lower (p<0.01) values of Hb concentration compared to values obtained for the control. The mean values of Hb with 30% bleeding were significantly (p<0.05) lower compared with the respective control values at days 4, 6 and 9.

**Total leukocyte count (TLC):** The initial mean values of TLC for the experimental groups were similar (= 10×10⁴ μL⁻¹) (Fig. 6). The control group showed fluctuating pattern for TLC during the experimental period. Both treated groups showed a sharp decline in TLC after 6 h post-bleeding. The group subjected to 30% bleeding had lower (p<0.05) TLC compared with the control group at 6 hrs post-bleeding. Then both treated groups showed gradual increase in TLC; the normal control values were attained at day 6. Thereafter, the control and treated groups had almost similar values of TLC until the end of the experimental period.

**Lymphocyte ratio:** Figure 7 shows that generally, the treated groups showed lower lymphocyte ratio compared with the control during the experimental period. The initial values of lymphocyte ratios ranged between 57 and 62% and there was no change in the ratio immediately after bleeding. The control group maintained an almost steady lymphocyte ratio (= 60%) during the experimental period. The treated groups showed lower (p<0.01) values at 6 h following bleeding. Thereafter, for both treated groups, the lymphocyte ratio increased to attain the control group values at day 2 for 15% bleeding and day 6 for 30% bleeding. The group subjected to 30% bleeding had lower (p<0.05) values compared to the control at days 1, 2 and 4. The 30% bleeding group had lower (p<0.01) values compared with control and 15% bleeding at day 4 post-bleeding.

**Neutrophil ratio:** The initial values of neutrophil ratio were close to each other (= 33%). Generally, the treated groups showed higher mean values of neutrophil ratio compared with the control (Fig. 8). There was no change in neutrophil ratios immediately after bleeding. The treated
groups showed higher (p<0.01) means values of neutrophil compared with the control at 6 h. The 30% bleeding group had higher (p<0.05) neutrophil ratios compared with the control group at days 1 and 2.

**Serum total protein:** Figure 9 shows the effect of bleeding level on serum total protein concentration. The initial values of total protein ranged between 7.30 and 7.73 g dL⁻¹. The control group showed fluctuations in total protein level during the experimental period. There was a decrease in total protein values with both bleeding levels. Immediately after bleeding, the 30% bleeding group showed lower (p<0.01) total protein value compared with the control group. The normal control value was re-established at 6 hrs for 15% bleeding and at 24 h for 30% bleeding.

**Serum albumin:** Figure 10 shows that there was no marked difference in serum albumin level immediately after bleeding. The control group maintained an almost steady albumin level (≈ 4.0 g dL⁻¹) during the experimental period. Both treated groups showed gradual decrease in albumin level at 6 and 24 h following bleeding. The 30% bleeding group had lower (p<0.05) albumin level compared with the control group at 6 h and days 1 and 2.

Figure 11 shows that the control group maintained an almost steady serum urea level during the experimental period. Both treated groups showed an immediate rise in serum urea level following bleeding, that was more pronounced with 30% bleeding and significantly (p<0.05) higher compared to the control group level at 6 h. The treated groups recovered the normal control level at day 4 following bleeding.
Fig. 12: Effect of bleeding on plasma glucose level in goats

Fig. 14: Effect of bleeding level on serum calcium concentration (Ca) in goats

Fig. 13: Effect of bleeding level on serum sodium concentration (Na) in goats

Fig. 15: Effect of bleeding level on serum magnesium concentration (Mg) in goats

**Plasma glucose:** The initial values of plasma glucose level ranged between 45 and 55 mg dL\(^{-1}\) and the control group had higher level compared to treated groups (Fig. 12). For all groups, there were marked similar fluctuations in glucose level and the treated groups tended to maintain higher glucose levels occasionally. The glucose level of treated groups was higher compared to respective control group values for 4 days.

**Serum Na:** Figure 13 shows that there were marked fluctuations in serum Na level for all experimental groups. Immediately post-bleeding there was decline in Na level in both treated groups, but the decline was more pronounced with 30% bleeding. At 6 h, the treated groups had higher Na level and then both groups showed gradual increase in Na level until day 2.

**Serum Ca:** The initial values of serum Ca were almost similar (≈ 10 mg dL\(^{-1}\)) for the control and treated groups of goats (Fig. 14). The control group maintained this value during the experimental period. Following bleeding, both treated groups showed gradual decline in Ca level for 6 h and the low values were maintained until day 4. Thereafter, for both groups, there was progressive increase in Ca level to attain the high control group level at week 2 for 15% bleeding and week 3 for 30% bleeding. During the experimental period, the 30% bleeding group maintained lower Ca level compared to respective values obtained for 15% bleeding group. The treated groups had significantly (p<0.05) lower mean values of Ca compared with the control group, immediately after bleeding and at 6, 24 and 48 h; also the treated groups had lower (p<0.01) mean values compared with the control at day 4. The 30% bleeding group had lower (p<0.05) Ca level compared with the control at days 6 and 9.

**Serum Mg:** The initial values of serum Mg were almost similar (≈ 2.5 mg dL\(^{-1}\)) for the experimental groups (Fig. 15). This level was almost maintained by the control group during the experimental period. Both treated groups
showed a sharp decrease in Mg level following bleeding and at 6 h, both groups showed a sharp increase to attain a value higher than the respective control group value. Thereafter, both treated groups showed undulating pattern until the end of the experimental period. The 30% bleeding group had lower serum Mg level immediately after bleeding (p<0.01) and after 24 h (p<0.05).

**DISCUSSION**

The results provide new information which suggests that haemorrhage influences thermoregulation. This was indicated by post-haemorrhage increase in rectal temperature, T, (Fig. 1). The increase in T, could be related to retardation of convective heat transfer from body-core to the periphery associated with reduction in blood volume. One of the primary reflex adjustments to haemorrhage involves an increase in total peripheral resistance in order to maintain blood pressure (Vatner, 1974). Also the post-haemorrhage pyrexia could be attributed to the effect of calorigenic hormones secreted in response to haemorrhage. The hormones which assume marked role in haemorrhaged animals include catecholamine and adenocorticotropic hormones (Rose et al., 1987). The calorigenic effect of bleeding could also be associated with the metabolism of fatty acids, increase in the activity of the membrane bound Na⁺,K⁺-ATPase and increase in concentration of 2,3-Diphosphoglycerate in erythrocytes that enhances delivery of oxygen to body tissues.

The two bleeding levels also resulted in an increase in respiratory rate, RR in goats (Fig. 2). Hyperventilation is a recognized physiological response to haemorrhage. It is presumed to be stimulated by hydrogen ions formed in tissues due to lowering of oxygen delivery. The respiratory centre located in the medulla is sensitive to hydrogen ion concentration and accordingly marked increase in respiratory frequency was triggered in goats immediately after bleeding. Loss of red blood cells decreases the \( O_2 \) carrying capacity of the blood and the blood flow in the carotid and aortic bodies is reduced (Ganong, 2003). The resultant anaemia and stagnant hypoxia, as well as acidosis that may occur, stimulate the chemoreceptors. Maltz et al. (1984) noted that in Bedouin goats, 23% bleeding was associated with forced breathing and increase in RR from 50 to 100 breaths min⁻¹.

The cardiovascular responses of goats to bleeding, manifested by marked increase in heart rate, HR (Fig. 3) could be related to stimulation of autonomic nervous system which increases the sympathetic activity induced by the baro-receptors. The present results in goats are in agreement with the tachycardia reported after 20% bleeding in adult sheep (Rose et al., 1987; Wintour et al., 1995) and rabbits (Clow et al., 2003).

The results indicate that loss of 15 and 30% of total blood volume resulted in significantly lower PCV level (Fig. 4) associated with decrease in Hb concentration (Fig. 5) after 6 h of bleeding. The immediate post-haemorrhage values of PCV of goats were apparently normal because erythrocyte and plasma volume were lost in similar proportions. The drop in PCV and Hb concentration was caused by the shifting of water from the interstitial fluid compartment to restore blood volume. Previous studies indicated that the PCV decreased significantly post-haemorrhage in sheep (Wintour et al., 1995). The return of PCV to pre-haemorrhage level occurred in approximately 6 days and 2 weeks for 15 and 30% bleeding groups, respectively. For Hb concentration, recovery occurred in 6 days and 5 weeks for 15 and 30% bleeding groups, respectively. These findings illustrate the influence of bleeding level on the capacity of the haemopoietic system to restitute normal haematologic values. The results are in general agreement with the findings of Lassen and Swanson (1995) and Tyler and Cowell (1996) which suggest that return to normal level of PCV occurs in approximately 2-4 weeks for most species of mammals.

Haemorrhage in goats was associated with decrease in total leukocyte count, TLC (Fig. 6). The decline in TLC values 6 h post-bleeding may be attributed to haemodilution. The value returned to normal level after 2 and 6 days for 15 and 30% bleeding, respectively. Duncan et al. (1994) noted that immature leukocytes may also appear in the blood, particularly in cases of severe blood loss.

Bleeding of goats resulted in an increase in the neutrophil ratio associated with decrease in lymphocyte ratio (Fig. 7, 8). The post-haemorrhage neutrophilic leukocytosis is related to a shift of neutrophils from marginal pool and bone marrow reserve to the circulation (Duncan et al., 1994). Epinephrine released in response to haemorrhage also mobilizes neutrophils from marginal pool into the circulation. The lymphopenia observed could be attributed to release of ACTH or cortisol, usually encountered after bleeding; ACTH induces dissolution of lymphocytes in tissue and increase in antibody concentration in blood (Swenson, 1993). The significant decrease in serum total protein and albumin immediately after bleeding and after 6 h (Fig. 9, 10) is related to haemodilution. Kovacs et al. (2000) reported that reduction in total protein and albumin levels occurs in post-operative blood loss in humans, whereas in dogs,
there was an increase in the plasma proteins in the beginning and gradual decreases in protein values in later stages after blood loss. The plasma proteins are usually replaced from mobilized fluid resource or intake (Cope and Litwin, 1962). The serum total protein level returned to normal values after 6 h and 1 day for 15 and 30% bleeding, respectively. The values of albumin returned to normal levels after 2 and 4 days for 15 and 30% bleeding, respectively. The current results for goats are in general agreement with the finding of Jain (1993) who reported that plasma proteins concentrations returned to pre-bleeding level within 5-7 days in animals.

The increase in serum urea level that was more pronounced with 30% bleeding (Fig. 11) may be attributed to decrease in renal plasma level. Also it could be associated with depression of glomerular filtration rate induced by reduction in blood volume. Similarly, in sheep, 20% haemorrhage caused an increase in blood urea nitrogen concentration (Wintour et al., 1995). Ware et al. (1982) noted that rats had showed an increase in plasma urea concentration as a result of 20% bleeding.

The haemorrhage-evoked elevation of plasma glucose concentration reported in this study that was influenced by level of blood loss (Fig. 12) is related to sympathoadrenergic activity and secretion of epinephrine, ACTH and cortisol which cause increase in glycogenolysis (Jarhult, 1975). In addition, Seredycz and Lautt (2006) indicated that in acute haemorrhage, insulin secretion is suppressed and insulin resistance is accounted for by elimination of the hepatic insulin sensitizing substance component of insulin action. This finding confirms previous results reported in sheep (Block et al., 1987) and in rats (Kadekar et al., 1998).

The current results indicate that bleeding in goats resulted in a decrease in serum Na level (Fig. 13). This response indicates haemodilution during acute haemorrhage which involves entry of extravascular fluid into the vascular space (Hjelmqvist et al., 1991). Similarly decrease in Na concentration has been reported in sheep subjected to 20% bleeding (Wintour et al., 1995). Walsh et al. (1980) reported that blood loss of 25 mL kg⁻¹ resulted in a decrease in Na level suggesting that compensatory fluid replacement originated in cells as well as interstitium. The marked post-haemorrhage fluctuations in serum Na level could indicate that feedback compensatory mechanisms related to renin-angiotensin system were intermittently stimulated.

Bleeding in goats resulted in a decrease in serum Ca concentration immediately post-haemorrhage, low values were maintained for 6 days and the normal control level was recovered after 2 and 3 weeks for 15 and 30% bleeding, respectively (Fig. 14). Hypocalcaemia observed after blood loss may be associated with haemodilution and vasodilatation which is influenced by haemorrhage. The decrease in Ca level may also be associated with hypoalbuminaemia (Kovacs et al., 2000), as albumin has an important role in transport of many cations such as Ca (Jain, 1993). About half of the plasma Ca is bound to albumin and it is not filtered at the glomerulus (Reece, 1993). In sheep subjected to 20% bleeding, Wintour et al. (1995) reported a decline in Ca level after haemorrhage. However, Ware et al. (1982) noted that Ca level was not changed within 90 min. after haemorrhage in rats subjected to 20% bleeding.

Bleeding was associated with a decrease in serum Mg concentration (Fig. 15). The decrease in Mg concentration may be attributed to vasodilatation which is caused by haemorrhage. The subsequent sharp increase after 6 h could be related to an increase in Na⁺-K⁺-ATPase. Mg acts as co-factor of cellular ATPase including the Na⁺-K⁺-ATPase (Hayes and Swenson, 1993). A decrease in Mg concentration after bleeding has been reported by Kovacs et al. (2000) in humans.

For most of the parameters investigated in this study, return to normal values occurred within 2 weeks for 15% bleeding, whereas the recovery occurred in 5 weeks for 30% bleeding. This pattern indicates that the recovery period was influenced by the bleeding level.

**CONCLUSION**

The goat model can be adopted for evaluation of the effects of acute blood loss in mammals. The most sensitive indicators of blood loss and recovery pattern seem to be respiratory rate, heart rate and erythropoietin values. Recovery from acute blood loss is related to the level of bleeding.

We assume that the findings reported in this study have valuable implications regarding haematology and surgery in both veterinary and medical sciences. Further investigations are needed to examine the effects of haemorrhagic shock in the goat model and to investigate the effects of dietary factors on recovery pattern from acute blood loss anaemia. Change were in acid-base parameters validated as accurate predictors of blood volume changes and therefore may be utilized in the assessment of conditions of ongoing haemorrhage.

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