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Physiological Responses of Goats (*Capra hircus*) to Haemorrhage as Influenced by Age

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Abstract: The aim of this study was to clarify the acute and long-term thermoregulatory and haematological responses to moderate haemorrhage (20% of initial blood volume) and to evaluate the influence of age in desert goats. The magnitude of haemorrhage was expressed as a percentage of initial total blood volume after plasma volume determination by dye dilution. Both age groups (3 months, 3 years) had higher rectal temperature (Tr), respiration rate (RR) and heart rate (HR) compared to the control in response to haemorrhage; the increase in Tr and RR was more pronounced in young animals. The total leukocyte count (TLC), packed cell volume (PCV) and haemoglobin concentration (Hb) were lower in haemorrhaged young and adult groups and the post-haemorrhage decline was more pronounced in adult goats. The decrease in lymphocyte and eosinophil ratios and increase in neutrophil and monocyte ratios in response to haemorrhage were more pronounced in adult goats. Haemorrhage resulted in lower serum total protein and albumin levels in young and adult goats. The haemorrhage induced rise in serum urea level was more pronounced in adult goats. The post-haemorrhage increase in plasma glucose level was more marked in young animals compared to the adults. The serum Na level decreased in both haemorrhaged age groups. The variables measured in goats exposed to haemorrhage returned to normal levels earlier in young animals. The results support the assumption that young animals have abilities to compensate the blood volume loss better than adults.

Key words: Goats · Haemorrhage · Age · Thermoregulation · Heart rate · Blood constituents

INTRODUCTION

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 blood loss anaemia and influence the growth of young animals and the productivity of adult goats during gestation and lactation.

The goat is extensively used as a mammalian research model in various disciplines, particularly physiological studies of caprine haematology have recently received considerable attention. Haematological investigations into the responses of goats to haemorrahage provide useful scientific knowledge that could be utilized in medicine, surgery and immunology. Information on the organization of the caprine immune system and immune response is limited. Such information is also essential for evaluation of efficiency of haemopoietic and other compensatory mechanisms involved in restoration of homeostasis and to prescribe the appropriate treatments.

Aging is associated with changes in metabolism and status of various nutrients. There is evidence that haemotologic variables such as haemoglobin and haematocrit may decline with aging in animals and humans [1]. During the postnatal development, the locations of active haemopoiesis change and the role of the liver in haemopoiesis decreases rapidly in the postnatal life of mammals [2]. The haemopoietic activity of the spleen is high in mice during the whole life span [3], but in rats it decreases during the age of maturation [4]. This suggests that the microenvironmental conditions in the bone-marrow and spleen are changing during the aging of rats [5].

Studies in sheep indicated that physiological responses to haemorrhage are age dependent [6]. In

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conscious adult animals and humans, haemorrhage leads to activation of a number of physiological mechanisms to promote the restoration of vascular volume and blood pressure [7]. During the foetal and neonatal development, these systems may be in various stages of maturity [8].

Previous studies have reported reduction in baroreceptor efficiency with aging, which might reduce the capacity to preserve homeostasis during hypovolaemia circulatory stress induced by haemorrhage [9]. The pathophysiological changes in the cardiovascular system with age have clinical implications. The crucial first line of defense comes into play within seconds during an acute hypovolaemic circulatory stress, where both the compensatory increase in effective circulating blood volume and the rate of compensation are important factors to preserve homeostasis; this might seriously impede the possibility of survival of acute blood loss in the aging [10].

The aim of this study was to clarify the acute and long-term thermoregulatory and haemotological responses to moderate haemorrhage and to evaluate the influence of age in desert goats.

MATERIALS AND METHODS

Animals and Diet: The animals used in the study comprised 6 young (~3 months) and 6 adult (~3 years) desert breed goats. The mean body weight (BW) of young and adult animals were 8.9±0.5kg and 25.7±0.5kg, respectively. All animals were non-gestating and non-lactating. The animals were examined clinically and were kept in the animal pens at the experimental farm of Sudan University of Science and Technology 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) and were offered tap water *ad libitum*. The study was conducted during September-October, 2007 at the Department of Physiology.

Experimental Design: Each age group of goats was randomly assigned to 2 groups of 3 animals each, control and treated. For all animals, the baseline data of haematological indices were determined. The treated goats in each age group were subjected to 20% bleeding. Graduated blood collection bags were used to withdraw the specific volume of blood from the jugular vein. The acute post-haemorrhage responses were investigated for 10 days. Then the long-term responses were monitored for 5 weeks.

Measurements of Rectal Temperature, Respiration Rate and Heart Rate: The measurements of rectal temperature (Tr) of the experimental animals were made to the nearest±0.1°C using a certified mercury-in-glass clinical thermometer (Hartman - United Kingdom). The respiration rate (RR) was measured by visually counting the flank movements. The heart rate (HR) was measured by monitoring with the aid of a stethoscope and stopwatch.

Measurement of Blood Volume and Blood Sampling: The total blood volume was measured utilizing plasma volume determined by Evans blue dye [11]. Blood samples were collected for analysis using 5ml plastic disposable syringes. Immediately 1 ml of blood was transferred to a clean dry test tube containing disodium ethylene diamine tetra acetate (Na_2- EDTA) as an anti-coagulant for blood analysis. 1 ml of blood was also transferred to another test tube containing sodium fluoride to inhibit the enzymatic reaction and was centrifuged at 3000 r.p.m. for 15 min. The plasma separated was used for glucose determination. The rest of the blood was allowed to stay for 2 hrs at room temperature and then centrifuged at 3000 r.p.m for 15 min. Serum samples were pipetted into clean vials and immediately frozen at -20°C for subsequent analysis.

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 [12, 13].

Serum and Plasma Analysis: The concentration of serum total protein was determined using Biuret reagent [14]. Serum albumin concentration was determined by the colorimetric method [15]. Serum urea concentration was determined by the enzymatic method test (Berthlot) using a kit (Spinreact, S.A., Spain).

The plasma glucose concentration was determined by enzymatic colorimetric method using a kit (Spinreact, S.A., Spain). The concentration of Na in serum was determined by flame photometer technique [16].

Statistical Analysis: The experiment was performed according to the complete randomized design (Factorial arrangement) (2X2X3). The data collected were subjected to appropriate analysis of variance (ANOVA) using the SAS package [17]. Duncan's Multiple range tests were used to detect statistical significance between treatment groups.

RESULTS

Thermoregulation and Heart Rate: Fig. 1 shows that the initial values of rectal temperature (Tr) of the goats ranged between 38.5 and 38.7°C and there were marked fluctuations of (Tr) during the experimental period. In young and adult haemorrhaged groups (Tr) increased significantly ($P<0.01$) immediately after bleeding and after 2 hr, respectively. The rise in (Tr) values was more pronounced in young animals compared to the adult values. The values of (Tr) returned to normal after 24 hrs in young animals and after 6 hr in adults.

Fig. 2 shows that the initial values of (RR) for experimental groups were similar (˜ 28 breaths/min). Following haemorrhage, the mean values of (RR) showed an increase in both treated groups. The (RR) values of treated young and adult groups were higher than respective control values for 5 hrs and 1 day, respectively; then the control and treated groups maintained a similar pattern of (RR) values until the end of experimental period. Immediately post-bleeding, the rise in (RR) values was more pronounced in young animals compared to the adult values. Conversely, at 2-6 hrs, the adult animals recorded higher values of (RR) compared to respective young animal's values. The treated groups showed significantly ($P<0.01$) higher values compared with the control immediately post-bleeding and at 1, 2, 4 and 5 hrs after haemorrhage. Haemorrhaged adult animals maintained significantly ($P<0.05$) higher values of (RR) compared with the adult control and both young animal groups at 3 hrs and 6 hrs after bleeding.

Fig. 3 shows that the initial values indicate that (HR) in young animals (109±12 beats/min.) were higher than the values obtained for the adult animals (67±6 beats/min.). In both age groups, there was a significant ($P<0.01$) increase in (HR)immediately following bleeding; the magnitude was almost similar in young and adult groups. The (HR) returned to normal values after 6 hr and 2 days in young and adult groups, respectively. Then the young and adult groups of animals maintained almost steady respective values of 100 and 65 beats/min. until the end of the experimental period. The treated groups of both age groups showed significantly ($P<0.01$) higher (HR) values compared with the control groups at 1, 2, 3, 4 and 5 hrs post-haemorrhage. The adult treated group had significantly ($P<0.05$) higher mean (HR) values compared with the control at 6 hrs and 24 hrs.

Haematologic Indices: Fig. 4 shows that the initial pre-haemorrhage values of PCV were 25.0±3.1 and 30.0±3.5% in young and adult goats, respectively. Immediately post-bleeding, the PCV in adults showed a slight increase. In contrast, in the young, the PCV decreased progressively until day 2. The return to baseline values occurred after 3 and 6 weeks in young and adult animals, respectively. The results indicate that the interaction of bleeding, age and time was significant ($P<0.01$).

Fig. 5 shows that the initial baseline (Hb) concentration values were 10.1±0.9 and 11.3±0.3g/dL in young and adult goats, respectively. Immediately post-haemorrhage, the (Hb) level of adult animals increased slightly. In contrast, the (Hb) level of young animals showed a decrease. The decline in (Hb) level was more pronounced in adult treated group compared with the young group. Then the values of (Hb) decreased progressively in both treated age groups for 2 days. The normal baseline values were re-established after 9 days and 4weeks in young and adult groups, respectively. The results indicate that the interaction between bleeding, age and time was significant ($P<0.01$).

Fig. 6 shows that the baseline values of (TLC) were 13.1±0.5 and $11.7\pm1.0\times10^3/\mu L$ in young and adult animals, respectively. The values of TLC did not change immediately post-bleeding in both age groups. However, the young animals showed a decrease in (TLC) for 24 hrs and the adults for 6 hrs. Then both treated groups showed progressive increase in (TLC) to maintain higher values after day 7.

The responses of lymphocyte ratio (%) are shown in Fig. 7. The initial values of lymphocyte ratio were almost similar (˜60%). Immediately post-bleeding, the lymphocyte ratio did not change in treated goats. Both treated groups showed a decline in the ratio of lymphocytes 6 hrs after haemorrhage, which was more pronounced in adults. The treated young and adult groups showed progressive increase in lymphocyte ratio after 6 hrs and 24 hrs, respectively. Lymphocyte ratio returned to normal value after 2 and 4 days, respectively in young and adult animals. All experimental groups maintained a similar pattern as from week 2 until the end of experimental period. The results indicate that the interaction between bleeding, age and time was significant ($P<0.01$).

The responses of neutrophil ratio are shown in Fig. 8. The initial values of neutrophil ratio of experimental groups were almost similar (˜33%). There was no marked difference in neutrophil ratio between groups immediately

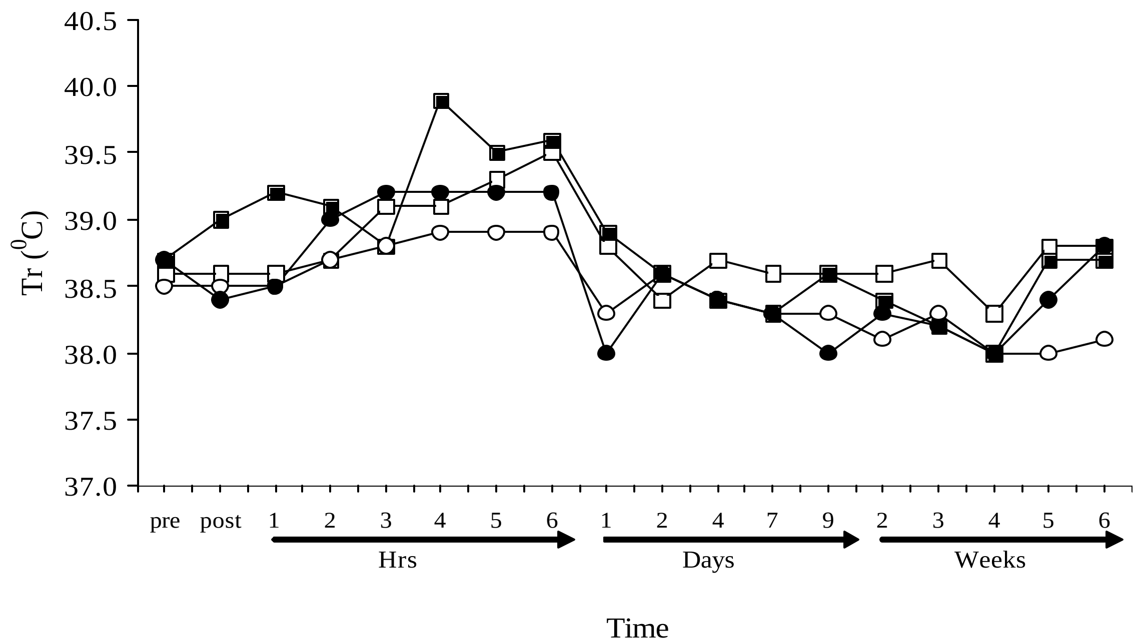

Fig. 1: Effects of 20% haemorrhage on rectal temperature (Tr) in young and adult goats

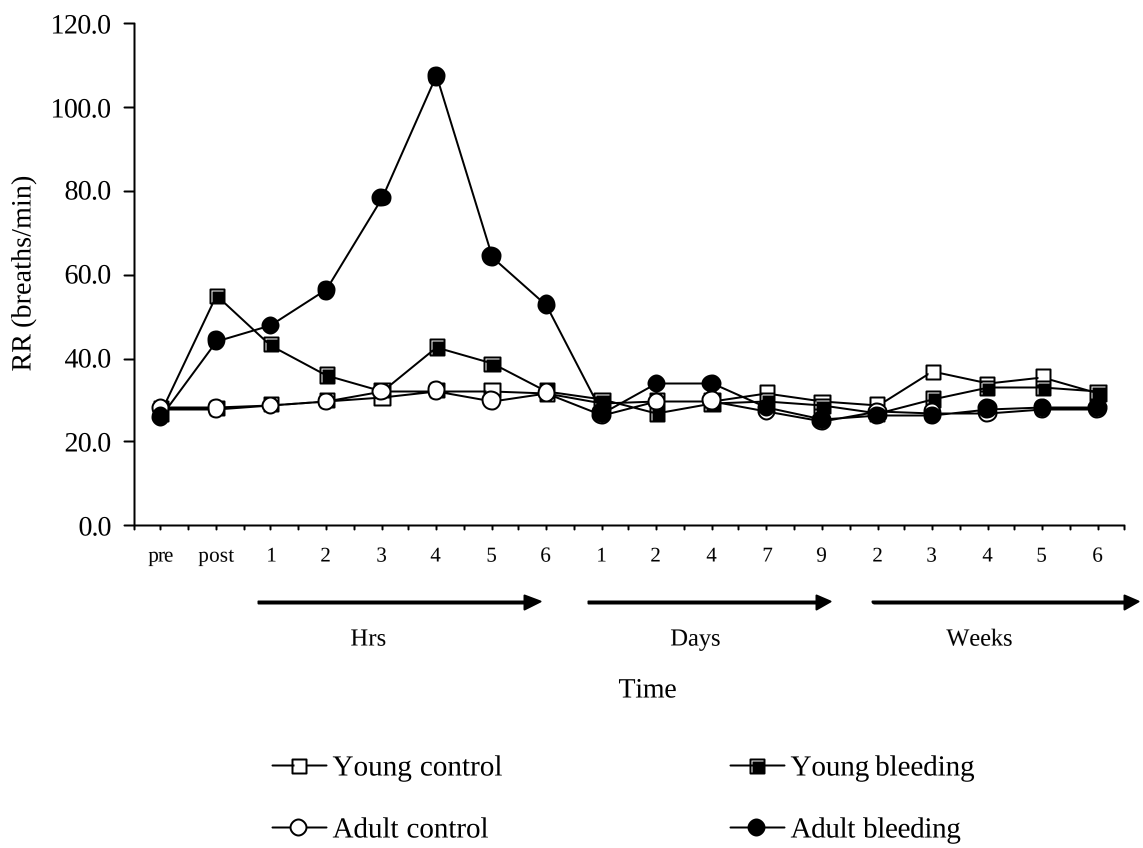

Fig. 2: Effects of 20% haemorrhage on respiratory rate (RR) in young and adult goats

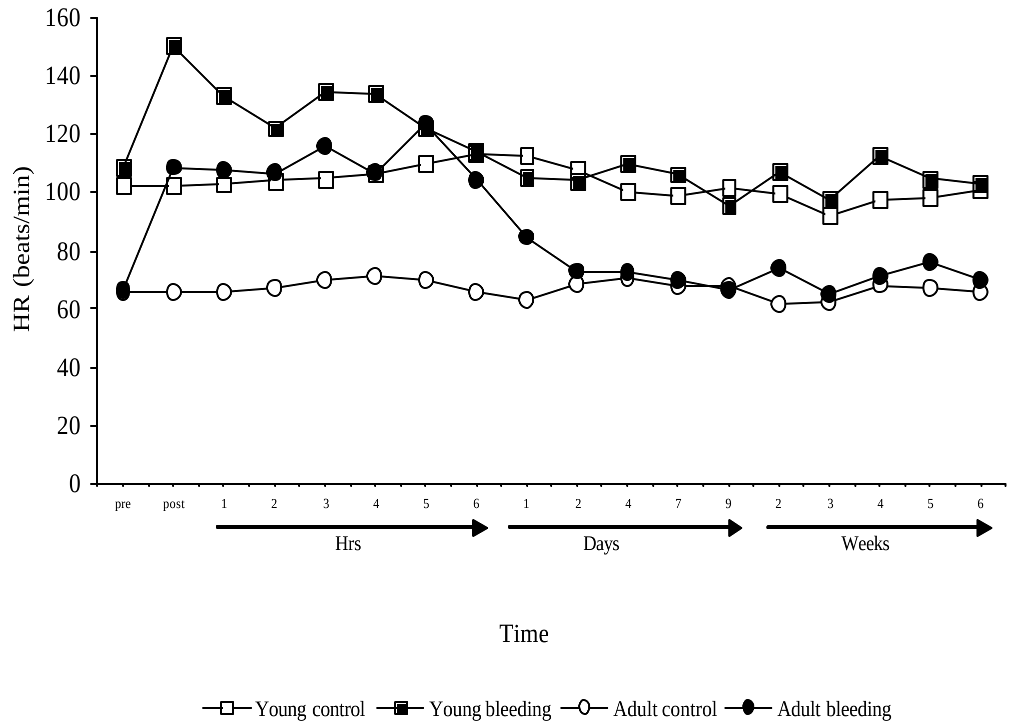

Fig. 3: Effects of 20% haemorrhage on of heart rate (HR) in young and adult goats

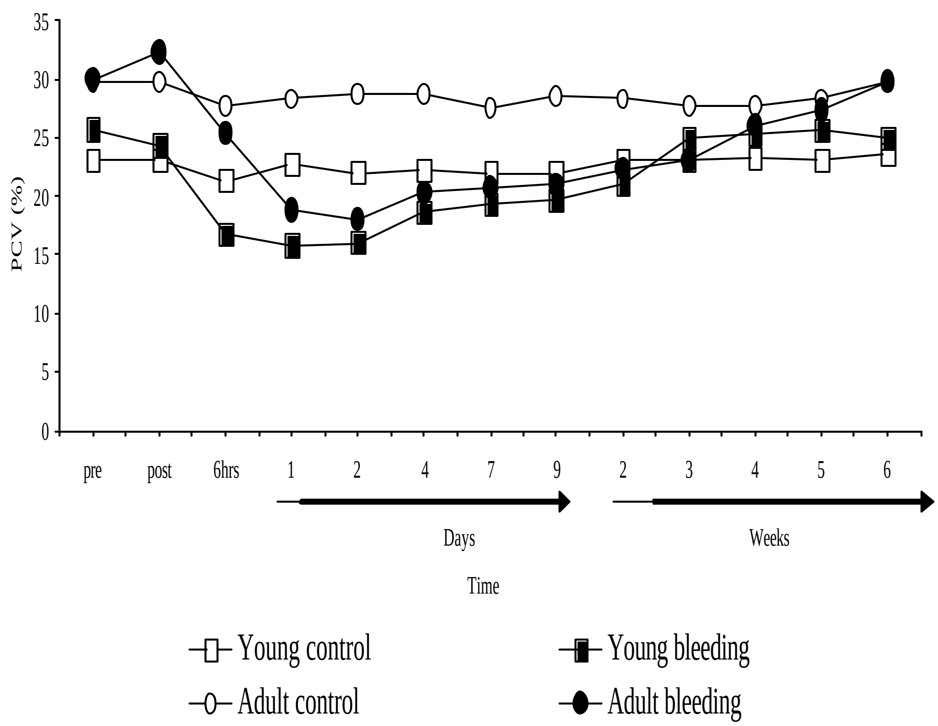

Fig. 4: Effects of 20% haemorrhage on packed cell volume (PCV) in young and adult goats

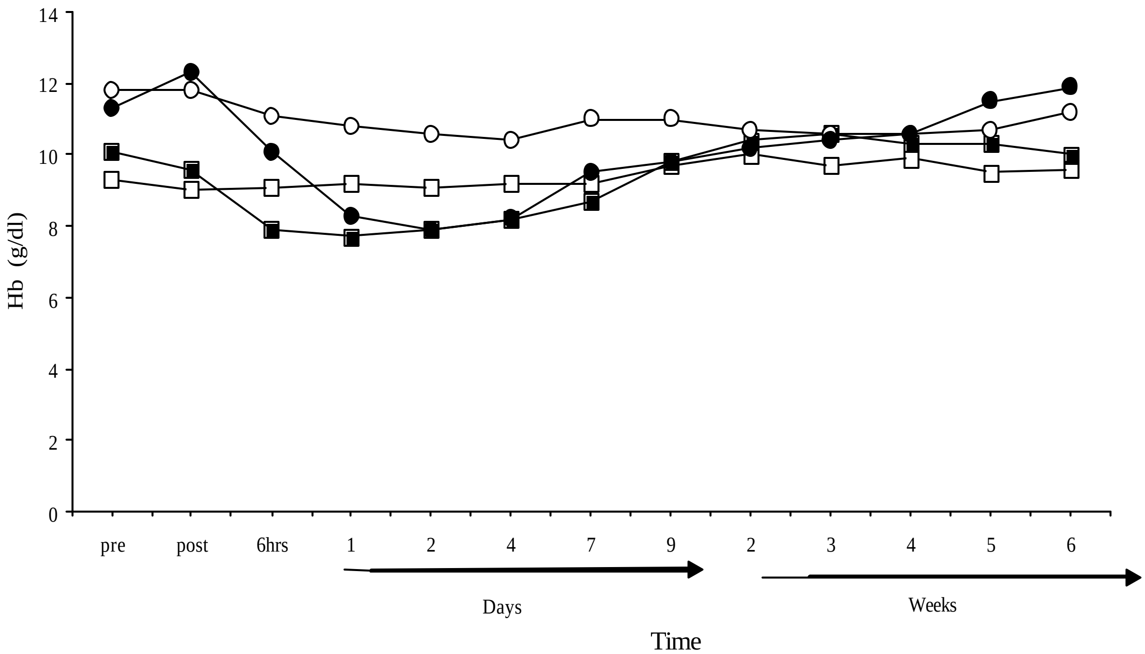

Fig. 5: Effects 20% haemorrhage on haemoglobin (Hb) concentration in young and adult goats

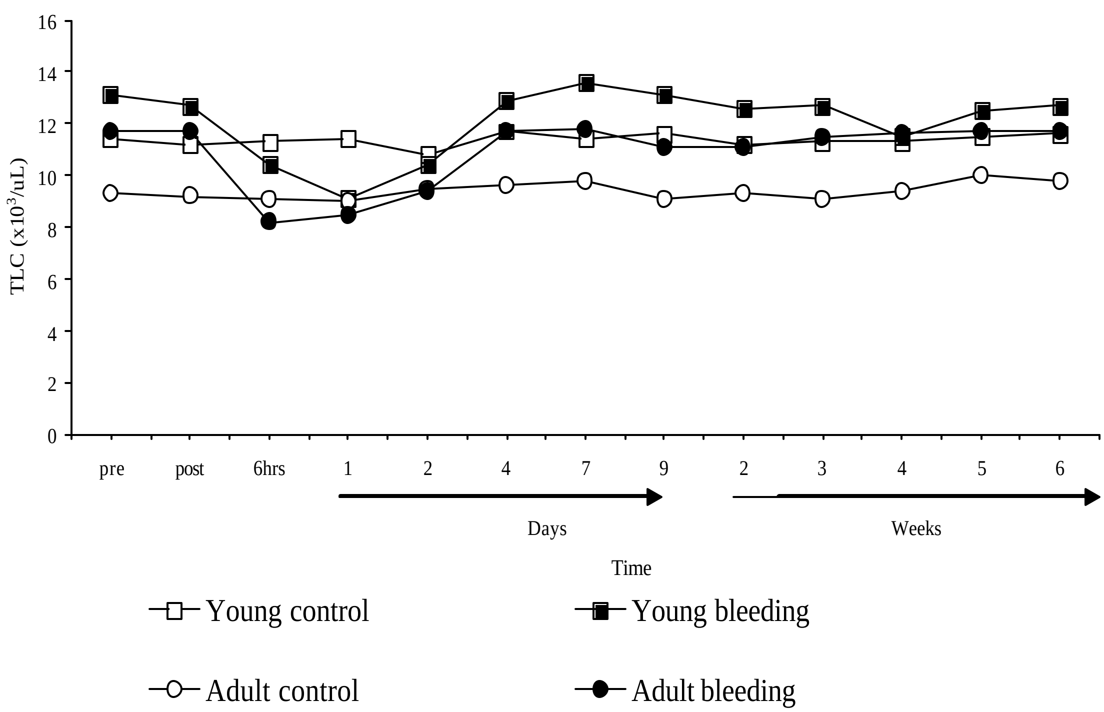

Fig. 6: Effects of 20% haemorrhage on total leukocyte count (TLC) in young and adult goats

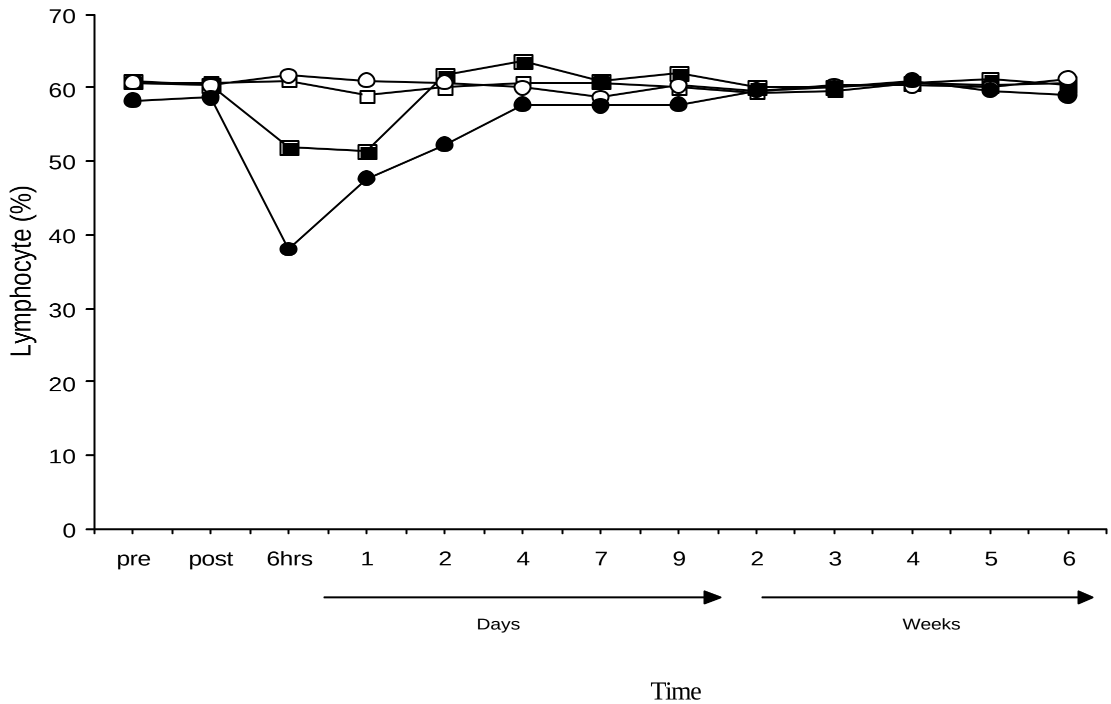

Fig. 7: Effects of 20%haemorrhage on lymphocyte ratio (%) in young and adult goats

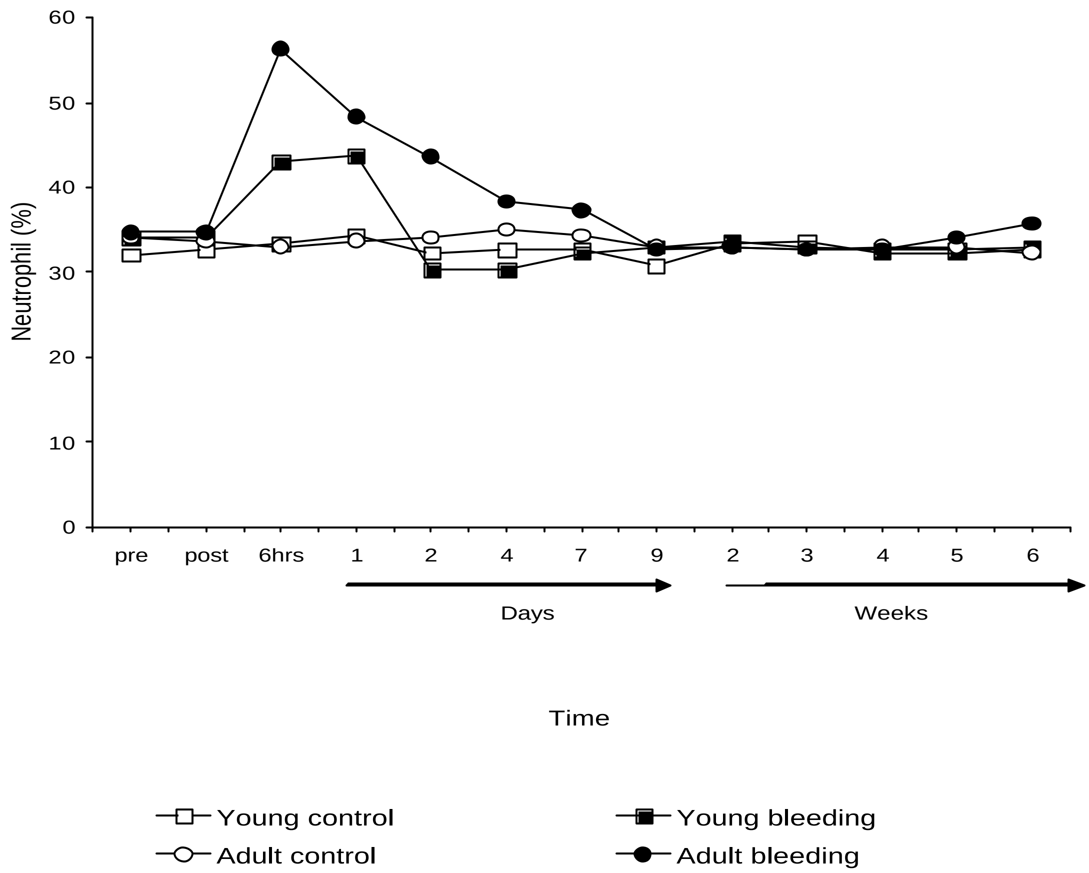

Fig. 8: Effects of 20% haemorrhage on neutrophil ratio (%) in young and adult goats

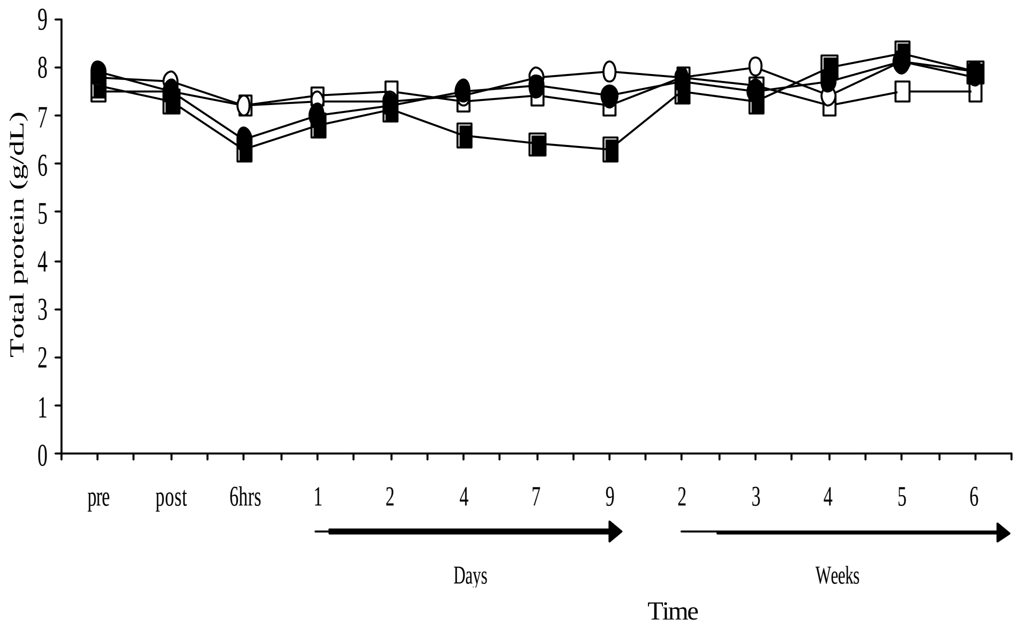

Fig. 9: Effects of 20% haemorrhage on serum total protein concentration in young and adult goats

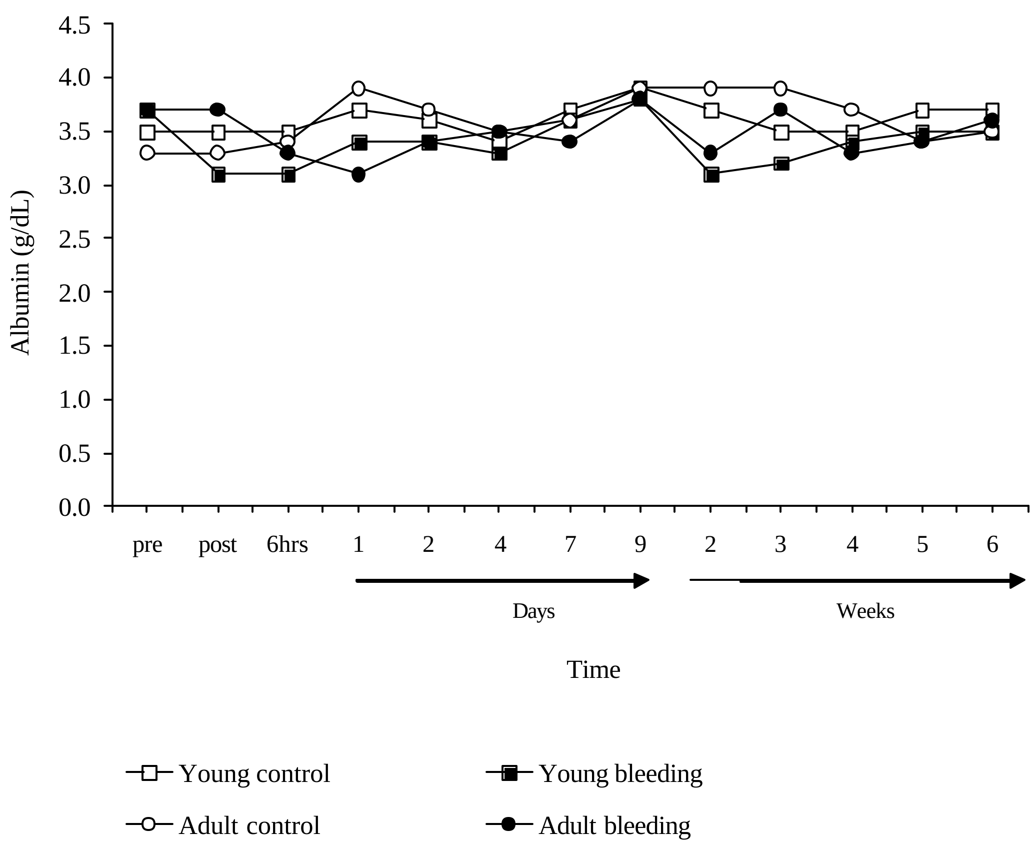

Fig. 10: Effects of 20% haemorrhage on serum albumin concentration in young and adult goats

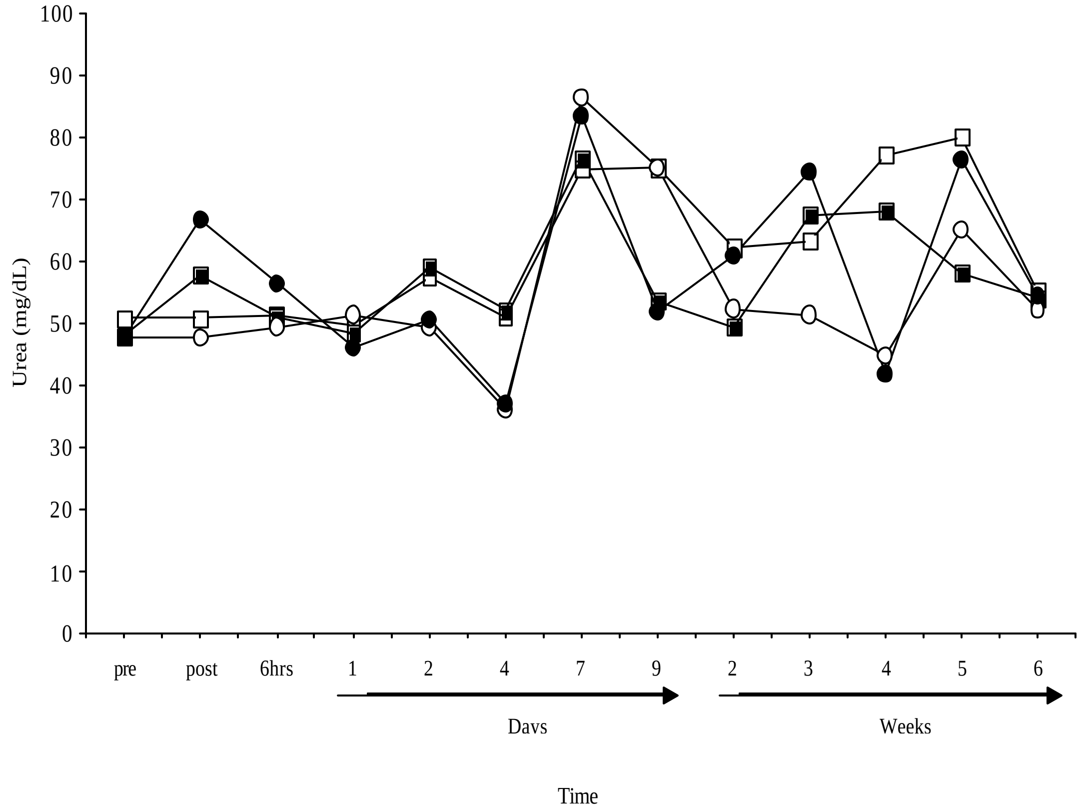

Fig. 11: Effects of 20% haemorrhage on serum urea concentration in young and adult goats

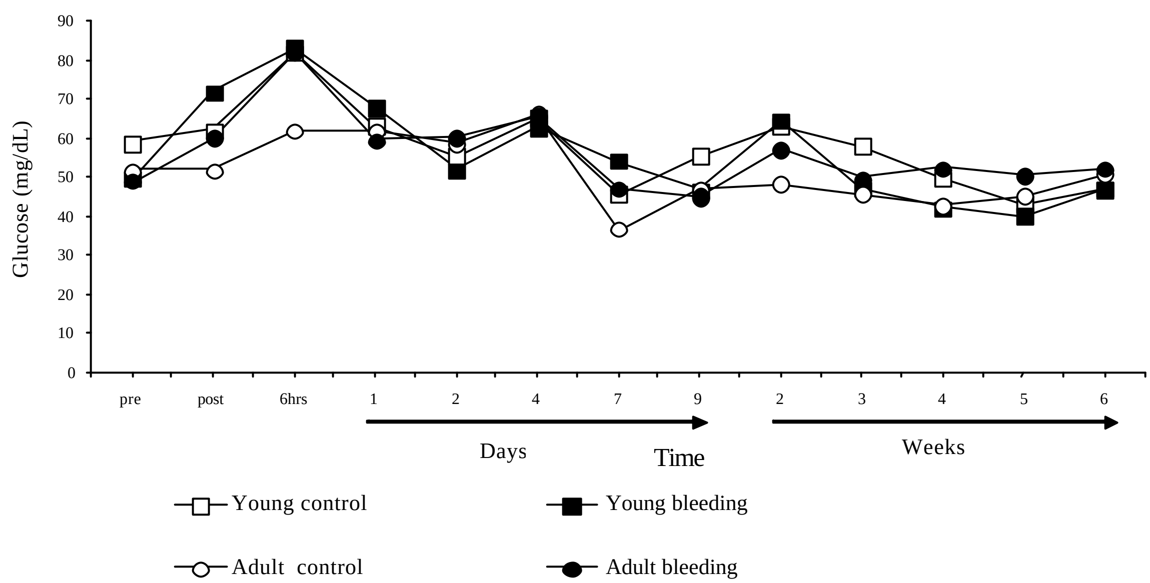

Fig. 12: Effects of 20% haemorrhage on plasma glucose level in young and adult goats

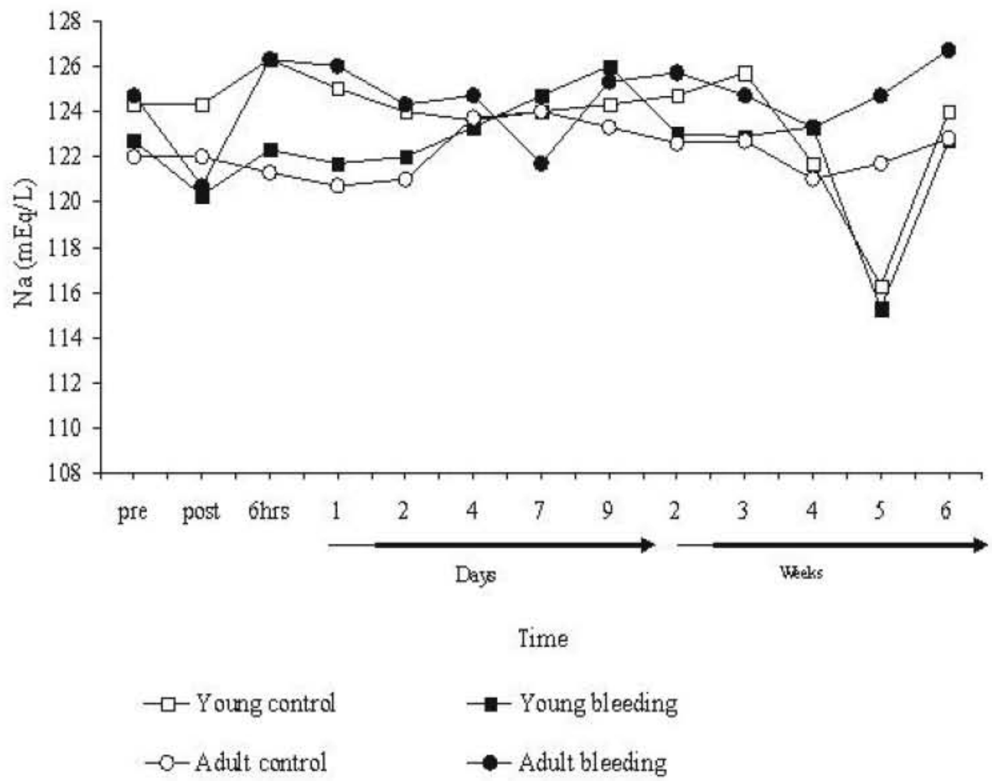

Fig. 13: Effect of 20% haemorrhage on serum sodium (Na) concentration in young and adult goats

following bleeding. However, both treated groups showed a marked increase in neutrophil ratio after 6 hrs, which was more pronounced in adults. The neutrophil ratio returned to normal values after 2 and 9 days in young and adult groups, respectively. The results indicate that the interaction between bleeding, age and time was significant ($P<0.01$).

Serum and Plasma Constituents: Fig. 9 shows that the initial values of serum total protein were almost similar (˜7.7g/dL). The total protein level did not change immediately post-bleeding in both treated groups, but the values decreased at 6 hrs. The young treated group showed progressive decline between day 2 and day 9. Then the general pattern showed steady values until the end of experimental period. The normal total protein values were recovered at day 4 and week 2 for young and adult groups, respectively.

Fig.10 shows that the initial values of serum albumin ranged between 3.3 and 3.7g/dL. Immediately post-bleeding, the values of serum albumin of adult group did not change, whereas there was a decrease in the young group. The values decreased in both treated groups at 6 hrs. The normal values of albumin were recovered at day 9 for both treated groups of goats. The pattern of control groups showed steady high values until the end of experimental period.

The effect of 20% bleeding on serum urea level is shown in Fig. 11. The initial values of urea were almost similar (˜50mg/dL). In both treated group, urea concentration increased significantly ($P<0.05$) immediately after bleeding, but the increase was more pronounced in adults. The return to normal values occurred after 24 hrs for both groups. Thereafter, the experimental groups showed marked fluctuations in urea level until the end of experimental period. Occasionally, treated adult animals maintained higher values compared to values obtained for young animals.

The responses of plasma glucose level to bleeding in young and adult goats are shown in Fig. 12. The initial values of glucose level for experimental groups ranged between 48 and 59 mg/dL. Immediately post-bleeding, the treated groups showed an increase in glucose level. Then the experimental groups with the exception of adult control showed a high value of 82mg/dL after 6 hr. Thereafter, there were marked fluctuations in plasma glucose level with a general decline pattern until the end of the experimental period.

The effect of haemorrhage on serum Na concentration is shown in Fig.13. The initial values of Na ranged between 122 and 124.5 mEq/L. Immediately post-bleeding, the mean values of treated groups decreased, the decrease was more pronounced in adult group. The normal values were almost recovered for both groups at day 4. The general pattern indicates that the treated adult group maintained higher serum Na level compared to the respective control values occasionally. In young animals, there was no consistent pattern.

DISCUSSION

In this study, the effects of age on physiological responses of goats to 20% haemorrhage have been assessed for 6 weeks. The results showed that the rectal temperature, (Tr) (Fig.1) and respiration, (HR) (Fig. 2) were influenced by 20% haemorrhage. Bleeding increased (Tr) significantly and the increase was less pronounced in adult goats. This may be attributed to the lower metabolic rate in adult animals associated with the larger body size. Many factors contribute to the accelerated decrease in metabolic rate as animals age, including a decrease in protein synthesis with aging [18] and a decrease in relative proportion of metabolically active tissues to total body weight. In previous studies [19], the heat production per unit body weight decreased as sheep aged. The ratio of basal metabolic rate to body weight in small animals is much greater than in large animals [20].

Physiological investigations have shown that an increase in (RR) is usually associated with rise in body temperature in goats. These mechanisms are integrated through the hypothalamus. The increase of (RR) in response to haemorrhage is likely to be related to decrease in ventilation. The response of ventilation is affected by mechano-receptors located in pulmonary circulation [21].

The cardiovascular responses of adult and young goats to bleeding were manifested in marked increase in heart rate (HR) in both age groups (Fig. 3). The results also indicate that the resting (HR) was higher in young goats as compared to values measured for adults. The rise in (HR) in response to haemorrahge was more pronounced in adult goats. This could be related to the fact that old animals are less able to maintain the (HR), probably as a consequence of age related changes in sympathetic activity [22]. The vagal inhibitory fibres are usually in a state of tone, the extent of resting vagal tone varies with age; in young animals, vagal tone is less marked than in mature individuals of the same species [23]. In adult sheep, the α-adreneric system as a whole has a limited response to haemorrhage [24].

The results indicate that bleeding resulted in significantly lower PCV level (Fig. 4) and a decrease in Hb concentration (Fig 5) in adult and young goats. The drop in PCV level in response to haemorrhage was more pronounced in adult goats. In young animals, the PCV fell immediately post-bleeding, but in adults, the PCV increased slightly suggesting a compensatory response of the spleen to haemorrhage in the adults. Previous studies indicated that the splenic response to haemorrhage is limited in sheep at the age of 12 weeks [25]. The α-adrenoreceptors that are involved in splenic contraction are functionally and numerically deficient in lambs [8]. Studies reported by Lipschitz et al. [26] demonstrated that elderly individuals with anaemia had lower erythroid precursors than the young and the 2, 3-DPG level in elderly anaemic subjects was significantly lower than the level usually found in younger subjects with similar anaemia.

The results showed a significant decrease in total leukocyte count (TLC) after bleeding (Fig. 6); the decline in TLC was more pronounced in adult goats. The leukocytes of old mammals may not be replaced as promptly as in younger age under haematopoietic stress such as blood loss; possible age-associated mechanisms include defect in progenitor cell production [27]. An overall reduction in haematopoiesis is reflected in lower peripheral leukocytes in elderly with anaemia [28]; also elderly people maintain significantly lower numbers of myeloid precursors than in the young control and a marked decrease in CFU-C levels was found [26].

The current results indicate that bleeding caused significantly lower lymphocyte ratio associated with a higher ratio of neutrophils in adult and young goats (Figs. 7 and 8). It has been reported that the decline in peripheral blood lymphocyte in old rats under haemotopoietic stress is partially controlled by mechanisms intrinsic to the bone marrow itself [29]. The current results indicate that the haemorrhage induced neutrophilia was more pronounced in adult goats (Fig. 8). Neutophilic leukocytosis due to increased neutrophil production appears to be a normal part of the aging process and this was more pronounced in aged than in young mice [30]. The increase in neutrophil production may be attributed to a change in haematopoietic microenvironment.

The results showed that bleeding caused significantly lower serum total protein and albumin concentrations (Figs. 9 and 10). The value did not change immediately post-bleeding in the adults, it decreased after 6 hrs. A considerable amount of protein may enters the vascular system together with water, thus preventing a dilutional decrease in protein concentration post-haemorrhage [24]. Some of the homeostatic processes involved in restoration of blood volume and plasma protein were shown to be functional in the newborn pigs [31]. In the present study, the goats were fed after bleeding, thus a potential source for the rapid fluid shift toward the intravascular compartment is the gastrointestinal contents.

The serum urea concentration values in the present study showed an increase in response to haemorrhage in both age groups (Fig. 11). The rise was more pronounced in adult than in young goats. The rise in urea level could be associated with decrease in renal blood flow, glomerular filtration rate (GFR) and filtration fraction probably being greater in adults than in young goats. This finding is in conformity with the observations made in lambs and young adult sheep subjected to 20% bleeding [7].

The responses of plasma glucose level to haemorrhage indicate a significant increase in both age groups immediately post-haemorrhage; the rise was more pronounced in young animals (Fig.12). Acute insulin resistance and hyperglycaemia may be important in the immediate response to haemorrhage [32]. Temporary increase in blood glucose level after haemorrahge in newborn lambs was associated with the rapid phase of blood volume restitution and peripheral vascular response to chatecholamines secreted during hypotension [24].

Bleeding was associated with an apparent decrease in serum Na concentration in both age groups (Fig.13). This response could be associated with haemodilution related to water flux from interstitium. Also it could be related to an increase in circulating arginine vasopressin (AVP) that promoted tubular water absorption. The renal responses to haemorrhage in lambs and young adult sheep include a decrease in GFR and filtration fraction [7]. The authors indicated that the decrease in electrolyte excretion in response to haemorrhage was greater in lambs compared to adult sheep.

The results support the assumption that young animals have abilities to compensate the blood volume loss better than adults. The current results in goats are in line with finding reported in previous studies suggesting a more rapid correction of blood loss at an early age in man [24], rats [33] and mice [34].

We conclude that the goat model can be adopted in investigations of physiological responses to haemorrhage. Thermoregulation and blood constituents were influenced by moderate haemorrhage in young and adult desert goats. The changes in many of the parameters investigated seem to be age dependent. The results obtained are pertinent and relevant to evaluation of blood loss in animals and humans. The mechanisms related to age dependent haematologic responses to haemorrhage warrant critical elucidation. The effects of pregnancy and lactation on physiological responses of goats to haemorrhagic anaemia also should be studied.

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