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

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Abstract: The objective of this study was to evaluate and compare the effects of 25% haemorrhage on physiological responses in spleen-intact and splenectomized adult goats. The magnitude of blood loss was expressed as percentage of initial blood volume after plasma volume determination by dye dilution. Haemorrhage resulted in significant increases in rectal temperature (Tr), respiration rate (RR) and heart rate (HR) values in normal and splenectomized animals. The values of packed cell volume (PCV), haemoglobin concentration (Hb) and total leukocyte count (TLC) were significantly lower in haemorrhaged normal and splenectomized animals. These parameters decreased immediately after haemorrhage in splenectomized animals and after 6 hrs in normal animals. In haemorrhaged animals, the lymphocytes, eosinophils and monocytes ratios showed lower values whereas neutrophils ratios were higher. The serum total protein and albumin concentrations decreased significantly in response to haemorrhage in normal and splenectomized animals. The plasma glucose level increased significantly in haemorrhaged animals. During recovery, haemorrhaged splenectomized animals maintained higher values of (Tr) and (HR) and lower values of (PCV) and (Hb) compared to haemorrhaged spleen-intact animals.

Key words: Goats · Haemorrhage · Splenectomy · Thermoregulation · Blood constituents

INTRODUCTION

Acute blood loss is a major haemorrhage that produces a variety of cardiovascular, haematologic and endocrine changes. Severe blood loss influences homeostasis and can lead to cardiovascular collapse, hypovolaemic shock and death [1]. Goats may experience considerable blood loss due to trauma and haemorrhage associated with surgery and gynaecological manipulations. Also, internal parasites and blood sucking insects may induce blood loss in certain occasions. Splenectomy may influence the capacity for restoration of normal composition and function of blood after haemorrhage. In ruminants, the spleen is considered as a reservoir of blood cells which can be mobilized by splenic contraction [2]. The spleen can also play a role in haematopoiesis, lymphopoiesis and haemoglobin processing [3].

In animals, splenectomy has been performed for studies involving physiology and trauma [4,5], diseases associated with blood parasites [6,7] and immunology [8,9]. Splenectomy may also be performed in animals for treatment of rupture [10], torsion [11] and suppurative splenitis [12]. The common diseases requiring splenectomy in humans include immune thrombocytopenic purpura (ITP), lymphoproliferative disorders, Hodgkin's disease and myeloproliferative disease [13]. Splenectomy alters both humoral and cellular immunity [14]. On removal of the spleen, the incidence of infection increases [15-18] with changes in many immunologic parameters [19]. A marked increase in the number of B-lymphocytes in the blood is constantly observed both in humans [20] and experimental animals [21,22], that affects the peripheral and the marginal pools of blood [23].
The effects of splenectomy have been previously investigated in several animal species, but relatively few investigations have been done in goats. Although the spleen is known to influence blood cell distribution when it contracts during stress, its role in responses to acute blood loss has not been adequately elucidated. This study was undertaken to evaluate the effects of splenectomy on the physiological responses of goats to moderate haemorrhage.

**MATERIALS AND METHODS**

**Animals and Ration:** Twelve adult nonpregnant goats with an initial mean body weight of 18.2 ± 0.5 kg were used for this study. The animals were kept in pens for an adaptation period of 2 weeks before experimentation so that they were accustomed to handling, experimental conditions and collection of the blood samples. They were examined clinically and blood samples were analysed. The animals were fed alfalfa hay (CP: 18%; ME: 7.9 MJ Kg⁻¹) and were watered *ad libitum.* The study was conducted at the Department of Physiology.

**Experimental Design:** For all experimental animals, the initial baseline physiological data were determined. The total blood volume was measured in all animals using Evans blue dye. The goats were then allowed a post-operative healing period of two weeks. The wound was dressed daily and the sutures were removed after 2 weeks. During the healing period, the goats were kept in an animal house and offered alfalfa hay and water *ad libitum.*

**Whole Blood Analysis:** The packed cell volume (PCV), haemoglobin concentration (Hb), total leukocyte count (TLC) and differential leukocyte count (DLC) were determined according to the standard methods [25,26].

**Serum and Plasma Analysis:** The concentration of serum total protein was determined using Biuret reagent as described by King and Wootton [27]. Serum albumin concentration was determined by the colorimetric method [28]. The plasma glucose concentration was determined by enzymatic colorimetric method using a kit (Spinreact, S.A., Spain).

**Statistical Analysis:** The experiment was performed according to the complete randomized design (Factorial arrangement) (2 x 2 x 6). The data collected are presented as mean ± standard deviation (SD) and significant differences within and between groups were assessed using analysis of variance (ANOVA) and paired t-test [29].

**RESULTS**

**Rectal Temperature (Tr):** The effects of splenectomy and haemorrhage on (Tr) are shown in Fig. 1. The initial values of (Tr) of experimental groups ranged between 37.0 and 37.5°C. The non-haemorrhaged control and splenectomized groups showed lower fluctuating values of (Tr). For the haemorrhaged normal and splenectomized groups, (Tr) showed marked increase in the first and second hour following bleeding. Thereafter, the control haemorrhaged group showed progressive decline in (Tr) until 24 hrs and then showed slight elevation that was maintained until day 9.
Fig. 1: Effect of splenectomy and 25% haemorrhage on rectal temperature (Tr) in goats

Fig. 2: Effect of splenectomy and 25% haemorrhage on respiratory rate (RR) in goats

Fig. 3: Effect of splenectomy and 25% haemorrhage on heart rate (HR) in goats
In splenectomized-haemorrhaged group, (Tr) values were higher compared to the respective values obtained for the control haemorrhaged group. The results indicate that there was no significant change in (Tr) values of splenectomized group, but the interaction between splenectomy and bleeding was significant (P<0.01).

**Respiratory Rate (RR):** Fig. 2 shows the effects of splenectomy and haemorrhage on (RR). The values of (RR) of non-haemorrhaged normal and splenectomized groups showed steady values during the experimental period, although the spleenectomized animals maintained lower values of (RR). Immediately following 25% bleeding, both groups showed a slight increase in (RR). However, the values obtained after 1 hr indicate that there was a sharp increase in (RR), the non-splenectomized animals having higher values of (RR). Thereafter, both haemorrhaged groups showed progressive decline in (RR) which was maintained at almost steady level after 24 hrs. However, normal haemorrhaged animals showed lower (RR) values at this stage. The results indicate that the interaction between splenectomy and bleeding was significant (P<0.05).

**Heart Rate (HR):** The effects of splenectomy and haemorrhage on (HR) are shown in Fig. 3. The experimental groups had initial pre-bleeding values of about 65 beats/min. For the control groups, (HR) was maintained with slight fluctuations until the end of the experimental period, but splenectomized-non-haemorrhaged animals had lower values. Both haemorrhaged groups (normal and splenectomized) showed a marked increase in (HR) following 25% bleeding, attaining values ranging between 105 and 110 beats/min. After 1hr, both haemorrhaged groups showed even higher values of 110 beats/min. and 120 beats/min for the normal and splenectomized groups, respectively. Thereafter, the control (non-splenectomized group) showed progressive decrease in (HR) until the third hour. For the splenectomized haemorrhaged group, high (HR) values were maintained for 6 hrs and then showed progressive decline to maintain almost steady level after 24 hr. The results showed that the interaction between splenectomy and bleeding was significant (P<0.01).

**Packed Cell Volume (PCV):** Fig. 4 shows that the initial pre-haemorrhage values of (PCV) for all groups ranged between 27 and 31%. Immediately following haemorrhage, there was no marked change in (PCV) level in treated groups. The non-haemorrhaged control group had almost steady high level of (PCV) until the end of the experimental period. Both normal and splenectomized groups showed progressive decline in (PCV) following (25%) bleeding until 24 hr. Thereafter, for both haemorrhaged groups, there was almost progressive increase in (PCV) level, the splenectomized group maintained lower values. The normal haemorrhaged group attained the control group level after 5 weeks, the splenectomized haemorrhaged group re-established normal values after 7 weeks.

**Haemoglobin Concentration (Hb):** Fig. 5 indicates that the initial pre-haemorrhage values of (Hb) for all groups ranged between 10.1 and 12.6 g/dL. Immediately following haemorrhage, there was no marked change in (Hb) level in normal treated groups, whereas, it deceased markedly in the splenectomized treated group. The non-haemorrhaged control group maintained almost steady level of (Hb) until day 6; thereafter, there were slight fluctuations in (Hb) concentration. Both normal and splenectomized groups showed progressive decline in (Hb) in response to haemorrhage to attain 7g/dL after 24 hrs. Then both haemorrhaged groups showed progressive increase in (Hb) concentration, the splenectomized group maintained lower values, to attain normal values after 5 weeks for the normal group and 7 weeks for the splenectomized group.

**Total Leukocyte Count (TLC):** Fig. 6 indicates that the initial values of (TLC) for all experimental groups were almost similar (~10×10³/µL). The control (non-haemorrhaged) groups maintained this level until the end of experimental period, except for a decrease in day 2 and apparent increases on day 9 and week 2. For the haemorrhaged groups, there was gradual decrease in (TLC) to 4×10³ /µL in day 2. Thereafter, both haemorrhaged groups showed sharp increase on days 2 and 9 to reach the highest values of ~12×10³/µL at week 2. It is evident that both groups re-established normal values of (TLC) after 3 weeks. However, the haemorrhaged groups maintained slightly lower values of (TLC) until the end of experimental period. The results indicate that the interaction between splenectomy and bleeding was significant (P<0.05).
Fig. 4: Effect of splenectomy and 25% haemorrhage on packed cell volume (PCV) in goats

Fig. 5: Effect of splenectomy and 25% haemorrhage on haemoglobin (Hb) concentration in goats

Fig. 6: Effect of splenectomy and 25% haemorrhage on total leukocyte count (TLC) in goats
Table 1: Effect of splenectomy and 25% haemorrhage on lymphocyte ratio (%) in goats

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre</th>
<th>Post</th>
<th>6 hr</th>
<th>Day1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Week2</th>
<th>Week3</th>
<th>Week4</th>
<th>Week5</th>
<th>Week6</th>
<th>Week7</th>
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</thead>
<tbody>
<tr>
<td>Normal Control</td>
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<td>59.7±1.5</td>
<td>59.7±1.5</td>
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</tr>
<tr>
<td>Bleeding</td>
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<td>61.3±5.7</td>
<td>58.0±7.8</td>
<td>51.7±4.9</td>
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<tr>
<td>Splenectomized</td>
<td>61.0±8.0</td>
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</table>

S.L. * ** ** **

S.L.: Significance level.
* : Significant at P<0.05.
** : Significant at P<0.01.

Table 2: Effect of splenectomy and 25% haemorrhage on neutrophil ratio (%) in goats

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre</th>
<th>Post</th>
<th>6 hr</th>
<th>Day1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Week2</th>
<th>Week3</th>
<th>Week4</th>
<th>Week5</th>
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<td>32.7±1.5</td>
<td>32.7±1.5</td>
<td>32.7±1.5</td>
<td>32.0±3.0</td>
<td>32.7±1.5</td>
<td>30.0±0.0</td>
<td>31.6±0.6</td>
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</tr>
<tr>
<td>Bleeding</td>
<td>29.3±4.5</td>
<td>31.7±7.0</td>
<td>40.8±6.7</td>
<td>42.7±6.4</td>
<td>35.±15</td>
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<td>29.3±3.2</td>
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<tr>
<td>Splenectomized</td>
<td>30.3±9.1</td>
<td>30.8±9.4</td>
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<td>28.3±0.6</td>
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</tbody>
</table>

S.L. N.S. ** * * *

S.L.: Significance level.
N.S.: Not significant.
** : Significant at P<0.01.

Table 3: Effect of splenectomy and 25% haemorrhage on monocyte ratio in goats

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre</th>
<th>Post</th>
<th>6 hr</th>
<th>Day1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Week2</th>
<th>Week3</th>
<th>Week4</th>
<th>Week5</th>
<th>Week6</th>
<th>Week7</th>
</tr>
</thead>
<tbody>
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<td>4.7±2.1</td>
<td>4.7±2.1</td>
<td>4.0±1.0</td>
<td>4.0±1.0</td>
<td>4.0±1.0</td>
<td>4.0±1.0</td>
<td>4.0±1.0</td>
<td>4.0±1.0</td>
<td>4.0±1.0</td>
<td>4.0±1.0</td>
<td>3.3±0.6</td>
<td>4.0±1.0</td>
</tr>
<tr>
<td>Bleeding</td>
<td>4.3±1.2</td>
<td>4.7±6.6</td>
<td>3.3±1.5</td>
<td>3.7±2.0</td>
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<td>5.3±1.5</td>
<td>4.0±1.0</td>
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<td>4.3±0.6</td>
<td>3.7±0.6</td>
<td>3.3±0.6</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>4.3±1.5</td>
<td>4.3±1.5</td>
<td>3.3±1.5</td>
<td>3.3±1.5</td>
<td>4.3±1.5</td>
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<td>4.3±1.2</td>
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</tr>
</tbody>
</table>

S.L. * N.S. N.S. N.S.

S.L.: Significance level.
N.S.: Not significant.
* : Significant at P<0.05.

Table 4: Effect of splenectomy and 25% haemorrhage on eosinophil ratio (%) in goats

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre</th>
<th>Post</th>
<th>6 hr</th>
<th>Day1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Week2</th>
<th>Week3</th>
<th>Week4</th>
<th>Week5</th>
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<td>3.3±0.6</td>
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<td>2.7±0.6</td>
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</tr>
<tr>
<td>Bleeding</td>
<td>3.0±1.0</td>
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<td>1.7±0.6</td>
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<td>2.8±1.0</td>
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<td>4.0±0.0</td>
<td>3.7±0.6</td>
<td>3.7±1.4</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>3.7±2.3</td>
<td>3.7±2.3</td>
<td>3.0±2.0</td>
<td>3.0±2.0</td>
<td>3.3±1.5</td>
<td>3.3±1.2</td>
<td>3.0±1.0</td>
<td>2.8±1.3</td>
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S.L. N.S. N.S. N.S.

S.L.: Significance level.
N.S.: Not significant.

Differential Leukocyte Count (DLC): Tables 1, 2, 3 and 4 show the effects of haemorrhage and splenectomy on (DLC). The lymphocyte ratio in haemorrhaged groups was significantly (P<0.01) lower compared to control groups values at 6 hrs and day 1, while the neutrophils ratio increased significantly (P<0.05) at 6 hrs and day1 post-haemorrhage. The eosinophil ratio decreased after haemorrhage and it increased after 4 days in treated groups. The monocyte ratio decreased after 6 hrs in treated groups, lower values were maintained for 2 days, then returned to normal level. The interactions between splenectomy and bleeding as regards the ratios of neutrophils and monocytes were significant (P<0.01).

Serum Total Protein: Fig. 7 shows that the initial values of total protein were not quite similar for the experimental groups. The non-haemorrhaged control groups of animals maintained almost steady similar values of serum total protein of about 7.5 g/dL until the end of experimental period. Both the normal and splenectomized animals

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Fig. 7: Effect of splenectomy and 25% haemorrhage on serum total protein concentration in goats

Fig. 8: Effect of splenectomy and 25% haemorrhage on serum albumin concentration in goats

Fig. 9: Effect of splenectomy and 25% haemorrhage on plasma glucose level in goats
showed initial similar responses to haemorrhage for 24 hr. Then, both groups showed significant (P<0.01) decline in total protein level to attain 5.5 g/dL on day 4. For both groups, serum total protein level increased to an almost steady level on days 6 and 9 and week 2. Thereafter, the normal haemorrhaged group maintained higher values of total protein compared to both control groups, whereas the splenectomized group maintained lower values compared to the other groups until the end of experimental period.

**Serum Albumin:** The effects of splenectomy and haemorrhage on serum albumin concentration are shown in Fig. 8. The initial values for experimental groups ranged between 4.2 and 4.6 g/dL. Following haemorrhage, there was no consistent pattern of serum albumin level as regards acute and long-term responses. However, the general pattern indicates that with few exceptions, haemorrhaged groups maintained higher serum albumin levels until day 4. However, as from week 2, the splenectomized control group tended to have higher values and the normal control maintained lower values compared to the other experimental groups.

**Plasma Glucose:** Fig. 9 shows that the initial mean value of plasma glucose level was (~ 55 mg/dL). The non-haemorrhaged control groups maintained similar glucose levels, with few exceptions, during the experimental period. The haemorrhaged groups had relatively higher glucose levels until week 2, with the spleen-intact haemorrhaged group maintaining higher levels compared to the splenectomized haemorrhaged group. However, at week 3, the haemorrhaged groups re-established the values of the control groups. Thereafter, the splenectomized haemorrhaged group maintained lower glucose levels until the end of the experimental period.

**DISCUSSION**

The study documented that moderate haemorrhage (25%) resulted in a significant increase in rectal temperature (Tr) in experimental groups of goats (Fig. 1). The rise in (Tr) is presumably associated with decrease in core-to-skin heat transfer due to reduction in blood volume. Also it is likely to be related to retention of heat as an increase in peripheral resistance is one of the primary adjustments to haemorrhage [30]. However, other researchers reported post-haemorrhage hypothermia [31]. This response was attributed to impaired cardiac output, lower oxygen consumption and lower metabolic heat production [32,33]. Apparently, the splenectomized groups of goats showed higher values of (Tr) compared with the spleen-intact groups (Fig. 1). This response could be related to lack of mobilization of blood from the spleen to the circulation after haemorrhage, thus reducing the capacity of convective heat transfer. The redistribution of splanchnic blood flow and mobilization of splanchnic blood volume can contribute significantly to support arterial blood pressure and cardiac output [34,35]. The carotid sinus hypotension mobilized 14% of splanchnic blood volume in dogs [36]. Greenway and Lister [37] reported that the mobilization of blood from splenic vessels after moderate haemorrhage involved active constriction of capacitance vessels mediated by a sympathetic reflex from arterial pressure receptors. Moderate haemorrhage in dogs mobilized 30% of spleen blood volume and severe haemorrhage from 55 to 81% [38].

The results indicate that in haemorrhaged groups, the respiratory rate (RR) increased sharply after 1 hr (Fig. 2). This response seems to be associated with oxygen supply. The decrease in circulating blood volume and the significant fall in (PCV) and (Hb) concentration (Figs. 4 and 5) resulted in inefficient oxygen delivery or relative hypoxia and consequently stimulation of peripheral chemoreceptors, the carotid and aortic bodies. Landgren and Neil [39] indicated that decreases in blood flow, following bleeding, even in the absence of decreases in arterial pressure or oxygen partial pressure, may stimulate carotid chemoreceptors. The increase in (RR) in haemorrhaged animals in the present study may also be associated with the baroreceptor reflex. In anaesthetized, chemodenervated dogs, decreases in carotid sinus pressure caused increases in total ventilation [40].

The current results indicate that the heart rate (HR) in goats increased markedly following induction of 25% haemorrhage (Fig. 3). This response is related to stimulation of autonomic nervous system which involves an increase in the sympathetic activity induced by the baroreceptors. In dogs and rabbits, a sympathoexcitatory response is evident until acute blood loss exceeds 25-35% [41]. Following haemorrhage, as cardiac output declines, cardiovascular reflexes cause a progressive increase in heart rate and increased resistance to blood flow in the musculocutaneous and splanchnic vascular beds. The increase in (HR) that occurs during nonhypotensive haemorrhage is attributed partly to increased cardiac sympathetic drive and partly to withdrawal of resting cardiac vagal drive, although the relative contribution of these two mechanisms varies among species [42-44].
The reported increase in (HR) in haemorrhaged animals represents a compensatory change that assists in the recovery process. The higher (HR) in splenectomized goats reported in the present study is consistent with previous results which indicated that splenectomized swine had elevated (HR) compared to sham-operated animals with differences persisting throughout haemorrhage [45] and the suggestion that the spleen maintains left ventricular performance during haemorrhage [46].

The (PCV) (Fig. 4) and (Hb) concentration (Fig. 5) were lower immediately post-bleeding in splemectomized goats, whereas there was no appreciable change immediately after haemorrhage in spleen-intact goats. This finding suggests that the splenectomized animals were deprived of storage source of erythrocytes that were mobilized in the spleen-intact animals. However, in both groups, there was progressive decline in the (PCV) and (Hb) concentration, the magnitude of decline being more pronounced in splenectomized animals. The pattern of recovery of these parameters also indicates that apparently splenectomized animals needed longer time for recovery of pre-haemorrhage values of (PCV) and (Hb) concentration compared to the spleen-intact group. The decrease in (PCV) and (Hb) concentration is related to haemodilution induced by flux of interstitial fluid. This response could also be associated with enhancement of renal reabsorption of water. Bleeding of 15-25% of the blood volume in goats caused approximately hundred fold increase in the plasma concentration of the antidiuretic hormone arginine vasopressin (AVP) [47]. The renin-angiotensin as well as sympathetic nervous system were shown to be involved in increased post-haemorrhagic vasopressin release [48].

The total leukocyte count (TLC) decreased in haemorrhaged animals (Fig. 6). The (TLC) was lower immediately post-bleeding in splenectomized goats, whereas there was no immediate change in intact goats and the values decreased after 6 hrs. The results support the observation which indicates that mobilization of blood volume from splanchnic region to circulation occurs as a result of splenic contraction. The results also demonstrated that the decrease in lymphocyte ratio was marked and the increase of neutrophil ratio was less pronounced in splenectomized animals; the ratios of monocytes and eosinophils decreased (Tables 1-4). These changes in the leukogram could be attributed to the absence of spleen which acts as a source of leukocytes. The lack of role of spleen could be implicated in the delay in the recovery period in lymphocyte and neutrophil ratios of splenectomized goats.

The decrease in serum levels of total protein and albumin (Figs. 7 and 8) after bleeding is clearly related to haemodilution due to the flux of interstitial fluid. Plasma protein restitution by transport through the lymphatics [49] plays a critical role in vascular refilling by maintaining transcapillary oncotic pressure gradient to favour continued fluid flux [50]. This may be associated with the role of spleen in compensation of blood volume. The blood volume in splenectomized dogs subjected to hypovolaemia was less than in intact dogs because the transvascular fluid shift replaced 20 to 35% of blood volume [51]. Grimes et al. [52] noted that there was a highly significant correlation between blood volume and plasma protein in splenectomized sheep.

The plasma glucose level showed an increase in response to haemorrhage in normal and splenectomized groups (Fig. 9). This increase in glucose level is attributed to secretion of hormones which induce glycogenolysis [53]. Furthermore, in such stressful conditions, the hyperglycaemia ensues due to either insulin deficiency or insulin resistance [54-56]. However, the general pattern indicates that in haemrrhaged groups, occasionally the splenectomized animals maintained lower glucose level. This could be associated with an increase in insulin in addition to the development of hyperglycaemia [57].

In conclusion, moderate haemorrhage in goats induced marked changes in thermoregulation as well as haematologic and blood metabolites responses. The findings illustrate that haemorrhaged splenectomized animals maintained higher values of rectal temperature (Tr) and heart rate (HR) and lower levels of (PCV) and (Hb) compared to hemorrhaged spleen-intact animals during post-haemorrhage recovery period.

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