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## EFFECTS OF BLOOD AND PLASMA TRANSFUSION ON VARIOUS HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN ANAEMIC CALVES

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### ABSTRACT

This experiment was performed to investigate the effects of blood and plasma transfusion on various haematological and biochemical parameters in anaemic calves. Ten apparently malnourished, anaemic calves were selected and divided into two equal groups. Group-A (n = 5) received blood and Group-B received plasma. The pre and post transfusion haematological and biochemical parameters recorded include total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin (Hb), packed cell volume (PCV), differential leukocyte count (DLC), Creatinine, ALT, AST and serum electrolytes (K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>) at day 1, 7, 14, 21 and 28. The post transfusion values of TEC, PCV and Hb increased significantly (P < 0.01) in both the groups. However, the values were superior in case of blood transfusion. The changes in TLC and DLC values in both the groups A and B were not significant during the experiment. The mean control values of Creatinine, ALT and AST were found to be increased up to day 14 in calves of both group A and B. However, the changes in these biochemical parameters were not statistically significant and the values remained within the normal range. The changes in the values of the serum electrolytes did not follow any definite pattern during the experimental period but remained within the normal range in both the groups. The whole blood and plasma transfusion was found to upgrade the haematological and biochemical parameters in anaemic calves. The blood and plasma transfusion can be an effective tool for clinical management of anaemic calves.

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## INTRODUCTION

Transfusion therapy has become an important tool in veterinary critical care and emergency medicine (Weingart et al., 2004). Blood transfusion is being practiced for centuries for saving life of human beings and animals. Blood transfusion has made considerable advancements in veterinary medicine in recent years. Transfusion aims to replace the missing component of blood in the case of anaemia, haemorrhage, haemolysis or ineffective erythropoiesis, to increase oxygen carrying capacity. Blood transfusion is an important remedy to manage animals with severe anaemia. Whole blood transfusion is used in conditions of life-threatening anaemia, such as acute traumatic hemorrhage, surgical blood loss, parasitism, hemolysis from drugs or toxins, immune-mediated anaemia, and neonatal isoerythrolysis (Tocci, 2010). The therapeutic use of whole blood, plasma and serum occupies an important place in modern veterinary therapy. One of the major constraints for development of cattle industry in Bangladesh is high rate of calf mortality and low rate of productivity. The calf mortality per year in Bangladesh is 15-20% (Anon, 1993). This includes an economic loss of taka 25 million per annum. The increased death rate is thought to be associated with malnutrition, parasitism, anaemia and declined body immunity (Biancardi, 1992). It may be due to colostrum deprivation, which hinders body immunity and growth. Thus young calves become weak, emaciated, anaemic and anorectic. Moreover disturbances in iron metabolism may result from poor absorption, which in turn leads to anaemia (Reddy et al., 1987). The hematopoietic system and electrolyte levels of these animals are seriously depressed; calves become anaemic and all blood parameters are significantly diminished. The animal, thus get to a stage when conventional treatment frequently fails and as a result death ensues. Transfusion medicine is the vital part of veterinary emergency and critical care medicine. In severe anaemia where there is extreme depletion of oxygen carrying capacity of the blood occurs and life is threatened, warrant need of blood transfusion is indicated. In our country large amount of blood is wasted every year at the abattoir. Sources of blood, therefore, are adequate. Now technique for the collection of blood from the donor, separation of components and its storage needs to be developed so as to institute transfusions therapy at the time of emergency (Alam and Hossain, 2005). Transfusion of either blood (Suffian and Hossain, 1999) or blood plasma (Alam and Hossain, 2005) has been in practice in Bangladesh over the last few years. They did their work to look into the impact of transfusion on haematological parameters in the vulnerable calves. Due to the lack of studies related to the impact of transfusion on biochemical parameters, the present study is, therefore, undertaken to know the effects of blood and plasma transfusion on various biochemical and haematological parameters in the anaemic calves and compare the therapeutic values of blood and plasma transfusion in anaemic calves. At the same time to determine the complications associated with blood and plasma transfusion (if any) in recipient calves.

## MATERIALS AND METHODS

### Experimental Animals

Ten indigenous calves of below 12 months were used as recipients. The calves were weak, emaciated, malnourished and of both sexes. They were clinically anaemic. They were housed in separate building in the animal shed of the Department of Surgery and Obstetrics, Bangladesh Agricultural University. They had an access to feed and water *ad libitum*. They were allowed to graze in the pasture 7-8 h a day. The calves were routinely dewormed and vaccinated. The experimental animals were randomly divided into two groups; Group-A: consisted of 5 calves and were used for fresh blood transfusion, and Group-B: consisted of 5 calves and were used for plasma transfusion. Five donors used for the collection of blood and plasma were apparently healthy, quiet, and amenable to handle. These animals were not previously transfused or vaccinated with biologics of bovine origin. They were free from ectoparasites and routinely dewormed. They were used once at a time for blood collection. After each collection the donors were administered with Inj. Hemovit Vet<sup>®</sup>, Renata Limited to prevent any stress due to blood donation. They were also allowed to drink sufficient water. The blood profile of each donor was assessed before blood collection. Cross-matching of the recipient blood to that with the donors was performed before transfusion.

### Collection of Blood and Separation of Plasma

Blood collection bag prepared by Green Cross Medical Crop, Korea were used for this experiment. Each blood bag contained 63 ml of anticoagulant CPDA (citrate phosphate dextrose adenine solution, USP) for collection of 450 ml blood. The animals were restrained on lateral recumbence; jugular vein was raised by digital pressure near the thoracic inlet. Raised vein was soaked with tincture of Iodine and the vein puncture was done by using 19-gauze needle which was connected with the blood bag. After collection, the blood transfused to the recipient afresh. Plasma was separated by centrifuging the blood immediately after collection. Eight centrifuge tubes with 15 ml of blood in each tube were set in the centrifuge machine and were spinned at 3000 rpm for 15 minutes. The tubes were then withdrawn and plasma was removed from above the packed red cells with the help of a sterile disposable dropper and transferred to a sterile plastic container.

### Transfusion of Blood and Plasma

The transfusion sets were prepared by Terumo Corporation, Tokyo, Japan. One bag (450 ml) of whole compatible blood was transfused in each occasion. Blood was administered through the jugular vein using a transfusion set which was connected with an eighteen gauze intravenous cannula. For the first two minutes blood was transfused at the rate of 120 drops per minute which was gradually raised to 140 drops per minute. Transfusion of blood was aided by gravitation force. For plasma transfusion, recipient calf was restrained in lateral recumbence. A 19 gauze butterfly needle attached with 50 ml syringe filled with plasma was placed in the jugular vein of the recipient animal. Then the plasma was transferred with gentle pressure on the plunger of the syringe at the rate of 5 ml/min for the first 2-3 minutes to monitor adverse sign. After that the rate was increased to 10 ml/ minute. When the plasma from one syringe was fully transfused, it was disconnected and another syringe filled with plasma was connected to the system. The same procedure was repeated until the desired amount was administered.

### Post Transfusion Blood Sample Analysis

Assessment of recipient blood was done 24 hours after transfusion and thereafter every 7 days up to 28 days. The pre-transfusion values of these parameters were considered as control and were compared to those obtained on different post-transfusion periods. Haematological parameters monitored were total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin (Hb), packed cell volume (PCV) and differential count (DLC) by the method of Sastry (1989). Among Biological parameters serum electrolytes ( K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup> ) level were determined by EasyLyte PLUS Na/ K / Cl ANALYZE and level of serum ALT, AST and Creatinine were determined by using Microlab Bio-chemistry Analyzer (Germany) by kinetic method .

### Clinical Parameters

Respiratory rate, pulse and rectal temperature of the experimental animals (recipients) were recorded an hour before transfusion, during transfusion and immediately after transfusion.

### Statistical Analysis

The collected data on various parameters were statistically analyzed using MSTAT statistical package to find out the variation resulting from experimental treatments following F variance test. The significance of difference between the pair of means was compared by Least Significant Difference (LSD) test at 1% and 5% level of probability.

## RESULTS

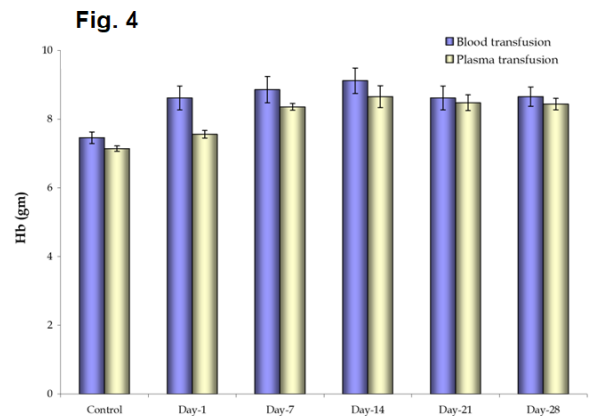
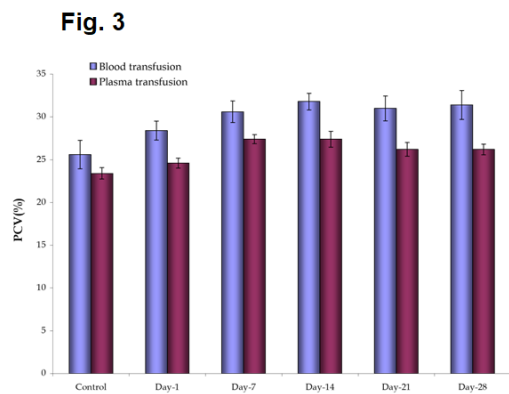
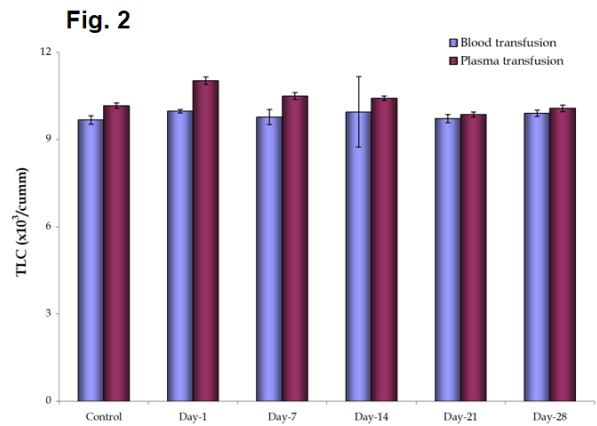
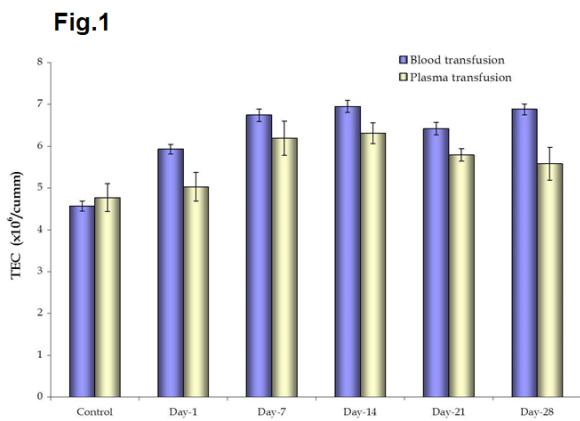
### Effects of Blood and Plasma Transfusion on Various Haematological and Biochemical parameters in Recipient Calves

#### Changes in Total Erythrocyte Count (TEC), Haemoglobin (Hb), Packed Cell Volume (PCV)

In blood and plasma transfused group, the pre-transfusion control values of TEC, Hb, PCV started increasing progressively from day-1 and the significant increases were recorded from day-1 ( $p < 0.01$ ). The elevated values of TEC, Hb and PCV sustained throughout the experimental period (Table 1 and Table 2). The TEC, Hb and PCV values seemed to be superior in case of blood transfused group in comparison to that of plasma transfused group (Figure1, 3 & 4).

**Changes in Total Leukocyte Count (TLC) and Different Leukocyte Count (DLC)**

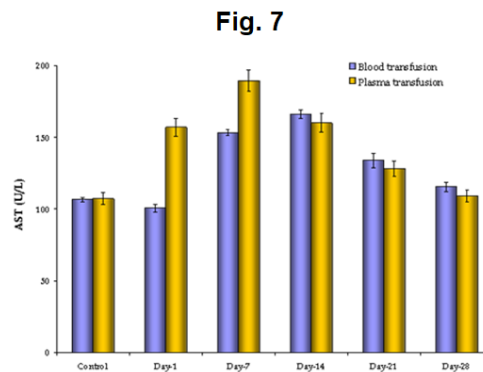
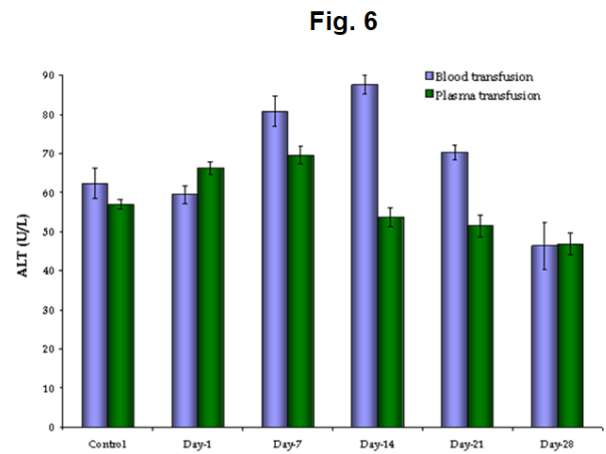
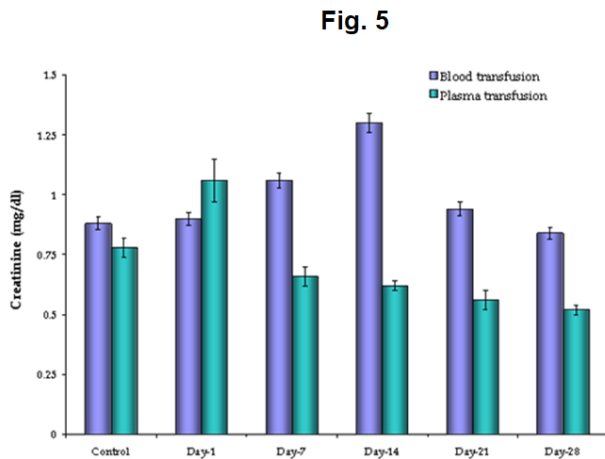
The mean control values of TLC, lymphocyte, monocyte, neutrophil and eosinophil showed little change throughout the experimental period in both blood and plasma transfused group. But the changes were not statistically significant ( $P>0.05$ ) (Table 1 and Table 2). When compared between two groups, the values of the TLC found superior up to day 14 in plasma transfused group (Figure 2).



**Figure 1.** Effects of blood transfusion and plasma transfusion on TEC; **Figure 2.** Effects of blood transfusion and plasma transfusion on TLC; **Figure 3.** Effects of blood transfusion and plasma transfusion on PCV; **Figure 4.** Effects of blood transfusion and plasma transfusion on Hb. (gm)

**Changes in Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Creatinine**

The mean control values of ALT and AST in both the groups showed little change during post transfusion period which was not statistically significant ( $P > 0.05$ ). The changes in creatinine values were also not significant ( $p < 0.05$ ) in both the groups (Table 3 and Table 4). When compared between two groups, plasma transfusion showed better result than that of blood transfused group (Figure 5, 6 & 7).



**Figure 5.** Effects of blood transfusion and plasma transfusion on Creatinine; **Figure 6.** Effects of blood transfusion and plasma transfusion on ALT; **Figure 7.** Effects of blood transfusion and plasma transfusion on AST

**Changes in Sodium, Potassium and Chlorine**

The mean control value of sodium in both blood and plasma transfused were inconsistent. The values of the sodium and potassium followed no definite pattern during the experimental period in both the groups (Table 3 and Table 4).

Table 1. Effects of Blood Transfusion on Various Hematological Parameters in Recipient Calves (n = 5)

Parameters	Pre transfusion values + (Control)	Post transfusion values (Mean ± SE)					Level of significance
		Day-1	Day-7	Day-14	Day-21	Day-28	
TEC (million/cumm)	4.57±0.124	5.93±0.114*	6.74±0.150**	6.95±0.144**	6.42±0.145**	6.88±0.132**	**
TLC (thousand/ cumm)	9.97±0.063	9.97±0.063	9.77±0.255	9.95±1.21	9.72±0.139	9.90±0.111	NS
Hb (gm %)	7.46±0.172	8.62±0.350*	8.86±0.382*	9.12±0.367*	8.62±0.347*	8.66±0.282*	*
PCV (%)	25.60±1.66	28.40±1.12*	30.60±1.25*	31.80±0.97*	31.00±1.45*	31.40±1.69*	*
Neutrophil (%)	34.40±0.964	31.00±0.652	33.00±0.822	30.40±0.83	31.80±0.669	29.60±1.02	NS
Lymphocyte (%)	62.20±0.669	64.60±0.579	63.80±0.400	66.20±0.669	64.00±0.436	66.00±0.692	NS
Monocyte (%)	3.00±0.181	2.80±0.140	3.20±0.157	3.40±0.116	2.20±0.113	1.60±0.094	NS
Eosinophil (%)	2.80±0.171	3.20±0.095	2.00±0.121	2.40±0.132	2.00±0.059	3.20±0.080	NS

± = Standard Error NS = Non Significant, \*\*=P<1% of Significance, \*=P<5% of Significance

**Table 2. Effects of Plasma Transfusion on Various Hematological Parameters in Recipient Calves (n = 5)**

Parameters	Pre transfusion values (Control)	Post transfusion values (Mean ± SE)					Level of significance
		Day-1	Day-7	Day-14	Day-21	Day-28	
TEC (million/cumm)	4.77±0.327	5.03±0.338*	6.19±0.414**	6.31±0.253**	5.79±0.147*	5.58±0.388*	**
TLC (thousand/ cumm)	10.16±0.091	11.02±0.134	10.49±0.122	10.42±0.077	9.86±0.094	10.07±0.108	NS
Hb (gm %)	7.14±0.075	7.56±0.112	8.36±0.098**	8.66±0.319**	8.48±0.233**	8.44±0.172**	**
PCV (%)	23.40±0.678	24.60±0.579*	27.40±0.533**	27.40±0.927* *	26.20±0.800*	26.20±0.620*	**
Neutrophil (%)	30.80±0.800	30.20±0.735	32.40±0.367	32.00±0.822	32.60±0.245	32.40±0.257	NS
Lymphocyte (%)	64.80±0.583	64.80±0.800	66.40±0.678	67.40±1.03	67.00±0.158	67.00±0.452	NS
Monocyte (%)	2.60±0.071	2.20±0.027	2.40±0.047	2.40±0.045	2.40±0.071	2.20±0.047	NS
Eosinophil (%)	2.60±0.100	2.60±0.122	2.40±0.127	1.80±0.138	1.80±0.122	2.40±0.100	NS

± = Standard Error NS = Non Significant, \*\*=P<1% of Significance, \*=P<5% of Significance

Table 3. Effects of Blood Transfusion on Various Biochemical Parameters in Recipient Calves (n = 5)

Parameters	Pre transfusion values (Control)	Post transfusion values (Mean $\pm$ SE)					Level of significance
		Day-1	Day-7	Day-14	Day-21	Day-28	
Creatinine (mg/dl)	0.88 $\pm$ 0.116	0.90 $\pm$ 0.071	1.06 $\pm$ 0.081	1.30 $\pm$ 0.145	0.94 $\pm$ 0.068	0.84 $\pm$ 0.040	NS
ALT (U/L)	62.36 $\pm$ 3.84	59.44 $\pm$ 2.21	80.80 $\pm$ 3.81	87.60 $\pm$ 2.34	70.26 $\pm$ 1.95	46.36 $\pm$ 6.08	NS
AST (U/L)	106.52 $\pm$ 1.27	100.63 $\pm$ 2.45	153.40 $\pm$ 2.11	166.04 $\pm$ 2.92	133.85 $\pm$ 5.31	115.40 $\pm$ 3.49	NS
K <sup>+</sup> (mmol/L)	4.38 $\pm$ 0.220	5.23 $\pm$ 0.090	5.11 $\pm$ 0.311	5.28 $\pm$ 0.235	4.79 $\pm$ 0.139	5.04 $\pm$ 0.176	NS
Na <sup>+</sup> (mmol/L)	144.10 $\pm$ 1.37	148.34 $\pm$ 1.71	151.24 $\pm$ 2.70	140.30 $\pm$ 2.38	141.66 $\pm$ 1.20	141.70 $\pm$ 1.77	NS
Cl <sup>-1</sup> (mmol/L)	102.68 $\pm$ 1.19	105.54 $\pm$ 1.29	108.04 $\pm$ 1.58	100.54 $\pm$ 1.83	101.36 $\pm$ 1.53	100.04 $\pm$ 1.59	NS

$\pm$  = Standard Error NS = Non Significant, \*\*=P<1% of Significance, \*=P< 5% of Significan



Table 4. Effects of Plasma Transfusion on Various Biochemical Parameters in Recipient Calves (n = 5)

Parameters	Pre-transfusion values (Control)	Post transfusion values (Mean $\pm$ SE)					Level of significance
		Day-1	Day-7	Day-14	Day-21	Day-28	
Creatinine (mg/dl)	0.78 $\pm$ 0.037	1.06 $\pm$ 0.087	0.66 $\pm$ 0.040	0.62 $\pm$ 0.020	0.56 $\pm$ 0.040	0.52 $\pm$ 0.020	NS
ALT (U/L)	57.00 $\pm$ 1.22	66.20 $\pm$ 1.64	69.52 $\pm$ 2.27	53.74 $\pm$ 2.43	51.38 $\pm$ 2.89	46.80 $\pm$ 2.80	NS
AST (U/L)	107.34 $\pm$ 0.918	156.96 $\pm$ 14.21	189.64 $\pm$ 14.65	160.20 $\pm$ 4.38	128.19 $\pm$ 4.30	109.15 $\pm$ 1.69	NS
K <sup>+</sup> (mmol/L)	4.72 $\pm$ 0.194	5.44 $\pm$ 0.020	6.17 $\pm$ 0.596	5.96 $\pm$ 0.017	5.29 $\pm$ 0.005	5.18 $\pm$ 0.034	NS
Na <sup>+</sup> (mmol/L)	146.86 $\pm$ 0.451	136.52 $\pm$ 0.790	169.60 $\pm$ 2.20	153.66 $\pm$ 0.710	149.24 $\pm$ 0.397	148.00 $\pm$ 0.707	NS
Cl <sup>-1</sup> (mmol/L)	102.40 $\pm$ 0.338	98.36 $\pm$ 0.397	123.10 $\pm$ 1.59	112.08 $\pm$ 0.525	101.76 $\pm$ 0.367	100.44 $\pm$ 0.312	NS

$\pm$  = Standard Error NS = Non Significant, \*\*=P<1% of Significance, \*=P<5% of Significance

## DISCUSSION

Microcytic hypo-chromic anemia in livestock may occur due to blood sucking parasites, gastro-intestinal lesions or dietary deficiency of iron. Fresh Blood was transfused to group A, and plasma transfused to group B. The results of the present study demonstrated that the transfusion of blood and plasma in calves could cause some statistically significant changes in haematological, and biochemical parameters. However, there were minor decreases below or increases above the reference ranges in some variables, but all values measured after transfusion were remained within the reference ranges (Kaneko et al., 2008). Mean control values of TEC progressively increased throughout the experimental period in calves of group A and B. The increase was highly significant ( $P < 0.01$ ) in both groups. Similar results have been reported by earlier workers (Suffian and Hossain, 1999; Alam and Hossain, 2005). When compared between two groups, TEC values seemed to be superior in case of group-A than group-B during the experimental period. Increased erythrocytes in case of blood transfusion may be due to increased supply of circulating blood constituents. The mechanism associated with increased TEC values after plasma transfusion is not available in the literature and this may be due to erythropoietin present in the donor's plasma. In the anemic calves the depleted erythropoietin level might be inadequate to trigger erythropoiesis and following plasma transfusion the erythropoietin level might be significantly increased to stimulate erythropoiesis (Alam and Hossain, 2005). Blood and plasma transfusion resulted in minor increase of the WBC in the present study. Fenwick et al., (1994) also found increased in WBC 12 hours after blood transfusion, which returned to baseline within 24 hours. The WBC was increased 12 hour after blood and plasma transfusion and decreased 14 days thereafter in this study. The leukocyte changes could be due to the presence of antibodies against the blood cells antigens, which triggered immune responses and consequent increase in the WBC (Nielsen et al., 1997). Hemoglobin and packed cell volume (PCV) values in calves of group A and B also significantly increased during the course of experiment and the values found superior in group-A than group-B. Transfusion of whole blood @ 15 mL/kg increased the PCV by 3.25% which was similar to the increase in sheep, goat, and horses that were undergoing homologous transfusion (Hunt and Moore 1990; McClure, 1997). However, the increased levels of Hb and PCV in recipient calves of group A and B might result from increased TEC values (Srivastova and Pandey, 1992).

Serum creatinine is the most commonly used indicator (but not direct measure) of renal function and there was temporary increase of mean control value of creatinine from day-1 up to day-14 in both calves of group of A and B but do not cross the reference ranges (Kaneko et al., 2008). When compared between two groups, plasma transfusion showed better result than that of blood transfusion. Elevated creatinine is not always representative of a true reduction in GFR. An increase in serum creatinine can be due to excessive intake of protein and creatine supplements. Dehydration secondary to an inflammatory process with fever may cause a false increase in creatinine levels. So, there are no abnormalities in kidney functions following blood/plasma transfusion. There were temporary increase of mean control value of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) up to day-14 in calves of both group A and B which return to baseline within 28 days of post-transfusion. Mild to moderate elevations of the liver enzymes are common and they are often unexpectedly encountered on routine blood screening tests in otherwise healthy individuals. Raised liver enzymes after blood transfusion are extremely common and potential causes of these raised liver enzymes after blood transfusion could be secondary to limited ischemic hepatitis after blood transfusion or concomitant medications. Other possibilities could include increased vascular permeability because of possible leukocyte antibodies as it occurs in patient with transfusion-related acute lung injury (Curtis and McFarland, 2006; Moore, 2006).

In present study, variations in AST, ALT activities were minimal and remained within the reference values for calves (Kaneko et al., 2008). No hepatic injuries following blood and plasma transfusion have been suggested. There was slight increase of mean control value of potassium in calves of both group A and B. In case of plasma transfusion increased value may be due to increased concentration of donor plasma (Rizos et al., 2017). Alteration in serum sodium and chloride concentration followed no consistent pattern in the cases of present study and similar findings were reported by Miller et al., (1954). This effect may be due to excretion of sodium and chlorine by kidney to adjust and maintain equilibrium. Transfusion reactions may vary in severity from a life threatening hemolytic crisis to mild urticaria. An adverse reaction to plasma proteins can cause a moderate release of histamine after antigenic stimulation. Severe reactions produce immediate hypotension, fever, vomiting, convulsion, salivation, tachycardia, hemolysis and hemoglobinuria followed by massive bleeding in the absence of factors (Byars and Divers, 1981). Milder reactions result in slight Jaundice. In the most severe form, death might occur within 3 days or so as a result of acute renal failure due to renal tubular damage. This incompatibility accompanied by urticaria may be prevented by intravenous administration of an antihistamine, mepyramine melete (Bliss et al., 1959). Tripathy and Dash (1984) tested 100 serum samples against blood cells of 5 bulls and observed 1% incompatibility.

## CONCLUSIONS

Blood and plasma transfusion appear to upgrade haematological parameters (TEC, Hb and PCV) in recipient calves. And the transfusion do not adversely affect kidney functions (no significant change in Creatinine level), any hepatic injury (no significant change in ALT and AST level). The transfusion cause mild change in the trace elements (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>) of recipient calves. As blood and plasma transfusion do not have any deleterious effects on recipient calves, it can be a tool for successful clinical management of anaemic calves.

## COMPETING INTEREST

The authors declare that they have no competing interests.

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