

THERAPEUTIC STUDIES ON ANEMIA IN CALVES

By

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CERTIFICATE

Mr. GIRADA KANTHI KIRAN has satisfactorily prosecuted the course of research and that the thesis entitled “**THERAPEUTIC STUDIES ON ANEMIA IN CALVES**” submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any university.

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No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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ABSTRACT

In the present study, an investigation was carried out on the incidence of anemia in the calves of white cattle for which blood samples collected from 105 calves (from those presented at different veterinary hospitals and of the calves from organized dairy farms) were processed for haemoglobin and packed cell volume after carrying out thorough clinical examination of the calves. All those animals showing haemoglobin of less than 8 g/dl and PCV of less than 25 per cent were considered as frankly anemic. Out of the 105 calves, 26 were found to be anemic with an incidence of 24.67%. The incidence of anemia was found to be highest in 0-4 months calves (30%) followed by 4-8 (27.91%) and 8-12 months (15.63%), while the incidence was 22.73, 25 and 25.64 per cent in HF cross, Jersey cross and non-descriptive breeds of calves respectively. Coccidiosis, tick infestation, a diet of milk and certain obscure

factors were found to be the causes of anemia in 53.85%, 30.77%, 7% and 7% of cases respectively.

Anemic calves after being treated for the primary etiology of anemia were divided into three groups, each consisting of 6 animals for studying therapeutic aspects of anemia. Calves of group I served as untreated control while that of group II and III were treated for anemia with blood transfusion and haematinics respectively. In these animals clinical, haematological and biochemical parameters were assessed before and at regular intervals up to 20 days after initiation of therapy. Another 6 apparently healthy calves free of any detectable ailment served as healthy control.

The calves of healthy control group were subjected to clinical and haematobiochemical tests once. The clinical symptoms observed in anemic calves were dullness, depression, moderate anorexia and slightly pale conjunctival mucous membranes. In calves of group I there was only slight improvement in the appetite and general activity, while considerable improvement was observed by day one in blood transfusion group and by day one of completion of hematinic therapy in group III. The conjunctival mucous membrane turned pink by day ten, one and five in the groups I, II and III respectively. There was no significant change in body temperature of group I calves while a significant decrease was observed in groups II and III. The mean pulse and respiratory rates which were found to be high in all three groups during anemia significantly decreased during the period of study. The decrease in these rates in the hemainic group was more.

The mean haemoglobin, PCV, TEC, MCV and MCH were found to be significantly ($P < 0.01$) low during anemia. In calves of group I though all

these parameters were found to have increased significantly ($P < 0.01$), the per cent increase was considerably low when compared with treated groups. All these hematological parameters showed a significant ($P < 0.01$) increase by day I in both the treated groups of II and III. However, in group II calves, the values started declining after day 3 to day 20, whereas in group III calves there was a gradual and sustained increase till the end of the study period.

The mean plasma glucose, total protein albumin and serum iron which were significantly ($P < 0.01$) decreased during anemia showed a significant ($P < 0.01$) increase in all the groups during study period. And the per cent increase of mean serum iron by the end of the study was highest in hematinic group.

Blood transfusion proved to be expensive, requiring technical expertise and in the calves treated with transfusion TEC and plasma albumin reached the healthy control values. In hematinic group hematology and plasma iron reached the levels of healthy control and this form of therapy was worked out to be cheaper without a need for technical skill. It was noticed that elimination of primary etiology of anemia alone could not bring about desirable response, in the calves thus necessitating therapy for anemia.

Based on the above results it is concluded that anemia in calves was commonly associated with internal and external parasitism and hematinic therapy can be undertaken when the haemoglobin is around 7g/dl for bringing about an adequate and persistent clinical and hematobiochemical response. As blood transfusion brought about immediate clinical and hemato-biochemical improvement (though hematological improvement was transient), it may be more useful in critical anemia with a much less haemoglobin though expensive.

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LIST OF ABBREVIATIONS AND SYMBOLS

A/G	=	Albumin Globulin ratio
ANOVA	=	Analysis of Variance
AT	=	After treatment
@	=	At a rate of
B	=	Blank
BT	=	Before treatment
CD	=	Critical Difference
CPD-A	=	Citrate Phosphate Dextrose-adenine
cmm	=	Cubic milimetre
df	=	Degees of freedom
dl	=	Deciliter
EDTA	=	Ethylene Diamine Tetraacetic Acid
°F	=	Degree Fahrenheit
Fig	=	Figure
fl	=	Femtolitre
g/dl	=	Grams/deciliter
Hb	=	Haemoglobin
hr	=	Hour
H.R	=	Heart rate
i.e	=	That is
lbs	=	Pounds
Kg	=	Kilogram
MCH	=	Mean Corpuscular Haemoglobin
MCHC	=	Mean Corpuscular Haemoglobin Concentration
MCV	=	Mean Corpuscular Volume
mg/dl	=	Milligram/deciliter
min	=	Minutes
ml	=	Mililitre
meq /l	=	milliequivalents per litre
No	=	Number
PCV	=	Packed Cell Volume
/	=	per
%	=	Per cent
Pg	=	Picogram
SE	=	Standard Error
T	=	Test
TEC	=	Total Erythrocyte Count
Temp	=	Temperature
Viz	=	Namely

CHAPTER-I

1. INTRODUCTION

Anemia may be defined as a decrease in the amount of haemoglobin per unit of blood which may or may not be accompanied by a reduction in the red cell count or volume of red cell count or volume of red cells in relation to plasma (Cattcott, 1968). Anemia is a sign and more commonly only one of the results of a generalized disease process (Jain, 1986). Hence once anemia is diagnosed, its etiology should be thoroughly investigated. Signs common to anemias include reduced exercise tolerance, pallor of mucous membranes, increased heart rate sometimes with murmurs, increased respiratory rate, depression and dementia. These symptoms are the result of reduced oxygen carrying capacity of the blood as well as a consequence of certain physiologic adjustments designed to increase the efficiency of the reduced circulating red cell mass and to reduce the work load of the heart (Jain, 1986).

As anemia is a reflection of some other primary disease, its incidence may depend upon the incidence of anemia producing disease(s). In farm animals, particularly in calves though blood loss anemia associated with external or internal parasitism or hemoprotozoal infections is more commonly encountered by a clinician other conditions like accidental trauma, prolonged surgical interference, warfarin poisoning etc also may produce anemia. It is likely that in clinical practice only the primary disease is paid much attention

leaving anemia, particularly if it is a mild or moderate condition. Mild anemia which usually passes undetected may be of a little consequence to the patient, provided the primary disease is treated successfully. But there is an imperative need to treat moderate anemia as it affects the patient's well being, performance, growth rate and resistance to infection and obviously the critical anemia to save the life of the affected animal.

The form and outcome of therapy of anemia may depend upon the kind of the primary disease or condition it is associated with and the ability to effectively treat it and also on the pathogenic mechanism involved in the production of anemia. Response to therapy is generally good in nutritional deficiency anemia as the bone marrow is fully capable of resuming normal erythropoietic activity as soon as adequate materials are supplied (Jain, 1986). In clinical practice, generally, therapy of anemia is in the form of provision of nutrients necessary for efficient erythropoiesis by means of hematinics or readily utilizable haemoglobin in the form of blood transfusion. A Systematic and readily available information regarding the different forms of therapy of anemia and knowledge about the incidence and etiology of anemia in calves is meagre. Hence the present study was undertaken with the following objectives.

1. To study the incidence of anemia in calves.
2. To establish the possible etiological cause(s)
3. To compare the efficacy of transfusion therapy and use of hematinics in clinical cases of anemia.

CHAPTER-II

2. REVIEW OF LITERATURE

2.1 INCIDENCE OF ANEMIA IN CATTLE CALVES:

Greenhalgh et al. (1972) carried out a survey on the incidence of anemia in farms adopting kale feeding practices in England and Scotland, during the year 1969. of the kale fed cows, 6.4% were classified as anemic which had haemoglobin content not more than 8g/dl. The respective proportions for England and Scotland were being 5% and 8%. About 36% of the cows showed disordered blood metabolism characterized by the presence of Heinz Ehrlich bodies in more than 5% of the erythrocytes. Hemoglobinuria was observed in less than 10% of the cows given kale.

Mollerberg (1975) observed that 30-35% of calves purchased in Sweden had iron deficiency anemia. The normal increase in plasma volume between birth and 24 hour age was 17% and that of blood volume was 14%.

Bunger et al. (1979) reported anemia in male calves. The calves were grouped according to the anemic status, i.e., on the basis of haemoglobin content and hematocrit values at 34th, 28th and 56th days of age. There was significant difference between groups on absolute and relative feed

consumption, on body weight and frequency of treatment of pneumonia or diarrhoea. In their study of anemia in male calves during a rearing period of 56 days on a semi-liquid diet, the criteria taken for anemia were haemoglobin being less than 10.5g/dl, hematocrit less than 33% and MCHC 30.5g/dl. Using these criteria the average duration of anemia was found to be 45, 30 and 44 days respectively on three premises. During the rearing period 89, 71 and 91% of calves were anemic for varying lengths of time. In 30% anemia lasted for more than 56 days.

Hellemond and Sprietsma (1979) observed anemia in ninety calves fed on milk substitutes and concluded that anemia developed in calves receiving less than 25ppm of iron was hypochromic and microcytic.

Bunger et al., (1980) studied the anemic status in male calves receiving concentrates ad libitum and fluid feed with milk from five Days of age. They found that anemia developed during the first month of life was not only physiological but was also due to iron deficiency.

Dilov et al.(1981) reported the incidence of anemia in young and new born calves. Twins born to heifers were particularly more prone to anemia. Both Simmental and Simmental-cross calves were more affected than the local breeds. The authors reported that the most susceptible group was calves aged upto 7 days and with highest rate of anemia in day old calves.

2.2 ANEMIA IN CALVES:

2.2.1 Symptomatology:

Martinovich and Woodhouse (1971) carried out a study on Heinz body hemolytic anemia in post parturient haemoglobinuria in cattle and reported symptoms like haemoglobinuria, hyperpnoea, pyrexia, icterus, anorexia and decreased milk yield (either singly or in combination). Anemia was also evident in their study by findings like anisocytosis, macrocytosis with polychromacia, basophilic stippling of erythrocytes and increase in numbers of nucleated blood cells in the peripheral blood.

Jain (1986) reported the signs common to anemias of diverse etiology as reduced exercise tolerance, pallor of mucous membranes, increased heart rate sometimes with murmurs, increased respiratory rate, depression and dementia. It was mentioned that a mild anemia usually passes undetected unless the animal is allowed to exert or is brought to the clinic for other

reasons. Clinical symptoms resulting from acute hemolytic anemia may be icterus, hemoglobinuria and fever.

Sharma et al. (1991) induced acute hemolytic anemia with phenylhydrazine hydrochloride in 1 week old bovine calves with a body weight of 30 kg and observed symptoms such as dullness, depression, gasping, involuntary muscular twitching, staggering gait, recumbency and mild pyrexia. They also observed pale visible mucous membranes, lacrymation, salivation, sunken eyeballs and starring coat as the anemia progressed.

Srivastava and Pandey (1993) induced the hemorrhagic anemia in male cross bred calves by withdrawing blood @ 25ml/kg body weight till the hemoglobin reached to 5 gm/dl and observed symptoms like dullness, depression, significantly increased respiratory and heart rates and thumping heartbeat.

Pradhan et al (1993) noticed symptoms like hurried respiration (100%), submandibular edema (44.4%), reduced appetite (100%), decreased milk yield (75-100%), high body temperature of 105-106 F(100%) in 18 milch cows between the age groups of 5 and 6 years suffering from anemia due to *Theileria annulata* infection.

Radostits et al (1994) mentioned the signs of anemia in domestic animals as pallor, muscular weakness, depression, anorexia, increased heart and pulse rate with a quality of large amplitude and labored breathing in the terminal stages. They also mentioned that other symptoms like edema, jaundice and haemoglobinuria may often accompany anemia though are not essential parts of the syndrome. Calf anemia associated with helminths like *Trichostrongylus*, *Ostertagia*, *Cooperia pneumatoduyrus* species is clinically reflected by pale and dry mucous membrane, submandibular edema, increased heart rate, loss of weight, soft faeces, reduced feed intake, emaciation etc..

Baxter (1996) reported the symptoms of persistent pyrexia, dullness and joint pain in a 2 weeks old calf born out of embryo transfer technology and whose blood smear revealed a hemolytic anemia.

2.3 HEMATOLOGICAL PROFILE

2.3.1 Haemoglobin

Baars (1971) estimated haemoglobin content of young calves and observed a wide variation in the values among calves. A haemoglobin level less than 6 gm/dl was found to be associated with retardation of growth.

Shetty et al (1977) worked on eight healthy male buffalo calves of 1-1.5 year age and reported the average mean haemoglobin value as 11.3 ± 0.84 g/dl.

Dhar and Gautam (1979) observed anemic changes in experimentally induced *Theileria annulata* infection in ten crossbred male calves. They observed a gradual fall of haemoglobin during the course of anemia and a maximum fall after peak parasitaemia. The mean values of haemoglobin during preinfection, acute phase of infection and chronic infection were 10.5 g/dl, 6.3g/dl and 7 g/dl respectively. The maximum fall in haemoglobin expressed was 54.7%.

Jain et al (1982) conducted hematological studies on fifty clinically normal lactating murrah buffaloes in India. The normal range and mean given for haemoglobin was 9-13.5 g/dl and 11.1 ± 0.96 g/dl respectively.

Parthasarthy et al (1985) in their study of the effect of blood transfusion in bovines and its hematological responses, induced anemia in 6 calves, aged 6-7 months. The mean haemoglobin values of the recipients in their study before and after infusion were 6.4 ± 0.93 g/dl and 7.83 ± 0.58 g/dl respectively.

Sharma et al (1991) induced acute hemolytic anemia with phenylhydrazine hydrochloride in one week old bovine calves with a body weight of 30 kg and observed a mean haemoglobin value of 6 ± 0.13 and 7 ± 0.1 g/dl at the stage of peak anemia.

Srivastava and Pandey (1992 a) induced anemia in 10 male crossbred calves of 6 M – 1 year age by making the animal bled @ 25ml/kg b.wt and reported a normal mean haemoglobin value of 10.2 ± 0.14 g/dl before bleeding and 5.02 ± 0.02 g/dl six hours after bleeding.

A range of haemoglobin of 8-15 g/dl with an average of 11 g/dl was reported in healthy cattle (Jain, 1986)

Srivastava and Pandey (1992 b) induced anemia in 10 male cross bred calves of 6months to 1year age by making the animal bled @ 20ml/kg b.wt. They reported a normal mean haemoglobin value of 11.56 ± 0.16 g/dl before bleeding and 5 ± 0.08 g/dl after induction of anemia.

Srivastava and Pandey (1993) induced hemorrhagic anemia in 15 cattle calves of one year old by bleeding the animals @ 25ml/kg b.wt. They reported

a mean haemoglobin of 10.68 ± 0.21 g/dl before bleeding and 5.06 ± 0.4 g/dl after induction of anemia.

Pradan et al (1993) reported a haemoglobin value of 4.15, 4.58 and 4.76 g/dl in 18 milch cows of 5-6 yrs age group, which has *Theileria annulata* infection and suffering from anemia.

Kaneko et al (1997) mentioned a normal haemoglobin value in cows as 8-15 g/dl with an average of 11 g/dl.

Aiello (1998) mentioned a normal haemoglobin range in bovines as 8-15 g/dl.

Samuel and Srinivasan (2001) studied anemia in 12 clinical cases of adult dairy cattle and reported the mean haemoglobin as 6.47 ± 3.1 g/dl, while it was 9.46 ± 4.717 g/dl in 6 apparently healthy cattle.

2.3.2 Packed cell volume (PCV)

Shetty et al (1977) have worked on the blood picture of 8 healthy male buffalo calves of 1-1.5 year age and reported the mean PCV as $28.24 \pm 2.06\%$.

Dhar and Gautam (1979) observed anemic changes in experimentally induced *Theileria annulata* infection in 10 crossbred male calves. During the course of chronic anemia a gradual fall in PCV was noted and maximum fall was observed after peak parasitaemia. The mean values of PCV during infection, acute phase of infection and chronic infection were 34%, 20.6% and 22.7% respectively. The maximum fall in PCV expressed in percentage was 56.7.

Jain et al (1982) performed hematological studies on 50 clinically normal lactating murrah buffaloes in India. The range and mean PCV quoted by them was 26-34% and $31 \pm 0.02\%$ respectively.

Verma and Quasimuddin (1981) undertook studies on the effect of oral and parenteral administration of iron in the treatment of bovine anemia, where they reported the pre therapeutic PCV to range from 21-24% in HF bulls of 4-6 years age.

Kumar et al (1984) studied clinical and hematological changes in buffaloes suffering from microfilariasis under field conditions. They observed that the animals developed anemia as the hemogram of the affected animals showed a significant difference in PCV. The mean PCV of the affected

animals was $28.45 \pm 0.85\%$ in contrast to $34.5 \pm 0.86\%$ recorded in healthy control buffaloes.

Parthasarathy et al., (1985) induced anemia in 6 calves aged 6-7 months and studied the effect of blood transfusion and its haematological responses. They recorded the mean haematocrit values of the recipients before and after infusion were $25.003 \pm 3.60\%$ and $27.83 \pm 3.37\%$ respectively.

Sharma et al., (1991) induced acute hemolytic anemia with phenylhydrazine hydrochloride in 1 week old bovine calves which are of 30 kg body weight and observed a mean PCV of 14.8 ± 0.62 and $15.0 \pm 0.71\%$ in two groups during peak anemia.

Srivastava and Pandey (1992 a) induced anemia in 70 male cross – bred calves of 6 month – 1 year age., by making the animal bled at the rate of 25 ml/kg Body weight. They reported a normal mean PCV of $34.74 \pm 0.35\%$ before bleeding and $13.98 \pm 0.05\%$ 6 hours after bleeding.

Srivastava and Pandey (1992 – b) induced anemia in 10 male cross – bred calves of 6 months – 1 year age by making the animal bled at the rate of

25 ml/kg body weight. He reported the normal mean PCV as $36.2 \pm 0.32\%$ before bleeding and $14.79 \pm 0.20\%$ after induction of anemia.

Jain (1993) mentioned a PCV range of 24 – 46% with an average of 35.0% in healthy cows.

Aiello (1998) mentioned the PCV range in healthy bovines as 24-46%.

Ramin *et al.* (2000) studied anemia in 22 cows and reported a PCV of 16.8%.

Samuel and Srinivasan (2001) studied anemia in two groups of adult dairy cattle consisting 6 in each and reported the mean PCV as 22.66 ± 0.67 and $22.33 \pm 0.95\%$ while it was $32.33 \pm 3.07\%$ in 6 apparently healthy cattle.

2.3.3 Total erythrocyte count (TEC)

Schalm(1965) mentioned a normal TEC of 13.10×10^6 /ml in 3 ½ to 4 1/2 months aged calves while a count of 10.65×10^6 /ml in 7 ½ -9 months aged calves.

Patel et al., (1971) studied the seasonal variation in certain biochemical and hematological constituents in the blood of calves, heifers and bulls. The

average total erythrocyte count was found to be 6.9 million /cmm and the counts were relatively higher in bulls and calves.

Dhar and Gautam (1979) observed anemic changes in experimentally induced theilaria annulata infection in the cross bred male calves. During the course of chronic anemia a gradual fall in TEC was noted and maximum fall after peak anemia. The mean values of TEC during pre-infection, acute phase of infection and chronic infection were 8.5×10^6 /cmm 5.38×10^6 /cmm and 6×10^6 /cmm respectively. The maximum fall in TEC recorded was 61.2%.

Jain et al (1982) performed hematological studies on fifty clinically normal lactating murrah buffaloes in India. The range and mean TEC quoted was 5.07 to 8.27 million/ml and 6.54 ± 0.77 million/ml.

Pandey and Misra (1983a) carried out study in the functional behavior of bone marrows of cow calves suffering from nutritional anemia induced by exclusive feeding of milk wheat straw ad libitum for a period of 2 months. The TEC at the peak of anemia varied from 4.1 ± 0.073 to $4.382 \pm 0.099 \times 10^6$ /cmm.

Parthasarathy et al., (1985) induced anemia in 6 calves aged 6-7 months and studied the effect of blood transfusion in bovines and its hematological responses. It was observed that the mean TEC values of the recipients before and after infusion were 5.06 ± 1.26 and $7.78 \pm 1.22 \times 10^6$ /ml.

Jain (1986) mentioned a TEC of $5-10 \times 10^6$ /ml with an average of 7×10^6 /ml in healthy cattle. He also reported a mean TEC of $9.5 \pm 1 \times 10^6$ /ul in fifteen calves of 3-16 weeks age. While the total erythrocyte counts in 3 ½ - 4½ months, 7 ½ -9 months and 11-12 months aged calves were 13.10×10^6 /ul, 10.65×10^6 /ul and 8.62×10^6 /ul.

Sharma et al (1991) induced acute hemolytic anemia with phenyl hydrazine hydrochloride in two groups of 1 week old bovine calves and observed a mean TEC of $5-1 \pm 01 \times 10^6$ / ul and $5-2 \pm 0.1 \times 10^6$ /ul at peak anemia.

Srivastava and Pandey (1992a) studied haemato-biochemical responses to whole blood transfusion in calves and induced hemorrhagic anemia and reported a mean TEC of $5.82 \pm 0.11 \times 10^6$ /mm³ before bleeding and 2 to $5 \pm 0.05 \times 10^6$ /cmm after induction of anemia.

Srivastava and Pandey (1992b) induced anemia in 10 male cross bred calves of 6 months to 1 year age by making the animal bled @ 25 ml/kg body weight. They reported the mean TEC of 6.30 ± 0.20 before bleeding and 3.27 ± 0.16 , 6 hours after bleeding.

Srivastava and Pandey (1993) induced haemorrhagic anemia in 15 cattle calves of 1 year old. They reported a mean TEC of $6.17 \pm 0.12 \times 10^6$ /cmm before bleeding and 2.75 ± 0.06 after induction of anemia.

Pradhan et al (1993) reported a TEC values of 3.7×10^6 /cmm , 3.25×10^6 /cmm and 3.8×10^6 in eighteen milch cows of 5-6 years age group, which had theileria annulata infection and suffering from anemia.

2.3.4 Erythrocyte indices:

Schalm(1965) mentioned a normal MCV, MCH and MCHC in 3 ½ - 4 ½ months aged calves as 27.6 fl, 8.45 pg and 30.6g% respectively where as these values in 7 ½ -9 months aged calves were 28.5 fl, 9.48 pg and 33.26g% respectively.

Dhar and Gautam (1979) experimentally induced *Theileria annulata* infection to young calves and based on the hematological findings divided the anemic status into pre-infection, acute and chronic. The pre-infection mean values for MCV, MCH and MCHC were 40.2 fl, 12.6 pg and 31.4 g/dl respectively. In chronic anemia the mean values of MCV, MCH and MCHC were 45.3fl, 11.8 pg and 29.4 g/dl respectively.

Bunger et al (1980) calculated MCHC values in anemic calves fed on semi liquid diet. The authors recorded MCHC as less than 30g/dl in anemic calves.

Jain et al (1982) performed hematological studies on fifty clinically normal lactating murrah buffaloes in India. The range of MCV, MCH and MCHC values quoted were 40.6 to 55.2 fl, 13.5 to 20.5 pg and 30.9 to 38.5 g/dl with their means as 48.2 ± 4.6 fl, 17.10 ± 1.85 pg and 35.2 ± 2.34 g/dl respectively.

Parthasarathy et al., (1985) induced anemia in 6 calves for studying the effect of blood transfusion and its hematological responses. The mean MCV, MCH and MCHC values of the recipients before infusion were 51.40 ± 10.10

fl, 13.10 ± 3.02 pg and 26.30 ± 6.15 g% respectively, where as these values after infusion were 49.4 ± 8.52 fl, 11.4 ± 2.43 and 23.4 ± 4.42 respectively.

Jain (1986) mentioned the normal MCV, MCH and MCHC ranges as 40-60 fl, 11-17 pg and 26-34 g% whose averages were 52 fl, 14 pg and 31 g% respectively. It was also mentioned that in young cattle with exceptionally high erythrocyte numbers, MCH may be as low as 8.5pg.

Jain (1993) mentioned the normal range of MCV, MCH and MCHC in cows to be 40-60fl, 11-17pg and 30-36 g% whose averages were 52 fl, 14 pg and 33 g% respectively.

Samuel and Srinivasan (2001) studied anemia in 12 clinical cases of adult dairy cattle and reported the mean MCV, MCH and MCHC values as 48.08 ± 0.33 fl, 13.36 ± 0.52 pg and 27.10 ± 1.03 g % respectively. They also reported that the normal mean MCHC was 32.55 ± 0.62 g% in 6 apparently healthy cattle.

2.4 BIOCHEMICAL CHANGES:

2.4.1 Total Plasma protein:

Shetty et al., (1977) worked on nine healthy male buffalo calves of 1-1.5 years age and gave the average mean plasma protein value as 6.15 ± 0.32 g%

Jain et al (1982) performed hematological studies on fifty clinically normal lactating murrah buffaloes in India. The range and mean plasma protein quoted by them was 6-9 g/dl and 7.8 ± 0.7 g/dl respectively.

Srivastava and Pandey (1992 a) induced anemia in 10 male cross bred calves of 6 months – 1 year age, and reported a mean total protein of 7.02 ± 0.09 g/dl before bleeding and 2.95 ± 0.03 after induction of anemia.

Srivastava and Pandey (1992 b) induced hemorrhagic anemia in 10 male cross bred calves of 6 months – 1year age and reported a mean total serum protein of 7.2 ± 0.11 g/dl before bleeding and 3.46 ± 0.03 g/dl after bleeding.

Srivastava and Pandey(1993) induced hemorrhagic anemia in 15 cattle calves of 1 year old by bleeding them @ 25 ml /kg body weight. They reported a mean total serum protein of 6.92 ± 0.12 g/dl before bleeding and 3.34 ± 0.04 g/dl after inducing anemia.

Jain (1993) mentioned the normal total protein range in cows as 6.8 to 8 g/dl with an average of 7.3 g/dl.

Kaneko et al (1997) tabulated the normal total protein level of various animals and the mean value of cows was found to be 7.1 ± 1.8 gm/dl with a range from 6.74 to 7.46 gm/dl.

Aiello (1998) mentioned the normal total protein range in bovines as 6.2 to 8.2 g/dl.

Samuel and Srinivasan (2001) studied anemia in 12 clinical cases of adult dairy cattle and reported the mean total protein values to be 6.28 ± 1.44 g/dl and 5.66 ± 0.35 g/dl. Where as a mean of 6.93 ± 0.18 was reported in 6 apparently healthy cattle.

2.4.2 Plasma albumin:

Kaneko et al (1997) mentioned the normal range of albumin as 3.03 - 3.55 g/dl with an average being 3.29 ± 1.3 g/dl in cows.

Aiello (1998) quoted that the serum albumin ranges from 2.8-3.9 g/dl in healthy bovines.

Samuel and Srinivasan (2001) studied anemia in 12 clinical cases of adult dairy cattle and reported the mean albumin values to be 2.56 ± 0.22 g/dl and 2.88 ± 0.34 g/dl. While the normal mean in 6 apparently healthy cattle was recorded on 3.5 ± 0.2 g /dl.

2.4.3 Plasma albumin globulin ratio (A/G):

Kaneko et al (1997) mentioned the normal range of albumin/ globulin ratio in cows to be 0.82 to 0.91 with an average being 0.86.

Aiello (1998) mentioned a normal range of albumin/ globulin ratio to be 0.82 to 0.91.

Samuel and Srinivasan (2001) studied anemia in 12 clinical cases of adult dairy cattle and reported the mean A/G ratio to be 0.69 and 1.03, whereas a mean of 1.02 was reported in 6 apparently healthy cattle.

2.4.4 Serum iron:

Bremner and Ronalds (1965) subjected healthy calves to a single blood withdrawal of 10,20,and 30 ml/ kg body weight and noted an increase in

plasma iron concentration after bleeding at 10ml/kg. A daily loss of 2 or 4 ml/kg increased the plasma iron concentration whereas a daily loss of 6 ml/kg depressed the plasma iron. When the bleeding was terminated, the plasma iron concentration reached an abnormally high concentration possibly because of an increase in dietary iron.

Goshal (1976) made an attempt to find out normal serum iron levels in twenty cows of north western deserts of Rajasthan, and reported the mean serum iron values for cows as 213.15 ± 14.08 ug/dl. They also reported that a subnormal serum iron level is evident in all species of animals suffering from anemia of hypochromic microcytic type, accompanied by a normoblastic, hyperplastic bone marrow in iron deficiency.

Saror (1980) estimated the serum iron in thirty seven calves of 15 weeks to 1 year of age and 42 calves of 1-3 years of age and gave their means as 25.3 ± 0.9 umol/litre and 149.34 ug /dl respectively.

Singh et al., (1980) reported the serum iron levels in healthy and haematuric bovines to be as 321.21 ± 7.4 ug/dl and 216.51 ± 6.98 ug/ dl respectively.

Pande et al., (1981) reported a seasonal variation of serum iron values in mehsani buffaloes of surat district, and gave their mean values as 268.8 ± 5.08 , 252.75 ± 18.95 and 179.98 ± 51.09 in monsson, winter and summer respectively.

Abdul Rahim et al (1983) studied the influence of extra supply of iron on some blood components and on meat colour at slaughter in fattening veal calves. During the last 3 weeks of fattening period the extra iron in diet lead to an increase in plasma iron concentration.

Jain (1993) mentioned the serum iron level in healthy cattle as 97 ± 29 ug/dl.

Kaneko (1997) tabulated the plasma iron levels of various domestic animals and mentioned a range of 57-162 ug/dl with an average of 97 ± 29 to be normal in healthy cattle.

Samuel and Srinivasan (2001) studied anemia in 12 clinical cows of adult dairy cattle and reported the mean serum iron values to be 61.63 ± 2.63 and 63.78 ± 2.52 ug/dl while a mean of 81.87 ± 6.72 ug/dl was reported in 6 healthy cattle.

2.4.5 Serum Calcium :

Shetty et al (1977) have worked on the blood picture of male buffalo calves of 1-1.5 years of age and reported the average mean plasma calcium to be 5.2 ± 0.08 Meq/lit.

Srivastava and Pandey (1992 a) induced hemorrhagic anemia in ten male cross bred calves of 6 months to 1 year age and reported a mean calcium value of 10.08 ± 0.06 mg/dl before bleeding and 6.85 ± 0.02 mg/dl 6 hours after bleeding.

Srivastava and Pandey (1992 b) in their other study on induced anemia in ten male cross-bred calves of 6 months to 1 year age reported the mean

serum calcium as 10.46 ± 0.2 mg/dl before bleeding and 7.21 ± 0.17 mg/dl after induction of anemia.

Pradhan et al., (1993) reported a calcium levels of 6.1, 6.25 and 6 mg/dl in eighteen milch cows of 5-6 years age group, which has *Theileria annulata* infection and suffering from anemia.

Srivastava and Pandey (1993) induced hemorrhagic anemia in 15 cattle calves of 1 year old by withdrawing blood @ 25 ml /kg body weight and reported serum calcium of 9.6 ± 0.27 mg/dl to be normal before bleeding and 6.98 ± 0.03 mg/dl after induction of anemia.

Kaneko et al (1997) mentioned the serum calcium level of various normal, healthy animals and the mean value of cows was found to be 11.08 ± 0.67 mg /dl with a normal range of 9.7 to 12.4 mg /dl.

Aiello (1998) reported that the serum calcium in cows ranges from 8.4 to 11.0 mg/dl.

2.4.6 Plasma Glucose:

Srivastava and Pandey(1992 a) induced anemia in ten male cross bred calves of 6 months – 1year and reported a mean blood glucose value of 61.64 ± 0.2 mg/dl before bleeding and $82.16 \pm .5$ mg/dl 6 hours after bleeding.

Srivastava and Pandey (1992 b) induced hemorrhagic anemia in ten male cross bred calves of 6 months -1 year age and reported a mean blood glucose level of 58.24 ± 1.17 mg/dl before bleeding and 76.64 ± 0.17 mg/dl 6 hours after bleeding.

Srivastava and Pandey (1993) induced hemorrhagic anemia in 15 cattle calves of 1 year old and reported a mean blood glucose of 55.82 ± 1.03 mg /dl before bleeding and 67.98 ± 1.11 mg/dl after induction of anemia.

Pradhan et al (1993) reported blood glucose levels of 30.4;38.1 and 34.12 mg/dl in eighteen milch cows of 5-6 years age group, which has *Theileria annulata* infection and suffering from anemia.

Kaneko et al (1997) postulated blood glucose levels of various healthy domestic animals where in the mean glucose value in cows was found to be 57.4 ± 6.8 mg/dl with a range from 45-75 mg/dl.

Aiello (1998) mentioned a normal serum glucose range in cows as 42.1 to 74.5 mg/respectively

2.5 Therapeutic aspects of anemia

2.5.1 Indications of blood transfusion:

Archer and Franks (1961) recommended blood transfusion in cows of blood loss due to accidental trauma as prolonged and major surgical interference. Blood transfusion was also indicated as a general supportive measure in specific diseases such as haemophilia, warfarin poisoning and hemolytic disease of the new born.

Foster (1967) indicated the use of whole blood transfusion in acute hemorrhagic shock, acute anemia, for pre and post – operative supportive therapy, as adjunctive therapy in combating infections, for increasing blood volume and cell count and as a source of protein and electrolytes in chronic debilitating diseases.

Wintrobe et al. (1974) mentioned that the most important use of whole blood was for the restoration of adequate blood volume after haemorrhage or trauma.

Balu and Krishnamurthy (1981) indicated that the morbidity and mortality rates can be minimized by blood transfusion in various conditions like massive infestation with blood sucking parasites, acute enteritis, dystochia, prolapse of uterus, mastitis, metritis etc. which invariably culminate in toxemia, dehydration and severe blood loss.

Balu and Krishnamurthy (1984) reported that blood transfusion in buffaloes may be useful as they succumb due to massive systemic derangements encountered in several medical, surgical, and gynaeco-obstetrical conditions.

Maiti and Sharma (1989) indicated blood transfusion in various species which are affected with acute hemorrhagic shock, in acute anemia and pre and post operative supportive therapy etc. they also indicated its importance in bovine babesiosis, theileriosis, anaplasmosis, enzootic influenza in calves and poisoning with sweet clover and bracken fern.

Sharma et al. (1991) mentioned that hemotherapy is the only remedy for combating critical anemia in bovine calves.

Srivastava and Pandey (1992a) reported that in severe anemia of calves, whole blood transfusion is unavoidable.

Pradhan et al. (1993) carried out the use of blood transfusion in cattle suffering from anemia associated with tropical Theileriosis.

Ramin *et al.* (2000) studied blood transfusion in 22 anemic cows and concluded that RBC, hemoglobin and PCV are the main parameters in blood transfusion which directly affect respiratory rate, pulse and temperature.

Sufiun *et al.* (2001) reported that blood transfusion may be an effective tool to improve the hematological profile of deficient calves.

Bhalerao (2002) reported a hemoglobin value of less than 8.26 gm% or <59% is anemic requires blood component therapy. He also indicated transfusion in hemostatic dysfunction with severe bleeding. Coagulopathy (disseminated intravascular coagulation, warfarin and hemophilia)

2.5.2 Cross matching:

Vongouth (1938) carried out a simple test before blood transfusion by mixing of few drops of the donor's blood with serum from patient in order to detect the presence of iso-agglutinin.

Archer and Frank (1961) stated that there was no equivalent of ABO blood group system in animals and there was no useful test for incompatibility. They also mentioned that single transfusion of unmatched whole blood is safe, regardless of the amount given.

Blood et al., (1989) recommended cross-matching of donors RBC with recipients serum and vice versa and test for hemolysis in animals before blood transfusion. This avoids transfusion reaction in positive cases. (Agglutination test).

Maiti and Sharma (1989) performed major and minor cross match test in animal before blood transfusion. Here major matching is done by addition of 2 drops of donor RBC with equivalent recipient's serum. While minor match is done by adding 2 drops of donor RBC with 2 drops of donor serum and both were incubated at room temperature for an hour, then centrifuged and examined for hemolysis.

2.5.3 Dosage and Administration:

Archer and Franks (1961) stated as a general rule that in large animals no more than 1 litre of blood should be given in 10 minutes period.

Hammonds (1969) stated that the amount of blood needed for transfusion depends upon the per cent of hemoglobin to be increased to desired levels.

Balu and Krishnamurthy (1984) reported that they have transfused a quantity of 300 ml of CPDG preserved blood in 3 six month old healthy buffalo calves.

Parthasarathy et al. (1985) reported that they have transfused one litre of blood @ 50 ml/min in six recipient calves aged 6-7 months respectively.

Sharma et al. (1991) stated that they have transfused blood through i/v at a flow rate of 60-80 drops/min. They also estimated the total blood volume in a 30 kg calf to be 1.71 litres as per Reynolds (1953) method and found it to be safe to transfuse 0.45 litre of blood as it constitutes barely 20% of the recipients total blood volume.

Srivastava and Pandey (1992) reported that they have administered blood in ten male cross bred calves of 6 month-1 year aged by gravity transfusion @ 500 ml/15 min. They calculated the amount of blood to be transfused as per Hammonds (1969).

Srivastava and Pandey (1993) mentioned that the amount of blood to be transfused to each recipient calves of 1 year old, was calculated by the method of Hammonds(1969) in order to raise the haemoglobin to 75 % and normal transfusion was done @ 500ml/lb min under gravity.

Nambi (1996) mentioned that the ml of blood required to raise hemoglobin by 1% is given by the formula $40 \times \text{body weight in pounds} / 100$.

Aiello (1998) mentioned that all domestic animals have a blood volume of approximately 7 % of their body weight except cats which has 4 % of their body weight. It was also mentioned that no more than 25% of donor's blood should be collected at a time.

Bhalerao (2002) reported a step-wise approach to the transfusion patient, in which he emphasized to determine the amount and type of current

or expected losses, obtain transfusion relevant history and determine which components are needed and calculate the dose.

2.5.4 Transfusion reactions:

Archer and Franks (1961) stated that there was considerable variation between different species in the reaction due to blood transfusion. The constant symptoms seem to be an increase in respiration and heart rate and defecation.

Kirk (1974) described transfusion reactions with different symptoms like rapid intravascular or extravascular destruction of erythrocytes resulting in nausea, vomiting, sweating, shivering, pruritis, dyspnoea, shock, tachycardia, hypotension, haemoglobinemia, haemogloninuria and acute renal failure respectively.

Balu and Krishnamurthy (1984) conducted blood transfusion in three buffalo calves with 0 day, 6 day and 15 day CPDG preserved blood. They observed only a mild and transient augmentation in heart rate and respiratory rate immediately after transfusion.

Parthasarathy et al. (1985) observed an increase in respiratory rate after transfusion in cow calves and observed no significant change in pulse rate and temperature following transfusion.

Maiti and Sharma (1989) performed blood transfusion therapy in domestic animals and some of the major transfusion reactions reported by them included frequent coughing, lacrymation, fever and in some cases ruminal tympany, hemoglobinuria and abortion.

Pradhan et al (1994) reported certain transfusion reactions in calves and cattle like respiratory distress, shivering of muscles, urticarial swellings, bellowing with pain, coughing, arching of back, frequent micturition and grinding of teeth.

Aiello (1998) mentioned that the most serious risk of transfusion is acute hemolysis though fortunately is rare in domestic animals. Other complications mentioned are sepsis from contaminated blood, hypercalcemia from too much citrate, urticaria, fever and vomiting.

Bhalerao (2002) mentioned that the transfusion reactions could be of immediate and delayed type. In immediate type of reactions he reported that

facial edema, pruritis, urticaria, dyspnoea, vomiting and diarrhoea are common. In delayed type he gave a note of immune mediated red cell destruction, disease transmission and citrate toxicity.

2.5.5 Transfusion with fresh blood:

Balu and Krishnamurthy (1984) evaluated the therapeutic use of citrate phosphate dextrose gluconate (CPDG) preserved blood in three buffalo calves of six month old.

Parthasarathy et al (1985) studied the effect of blood transfusion in six calves of 6-7 months age and reported that the changes in TEC, Hb and PCV were highly variable and not statistically significant. MCV and MCH changes were not significant while MCHC increased significantly following transfusion. The changes in pulse, respiratory rate and temperature did not alter markedly following transfusion. They reported a reduction in RBC and attributed this to progressive elimination of transferred RBCs within 2-3 days.

Maiti and Sharma (1989) studied about blood transfusion therapy in domestic animals and mentioned that transfusion of 7.5 ml of blood/lb will raise the RBC count by about 1 million/cmm and hemoglobin by about 3 gm.

Sharma et al (1991) studied about hemotherapy of induced acute hemolytic anemia in bovine calves. They reported that hemotherapy at peak anemia elicited a progressive improvement and the hemogram was virtually normal on day 12. They mentioned that the clinical recovery was faster than improvement of hematological profile which was evident by prompt stabilization of the body temperature of anemic calves that received blood transfusion.

Srivastava and Pandey (1992 a) studied about hematobiochemical responses to whole blood transfusion in anemic calves of 6 months to 1 year age. They reported that blood transfusion played a role in improvement of different parameters like haemoglobin, TEC, PCV, total protein and calcium which reached almost normal level by day 11.

Srivastava and Pandey (1992 b) made a study on biochemical responses to plasma transfusion in anemic calves of 6 months to 1 year of age. They reported that the clinical and hematobiochemical response to plasma transfusion was found to be encouraging in the calves making complete clinical recovery by day 11 with an 80% recovery rate.

Pradhan et al (1993) performed blood transfusion along with two different oral hematinics i.e. hematol and livol, parentral haematinics with blood transfusion or both parentral and oral haematinics along with blood transfusion. They considered the superiority of the first group based on the clinical signs, early regain of normal haemo-biochemical values, and cheapness of treatment cost.

2.5.6 Hematinic therapy:

Rice et al (1967) studied the effect of injectable iron on blood hematocrit, haemoglobin and weaning weight of beef calves. They injected an iron dextran of 26.4mg/kg b wt in calves from birth to two months of age, which increased blood haemoglobin and hematocrit for a period of time during which untreated calves had low levels.

Getty et al (1968) noted that calves given ferrous sulfate by mouth or iron dextran by intra muscular injection showed a significant increase in haemoglobin and PCV compared to calves receiving no iron.

Volkar et al (1972) demonstrated the existence of iron deficiency anemia in white veil calves even during early stages of fattening which could be prevented by parenteral injection of iron. The authors reported higher growth rate in iron treated calves.

Malloerberg et al (1975) undertook ferrokinetic studies in normal and iron deficient anemic calves. They concluded that total daily requirement of iron was 160-180mg.

Verma and quasimuddin (1981) carried out treatment on Holstein friesian bulls of 4-6 years of age by giving 500mg of iron dextran intramuscularly on alternate days for 45 days to one group, 500mg of iron dextran once a week to other group and 13gm of ferrous sulfate daily for 45 days to third group with parenteral iron the haemoglobin raised almost to normal by 24th day while with oral iron, haemogram started showing improvement from 8th day onwards and reached normal level.

Ghargariu et al (1984) recommended intramuscular injection of iron in two doses of 500 mg per week to calves of 3-6 weeks of age.

Pradhan et al (1993) studied the role of hematinic to maintain the haemoglobin after blood transfusion in 18 calves of 5-6 years of age. They reported that iron dextran given @ 6ml/animal intra muscularly for 5 occasions has a considerable effect in improving the hematological profile.

Adams (2001) recommended that severe iron deficiency be treated first by parenteral preparation followed by continued oral supplementation for approximately one month or for as long as iron imbalance exists. He also mentioned the oral iron dose to be 8-15 gms per day for 2 weeks or more in cattle and 0.5-2 gm per os / day for 2 weeks in sheep respectively.

Samuel and Srinivasan (2001) studied the effect of hematinics in 12 clinical cases of anemia in adult cattle. They reported a considerable improvement in hematological profile with oral hematinics containing 10gm of ferrous sulfate, 200ug of copper sulfate and 40mg cobalt sulfate daily followed by injection of iron sorbitol combination with folic acid and vitamin B-12 10ml intra muscularly twice a week.

CHAPTER-III

3. MATERIALS AND METHODS

3.1 MATERIALS:

3.1.1 Selection of cases:

In this study, from among the calves of white cattle irrespective of breed, sex and age which were brought for treatment to the college clinic, city veterinary hospital and ambulatory clinic of Thondawada and of the organized dairy farms. 105 calves, with their body weights ranging from 30-100 kgs were screened preliminarily for the presence or absence of anemia, through by clinical examination and by estimating hemoglobin and packed cell volume. Twelve such anemic calves were selected for therapeutic studies; another group of 6 calves with anemia were selected to serve as untreated control.

3.1.2 Collection of clinical materials:

Faecal samples and blood smears were collected to establish the cause of anemia while whole blood with and without anticoagulant was collected for plasma and serum separation, which were subsequently used for hematological and biochemical studies.

3.1.3 Glassware and chemicals:

All the glassware used in this present study were procured from M/s Borosil, Mumbai and M/s Qualigens, Mumbai. Kits for biochemical estimation like that of glucose, total protein and albumin and calcium were procured from Sigma Diagnostics, Gujarat marketed by Qualigens fine chemicals, Glaxo, Mumbai, BDH and Sarabhai chemicals. Kits for plasma iron estimation were supplied from Span Diagnostics Pvt. Ltd, Surat.C.P.D.A bags for blood collection were procured from Mithra Industries Ltd. Delhi.

3.1.4 Therapeutic agents:

The drugs used in the therapeutic trial with their trade names, presentation and manufacturer are as follows:

Trade name	presentation	manufacturer
1. Imferon	50 mg/ml	Shreya life sciences Pvt
Each ml contains	(2 ml ampoule)	Ltd.B-9/2, M.I.D.C.,
Iron dextran equivalent		Waluj-431136
to elemental iron 50mg		(Aurangabad)
2. Haematinic mixture		—————
Ferrous sulfate- 8gm		
Copper sulfate- 250 mg		
Cobalt sulfate- 25mg		

3.2 METHODS:

3.2.1 Diagnosis of anemia:

Anemia was diagnosed by thorough clinical examination of the calves and by estimating the haemoglobin and packed cell volume of their blood samples.

3.2.2 Diagnosis of probable etiology of anemia:

After obtaining detailed anamnesis, the anemic calves were thoroughly examined clinically including for external parasitism. Their blood smears and faecal samples were examined for any hemoprotozoa and internal parasitism. Details of the diet given was also explored

3.2.3 Clinical examination:

All the anemic cases taken up for therapeutic trials including untreated control were treated for the etiology of anemia and were subjected to detailed clinical examination as described by Kelly (1974), before and after initiation of therapy for anemia.

3.2.4 Collection of blood samples:

10 ml of blood was collected from jugular vein into glass vials containing disodium EDTA @ 1mg/ml.

3.2.5 Separation of serum:

About 10 ml of blood was collected into sterile test tubes devoid of any anticoagulant. These samples were kept in slant position making an angle of 20-30 degrees, undisturbed for 2-3 hrs. Then they were subjected to centrifugation @2000rpm for 15 min and serum was separated. Duplicate samples of each serum sample were prepared and stored in appendorf tubes by the addition of thiomersol/merthiolate(0.001%) @0.05ml/ml of serum and were kept in freezing chamber till use.

3.2.6 Haematological methods:

The hematological examination of the blood sample was carried out as follows:

3.2.6.1 Haemoglobin (Hb):

Haemoglobin estimation was done by acid hematin method using sahli haemoglobinometer. 0.1N HCL was pippered out up to the mark 2 of haemometer tube and about 0.02ml of freshly collected whole blood was taken into haemometer tube. The acid was gently mixed and left for 10 min (Kolmer *et al.*, 1959). Adding more N/10 HCL so as to obtain a colour, which matches with the standard colour tubes provided on both the sides, diluted the acid

haematin formed. The results were directly read and expressed as gram percent.

3.2.6.2 Packed cell volume (PCV):

Packed cell volume was determined by using clay Adam microhaematocrit centrifuge @14,000 rpm for 5 min. The microhaematocrit tubes were removed and read in a microhaematocrit reader as per the procedure of Jain (1986). The packed cell volume was expressed in per cent (%).

3.2.6.3 Total erythrocyte count:

Total erythrocyte count was estimated by utilizing the Neubaur counting chamber as per the method described by Jain (1986) and expressed as millions per cubic millimeter (cmm).

3.2.6.4 Erythrocyte indices:

The erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Jain (1986) and were expressed in fl, pg and g/dl respectively.

$$\text{MCV} = \frac{\text{PCV (\%)}}{\text{RBC (millions/cmm)}} \times 10$$

$$\text{MCH} = \frac{\text{Hb(g/dl)}}{\text{RBC (millions/cmm)}} \times 10$$

$$\text{MCHC} = \frac{\text{Hb(gm/dl)}}{\text{PCV (\%)}} \times 100$$

3.2.7 BIOCHEMICAL METHODS:

Biochemical parameters were estimated by using either plasma or serum depending upon the specificity. Plasma or serum was obtained by either centrifuging the whole blood or blood clot @2000 rpm for 15 min.

3.2.7.1 Total Plasma Protein

The quantitative determination of total proteins in plasma was carried out by the Biuret and BCG Dye method. The kit employed was of Quailgens diagnostics and was expressed in g/dl plasma (%).

The reagents supplied along with the kit were as follows:

1. Biuret reagent
2. Buffered dye reagent
3. Protein standard.

Test procedure:

Three clean dry glass test tubes were taken and marked as blank (B), Standard (S) and test (T).

	(B)	(S)	(T)
Biuret Reagent	1ml	1ml	1ml
Distilled Water	2ml	2ml	2ml
Protein Standard	----	0.05ml	----
Serum/Plasma	----	----	0.05ml

The contents of the tubes were mixed well and incubated at 37° C for 10 min. The absorbance of 'S' and 'T' against blank 'B' were measured in a spectrophotometer (ELICO-EL.24) at 555 nm wave length.

Calculations

Total protein in the plasma samples was calculated using the formulae as given below.

$$\text{Total protein (g\%)} = \frac{\text{Absorbance of (T)}}{\text{Absorbance of (S)}} \times \text{Conc. of total protein}$$

Where concentration of total protein= 6.32% (stamped on vial).

3.2.7.2 Plasma albumin

For the quantitative determination of albumin in plasma, the same kit that was used for assay of total protein was employed and the values were expressed in g/dl of plasma (%).

Test procedure

Three clean dry glass test tubes were taken and marked as blank (B), standard (S) and test (T).

	(B)	(S)	(T)
Buffered dye reagent	1.0ml	1.0ml	1.0ml
Distilled water	2.0ml	2.0ml	2.0ml
Albumin standard	---	0.01ml	---
Serum/Plasma	---	---	0.01ml

The contents were well mixed and the absorbance was measured immediately in an UV spectrophotometer at 630nm.

Calculations:

$$\text{Albumin in gm\%} = \frac{\text{Absorbance of (T)}}{\text{Absorbance of (S)}} \times \text{conc. of albumin}$$

(Concentration of albumin standard = 4 gm %)

3.2.7.3 Albumin-Globulin ratio (A: G)

Albumin to globulin ratio was obtained by dividing the percent of albumin with the percent of globulin in the plasma.

$$\text{A:G ratio} = \frac{\text{Albumin (g \%)}}{\text{Globulin (g \%)}}$$

3.2.7.4 Serum iron:

Principle

Serum proteins are precipitated with a reagent containing hydrochloric acid (to dissociate iron), thioglycolic acid (to reduce iron to ferrous state) and trichloro acetic acid (to precipitate proteins). The iron in ferrous state reacts with chromogen, to give pink coloured complex, which is measured calorimetrically.

Sample:

Serum 4ml

Reagents (supplied in the kit):

Reagent 1 : protein precipitant

Reagent 2 : chromogen

Reagent 3 : magnesium carbonate (light)

Reagent 4 : stock iron standard, 10 mg%

Working iron standard solution preparation:

Reagent 4(stock iron standard, 10 mg%) was diluted @1 to 50 with distilled water and prepared fresh before use.

Test procedure (for calorimeter)

Three clean dry test tubes were taken and marked as experimental blank, standard and test.

Step –1, deproteinization of test sample:

	Exp.blank (EB)	Standard (S)	Test (T)
Serum	----	---	2.0ml
Working iron standard	----	2.0ml	---
Distilled water	2.0ml	---	---
Reagent 1(protein precipitant)	2.0ml	2.0ml	2.0ml

The tubes were mixed well and allowed to stand at room temperature for 10min then the test (T) was centrifuged and incubated at 37 °C for 30 min. absorbance of test and standard against experimental blank was read in a spectrophotometer at 570nm.

Step-2 colour development

	Blank (B)	Exp. Bank (EB)	Standard (S)	Test (T)
Supernatant (from step-1)	--	2.0ml	2.0ml	2.0ml
Distilled water	3.0ml	1.0ml	1.0ml	1.0ml
Reagent-2 (chromogen)	2.0ml	2.0ml	2.0ml	2.0ml

The tubes were mixed well and incubated at 37 °C for 30 min. absorbance of test and standard against experimental blank was read in a spectrophotometer at 570nm.

Calculations:

$$\text{Serum iron in ug/100} = \frac{\text{O.D.Test(T)} - \text{O.D.Exp. Blank}}{\text{O.D.Std.(S)} - \text{O.D.Exp.Blank}} \times 200$$

3.2.7.5 Serum Calcium:

The calcium level in serum was estimated as per O-cresolphthalein complexone method by Gitelam(1967). The values were expressed in mg per 100ml of serum.

Reagents:

Diagnostic reagent kit supplied by qualigens-diagnostics was used for determination of serum calcium

1. Buffer solution
2. Colour reagent
3. Calcium standard 10 mg%.

Preparation of working reagent

Equal volumes of buffer solution and colour reagent were mixed as per daily requirements.

Test procedure

Three test tubes labeled blank (B), standard (S) and test (T) were taken and 2.5ml of working reagent was added. To the test tube (S) 0.02ml of calcium standard was added and to the tube (T) 0.02ml of serum was added, mixed well, allowed to stand at room temperature for 5 minutes. Then the absorbance of standard (S) and test (T) were measured against blank (B) on a spectrophotometer at 570nm within 30 minutes.

Calculations

$$\text{Serum calcium concentration (mg \%)} = \frac{\text{OD of (T)}}{\text{OD of (S)}} \times 10$$

3.2.7.6 Plasma glucose:

Plasma glucose level as estimated by GOD/POD method (Henry, 1974) using the kit supplied, was expressed as mg% of blood.

Principle:

Glucose is oxidized by the enzyme glucose oxidase (GOD) to give D-gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of enzyme peroxidase (POD) oxidizes phenol, which combines with 4-amino antipyrine to produce a red colored quinoneimine dye. The intensity of the color developed is proportional to glucose concentration in the sample.

Reagents:

The reagents supplied in the kit were:

Reagent 1: Enzyme Reagent (glucose oxidase and peroxidase)

Reagent 2: Buffer Solution

Reagent 3: Glucose Standard (100 mg%)

Working enzyme reagent:

The enzyme reagent vial was mixed with the buffer solution.

Procedure:

Three clean dry test tubes were taken, labeled as blank, standard and test. The protocol is as follows.

	(B)	(S)	(T)
Working enzyme reagent	1.0 ml	1.0 ml	1.0 ml
Distilled water	10ul	---	---
Glucose standards (3)	---	10ul	---
Plasma	---	---	10ul

The tubes were mixed well and incubated at 37°C for 15 min.

Absorbance of test and standard against blank was read in a spectrophotometer at 505 nm.

Calculations:

$$\text{Glucose in mg\%} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

3.2.8 THERAPEUTIC TRIALS:

Anemic calves after being treated for the probable cause of anemia were divided into three groups each consisting of six animals, of which two groups were given therapy for anemia while another group served as untreated control. Six healthy calves were assessed initially clinically and hemato-biochemically which served as healthy control group.

3.2.8.1 Selection of donor

Six apparently healthy cows weighing around 350kg and which were free from any external, internal and hemoprotozoan infections were selected as donors.

3.2.8.2 Cross matching of blood

Matching and cross matching of blood samples between donors and recipients was carried out according to the method of Jain (1986) prior to every transfusion.

3.2.8.3 Dosage and administration :

The dosage was calculated based on the percentage of haemoglobin present in the recipient and the percentage to which it should be raised i.e. upto 75% of the normal level. The amount of blood to be transfused for the animal was arrived at as per the method of Hammonds (1969) and Nambi (1996)

$$\frac{*40 \times \text{Body weight in pounds}}{100} = \text{ml of blood required to raise the haemoglobin level by 1\%}.$$

* Since an animal contains about 40ml of blood per pound of body weight

Blood was administered through external jugular vein (plate 6) using the infusion set which was meant for blood transfusion (containing a nylon net filter). Blood was transfused @ 60-80 drops/min as per Sharma et al. 1991.

3.2.8.4 Untreated control (Group I)

This group comprised of six anemic calves, and were not given any therapy for anemia.

3.2.8.5 Transfusion with fresh blood (Group II)

The six anemic calves of this group II were transfused with the required quantity of blood collected from six different donor cows in CPD-A blood bags. (Plate 6)

3.2.8.6 Hematinic therapy (group III)

In the animals of this group, hematinic therapy was carried out as suggested by Siegmund (1983) i.e administration of parenteral iron followed by oral hematinics. Parenteral iron was given in the form of Imferon, an iron dextran injection @3.4mg/gm of Hb deficiency/ Kg weight as per siegmund (1983) and was given in divided doses intramuscularly three times, on every alternate day. The parenteral administration of iron was followed by oral haematinic given in a dose range of 0.5-2.0 grams daily for a week.

To have parity and to eliminate the dietary influence on the recovery from anemia a similar dietary schedule was followed in all the three groups as mentioned below. Calves of group II were being fed with paddy straw, green fodder and concentrate mixture regularly. Hence green fodder and concentrate mixture were added to the diet (paddy straw) of calves of group III while they received hematinic therapy for anemia for a period of 12 days besides during their anti coccidial therapy.

Calves of group I which did not receive any therapy for anemia were also fed with green fodder and concentrate mixture besides the regularly fed paddy straw (for the same duration as that of calves of group III). The animals of group II and III were monitored on day zero i.e. before institution of therapy for anemia and on days 1, 2, 3, 5, 10 and 20 following blood transfusion and completion of hematinic therapy. Animals of untreated control were also monitored on similar days as that of hematinic group.

3.2.9 STATISTICAL ANALYSIS:

The statistical analysis was carried out as per the methods described by Snedecor and Cochran (1989). The tests employed in the present study included students' t' test and ANOVA one way classification.

CHAPTER IV

4. RESULTS

The results of incidence of anemia, its probable etiology, clinical symptomatology, hemato-biochemical changes and therapeutic aspects of anemia in calves of white cattle are presented in this chapter.

4.1 INCIDENCE OF ANEMIA:

About 105 blood samples, which included 46 samples of the calves from three different hospitals and 59 from organized dairy farms were processed for haemoglobin and packed cell volume to find out the anemic status during the period from January to June, 2003. Out of the total 105 calves, 26 of them had a haemoglobin and PCV concentration ranging from 6.8 to 7.6g/dl and 19 to 21% respectively and were considered to be frankly anemic. Thus, the overall incidence of anemia was found to be 24.76%. Later the percentage of incidence with respect to different age groups and breed was also analyzed and presented in Table 1a and 1b. The results revealed that the incidence of anemia was 30%, 27.91% and 15.63% (Table 1a) (Fig. 1a) in the calves of age groups 0-4, 4-8 and 8-12 months respectively, while the incidence was 22.73, 25 and 25.64% in HF cross, Jersey cross and non descriptive breeds (Table 1b) (Fig. 1b) of calves respectively.

4.2 PROBABLE ETIOLOGY

Exploration of probable etiology responsible for anemia revealed that coccidiosis (Plate 1), tick infestation (Plate 2), a diet of milk and certain obscure factors were the causes of anemia in 53.85%(14/26), 30.77%(8/26), 7.67%(2/26) and 7.67%(2/26) of the anemic calves respectively.

4.3 CLINICAL ANEMIA:

Therapeutic aspects of anemia were investigated in the present study for which the anemic calves after being treated for the etiology of anemia were divided into three groups randomly each consisting of 6 animals. The calves of untreated control group (Group I) were not treated for anemia, while 6 calves (Group II) received blood transfusion (Plate 6) and another 6 calves (Group III) received hematinics parenterally followed by oral route. Clinical symptomatology, hematological changes and biochemical analyses before and at regular intervals after therapy for anemia were studied. The calves of healthy control group were mostly selected from organized dairy farms and those animals present in and around the premises of Tirupathi for comparison.

4.3.1 SYMPTOMATOLOGY

The animals of healthy control group were active, bright, alert and in good physical condition with normal appetite and lustrous skin coat. The

visible conjunctival mucous membrane was pink in colour. Their faecal and blood smear examination revealed absence of any parasitic ova and hemoprotozoans.

All the calves of untreated control (Group I) and hematinic group (Group III) after being treated for the probable etiology of coccidiosis exhibited certain general symptoms like moderate anorexia, weakness, dullness, lethargy, depression (Plate 5) with slightly pale conjunctival mucous membrane. Rough hair coat and unsteady gait was observed in 2 calves of untreated control. Rough hair coat was also observed in 2 calves of group III.

In calves of blood transfusion group (Group II), the general symptoms observed were similar to that of the previous two groups i.e. they were weak, dull, moderately anorectic, lethargic, depressed and had a slightly paler conjunctival mucous membrane. The physical body condition of these calves was comparatively better than the calves of group I and III. Here the primary etiology, tick infestation was successfully treated with deltamethrin.

Monitoring of animals of group I during the study period revealed that there was a partial improvement in appetite and general activity by day-3. The gait in the two affected animals improved by 10th day of study with pink

conjunctiva, while the rough hair coat showed only slight improvement by the end of the study.

Calves of group II following blood transfusion became alert and active with pink mucous membrane by next day. Their appetite partially improved by day-1 and became completely normal by 3rd day. Following completion of hematinic therapy, the calves were observed to be bright and alert with normal appetite on day one. However the mucous membranes turned pink by day five. The rough hair coat in the two calves showed a considerable improvement by day ten.

4.3.1.1 TEMPERATURE

The mean temperature of healthy control group was 101.57 ± 0.19 (Table 2). The mean temperature of untreated control group was found to be $101.97 \pm 0.14^\circ\text{F}$ (Table 4) on zero day of anemia and there was no significant change in the mean temperature values till the end of the study period.

Calves of group II showed a mean pre therapeutic value of 102.80 ± 0.12 , and after transfusion with fresh blood, the mean temperature decreased significantly ($P < 0.01$) by day-10 with a value of 102.33 ± 0.08 by day 10 and was maintained almost at that level even on day 20.

In calves of hematinic group the mean pre therapeutic temperature was found to be 102.60 ± 0.16 . Following treatment with the hematinics, the temperature showed significant ($P<0.01$) decrease by day-10 with a mean value of 101.83 ± 0.06 which was almost maintained even on day 20.

4.3.1.2 PULSE RATE

The mean pulse rate of healthy control group was 71.33 ± 1.33 /min (Table 2). From table 4, it is seen that the mean pulse rate of untreated control group was 78.33 ± 1.31 /min on zero day of anemia. The mean pulse rate was found to have decreased significantly ($p<0.01$) by day -1 reaching 73.00 ± 0.68 which decreased further significantly ($P<0.01$) showing a mean value of 70.00 ± 0.52 by the end of the study period.

In animals of group II, the mean pre therapeutic pulse rate was 84.00 ± 0.89 /min. After transfusion with fresh blood, the mean pulse rate decreased significantly ($P<0.01$) with a value of 78.33 ± 0.80 by day-1 which was maintained around this level till day-20.

In the hematinic group of animals the mean pre therapeutic pulse rate was found to be 83.83 ± 1.97 . Upon treatment with hematinics the mean pulse

rate decreased significantly ($P<0.01$) by day-1 with a value of 75.17 ± 1.70 . The value showed a further significant ($P<0.01$) decrease by day-5 reaching 69.50 ± 0.96 and persisted around this till the end of the study.

4.3.1.3 RESPIRATION RATE

The mean respiratory rate recorded in healthy control group was $45.67\pm 1.58/\text{min}$ (Table 2). From the given table no 4, it was observed that the mean respiratory rate of group I was found to be 51.00 ± 1.24 on zero day of anemia, which decreased significantly ($P<0.01$) by day-3 with a mean of 45.33 ± 1.12 and was maintained around that level till the end of the study.

Calves of blood transfusion group showed a mean pre therapeutic respiratory rate of 58.00 ± 1.86 . Following transfusion with fresh blood, the mean respiratory rate decreased significantly ($P<0.01$) with a value of 51.33 ± 1.52 by day-2, and the mean continued to decrease till the end of the study which reached a value of 48.67 ± 1.23 .

In the calves of hematinic group, the mean pre therapeutic respiratory rate was found to be 56.17 ± 1.56 . Following completion of treatment with hematinics, the mean respiratory rate value showed a significant ($P<0.01$)

decrease by day-1 with a value of 49.17 ± 1.56 . The rate showed a further significant ($P < 0.01$) decrease by day 10 and 20 with a mean value of 40.17 ± 0.98 and 39.17 ± 0.75 respectively.

4.3.2 HAEMATOLOGICAL OBSERVATIONS:

4.3.2.1 HAEMOGLOBIN (Hb)

In healthy control calves, the mean haemoglobin recorded was 11.37 ± 0.2 (Table 2). From the table no 5, it is seen that the mean haemoglobin value of the group of untreated control was 7.47 ± 0.15 g/dl on zero day of study period of anemia and it was found that the mean haemoglobin reached 8.03 ± 0.4 by day one with a significant ($P < 0.01$) difference and was maintained around that level till day ten. The mean value significantly ($P < 0.01$) increased further and reached 8.97 ± 0.16 by day-20.

Animals of group II showed a mean pre therapeutic value of 7.02 ± 0.12 . Following transfusion with fresh blood, the mean haemoglobin increased significantly ($P < 0.01$) with a value of 10.27 ± 0.16 and persisting around that level till day three. There after the mean haemoglobin started declining (Fig. 2) and reached a significantly ($P < 0.01$) low level by day ten and twenty

showing values of 8.98 ± 0.17 and 8.60 ± 0.17 respectively, however these values were

significantly ($P<0.01$) higher compared to pre therapeutic mean haemoglobin value.

In animals of hematinic group, the mean pre therapeutic haemoglobin value was 6.98 ± 0.22 . Following treatment with hematinics over a period of 12 days, the mean haemoglobin value showed a significant ($P<0.01$) increase by day one with a value of 8.28 ± 0.16 , which continued to increase gradually reaching 8.93 ± 0.17 by day five. The mean haemoglobin value showed a further significant ($P<0.01$) increase by day ten and twenty exhibiting mean values of 9.90 ± 0.18 and 10.57 ± 0.2 respectively.

4.3.2.2 PACKED CELL VOLUME (PCV)

The mean PCV recorded in healthy control group was 32.67 ± 0.4 (Table 2). The mean PCV of the untreated control as per table 5, was found to be $20.17\pm 0.48\%$ on zero day of anemia which reached to the mean PCV 21.6 ± 0.21 by day one showing a significant ($P<0.01$) difference. The mean value significantly ($p<0.01$) increased further (Fig. 3) and reached a value of 23.17 ± 0.31 by day three, which was maintained around this level up to day-10. By the end of study there was a further significant ($p<0.01$) improvement as the value reached 26.00 ± 0.52 by day-20.

Calves of group-II showed a mean pre therapeutic value of $19.33 \pm 0.42\%$. After transfusion with fresh blood, the mean PCV increased significantly ($P < 0.01$) with a value of 30.17 ± 0.54 by day-1 and were maintained up to day-3. Thereafter the value started decreasing and reached a significantly ($P < 0.01$) low level by day-10 and 20 with a mean value of 25.83 ± 0.65 and 25.00 ± 0.37 respectively, but these were found to be significantly ($P < 0.01$) higher when compared to pre therapeutic value.

In calves of hematinic group, the mean pre therapeutic PCV value was found to be 20.24 ± 0.75 . Following treatment with parenteral iron followed by oral hematinics, the mean PCV value showed a significant ($P < 0.01$) increase by day-1 with a value of 27.12 ± 0.71 . The mean continued to increase gradually reaching 28.24 ± 0.89 by day-5. The value showed a further significant ($P < 0.01$) increase by day-10 with a mean value of 31.22 ± 0.68 and was maintained till the end of the study.

4.3.2.3 TOTAL ERYTHROCYTE COUNT (TEC)

The mean TEC recorded in healthy control was 7.45 ± 0.17 (Table 2). From the table no 5, it is evident that the mean TEC value of untreated control was found to be 5.82 ± 0.15 on zero day of anemia. It was observed that the

mean TEC value increased significantly ($P<0.01$) by day-2 with a mean of 6.27 ± 0.13 . This was maintained around that level up to day-5. The mean value significantly ($P<0.01$) increased further and reached 6.85 ± 0.10 by the end of the study.

Animals of group II showed a mean pre therapeutic value of 6.01 ± 0.15 . Following transfusion with fresh blood, the mean TEC increased significantly ($P<0.01$) with a value of 7.40 ± 0.20 by day-1 and was maintained around that level till day-10. Thereafter, the mean TEC value started declining and reached a value of 6.86 ± 0.14 by day-20. However, this value was significantly ($P<0.01$) higher when compared to pre therapeutic value. (Fig. 4)

In the calves of hematinic group, the mean pre therapeutic TEC was found to be 5.84 ± 0.17 . After treatment with parenteral and oral hematinics, the mean TEC value showed a significant ($P<0.01$) increase by day-1 with a value of 6.53 ± 0.16 . The mean continued to increase gradually till day-10 which reached a value of 7.03 ± 0.18 . The mean TEC value showed a further significant ($P<0.01$) increase by day-20 exhibiting a value of 7.42 ± 0.07 .

4.3.2.4 ERYTHROCYTE INDICES:

4.3.2.4.1 MEAN CORPUSCULAR VOLUME (MCV)

The mean MCV noticed in healthy control calves was 43.9 ± 0.63 (Table 2). As per the table no 6, it was observed that the mean MCV of untreated control was found to be 34.71 ± 0.60 on zero day of anemia. The mean MCV did not change significantly ($P < 0.01$) till the end of the study.

Calves of group II showed a mean pre therapeutic value of 32.22 ± 0.53 . After transfusion with fresh blood, the mean MCV increased significantly ($P < 0.01$) by day -1 with a value of 40.92 ± 1.35 and persisted around that level till day-3. There after the mean MCV values started declining and reached a significantly ($P < 0.01$) low level by day-20 with a value of 36.52 ± 0.93 , However, this value was found to be significantly ($P < 0.01$) higher compared to pre therapeutic mean value.

In animals of hematinic group, the mean pre therapeutic MCV value was 33.07 ± 1.10 . After treatment with hematinics, the mean MCV showed a significant ($P < 0.01$) increase by day-1 with a value of 37.28 ± 0.84 . The mean MCV continued to increase gradually reaching to a value of 40.13 ± 0.95 by day-5, which showed a further significant increase by day-10 exhibiting a

mean value of 42.36 ± 0.73 and was almost maintained at that level till the end of the study.

4.3.2.4.2 MEAN CORPUSCULAR HAEMOGLOBIN (MCH)

In healthy control calves the mean MCH recorded was 15.26 ± 0.1 (Table 2). As per the table no 6, it is seen that mean MCH value of untreated control was 12.86 ± 0.27 on zero day of anemia and there was no significant ($P < 0.01$) change observed in the MCH value till the end of the study.

Animals of group II showed a mean pre therapeutic MCH value of 11.70 ± 0.22 . Following transfusion with fresh blood, the mean MCH increased significantly ($P < 0.01$) with a value of 13.92 ± 0.43 by day-1. This value was maintained around that level till the end of the study.

In animals of hematinic group, the mean pre therapeutic MCH value was 11.78 ± 0.25 . Following treatment with hematinics, the mean MCH value showed a significant ($P < 0.01$) increase by day-5 with a value of 13.10 ± 0.32 which was almost persistent at that level till the end of the study period.

4.3.2.4.3 MEAN CORPUSCULAR HAEMOGLOBIN

CONCENTRATION (MCHC)

The mean MCHC observed in healthy control calves was 34.8 ± 0.25 (Table 2). The mean MCHC value of group I was found to be 37.04 ± 0.53 on zero day of anemia (Table 6) and it reached to 34.71 ± 0.40 by day-10 revealing a significant ($P < 0.01$) difference.

In animals of group II which received blood transfusion there was a significant ($p < 0.01$) decrease observed from pre therapeutic mean value of 36.34 ± 0.66 to 33.14 ± 0.21 by day-3. And a significant ($p < 0.01$) increase by day- 10 with a mean of 34.82 ± 0.34 which was almost persisting at that level on day-20 also.

In animals of group III which received hematinics, there was a significant ($p < 0.01$) decrease from the pre therapeutic mean of 35.79 ± 0.88 to 33.63 ± 0.48 by day-1 which was maintained around that level till the end of the study.

4.3.3 BIOCHEMICAL OBSERVATIONS:

4.3.3.1 TOTAL PLASMA PROTEIN

The mean plasma protein recorded in healthy control group was 7.85 ± 0.1 (Table 3). The mean total plasma protein values in the calves of group I, II and III are depicted in Table 7, and expressed in g/dl of plasma.

The mean protein value of group I was found to be 5.86 ± 0.10 on zero day of anemia and this reached to 6.52 ± 0.14 by day-1 exhibiting a significant ($P < 0.01$) difference. This persisted till day-5 with a mean value of 6.88 ± 0.15 . The mean value further increased significantly ($P < 0.01$) by day-10 with a value of 7.21 ± 0.13 which was maintained till day-20.

In animals of group II which received blood transfusion, significant ($P < 0.01$) increase was observed from pre therapeutic mean value of 6.32 ± 0.15 to 6.95 ± 0.13 by day 1. There after the mean protein value increased till the end of the study, but was statistically insignificant.

In animals of group III which received hematinics there was a significant ($P < 0.01$) increase from the pre therapeutic mean of 5.97 ± 0.17 to 6.83 ± 0.11 by day-1. There was a further significant ($P < 0.01$) increase on day-5 with a mean protein value of 7.33 ± 0.10 , which was persistently maintained till the end of the study.

4.3.3.2 PLASMA ALBUMIN

In calves of healthy control group, the mean plasma albumin recorded was 3.78 ± 0.05 (Table 3). It was evident from the table no 7, that the mean albumin level of untreated control on zero day of study period was significantly ($P < 0.01$) lower (2.94 ± 0.04). However there was a significant ($P < 0.01$) increase in albumin level by day-1 (3.23 ± 0.01) and these levels were almost maintained till day 3. Further, the albumin level increased significantly ($P < 0.01$) by day-5 and 20 with mean values of 3.39 ± 0.04 and 3.69 ± 0.04 respectively.

In blood transfusion group, the pre therapeutic mean plasma albumin level was 2.79 ± 0.07 . After transfusion with fresh blood the albumin level significantly ($P < 0.01$) increased up to 3.31 ± 0.01 by day-1. The value showed a further significant ($P < 0.01$) increase by day-10 with a mean value of 3.66 ± 0.05 which was maintained even on day-20.

In the animals which received hematinics, the mean albumin significantly ($P < 0.01$) increased up to 3.29 ± 0.06 by day-1 from a pre therapeutic mean of 2.75 ± 0.03 and this was maintained up to day-3. Further,

albumin level was significantly ($P < 0.01$) increased on day -5 and day-20 with a mean of 3.52 ± 0.04 and 3.82 ± 0.04 respectively.

4.3.3.3 ALBUMIN-GLOBULIN RATIO

In calves of healthy control the mean A/G ratio observed was 0.93 ± 0.01 (Table 3). The mean albumin/globulin ratio of untreated control as per table 7, was found to be 0.88 ± 0.04 on zero day of anemia, which did not differ significantly during the study period.

Calves of group II which showed a mean pre therapeutic value of 1.14 ± 0.08 did not differ significantly ($P < 0.01$) after transfusion with fresh blood at any time during the study period.

In the calves of hematinic group the mean pre therapeutic A/G ratio was found to be 1.04 ± 0.05 . After treatment with hematinics there was no significant ($P < 0.01$) change in the A/G ratio till the end of the study.

4.3.3.4 SERUM IRON

The mean serum iron values in calves of group I, II and III are depicted in Table 8 and expressed as ug/dl.

The mean serum iron value recorded in healthy control group was 122.54 ± 2.77 (Table 3). The mean serum iron value of untreated control was found to be 75.50 ± 2.67 (Table 8) on the zero day of anemia and this reached 86.85 ± 2.52 by day one revealing a significant ($P < 0.01$) difference. This level was almost persistent till day-10, showing a further significant ($P < 0.01$) difference and reaching to a mean of 99.36 ± 1.53 by the end of day-20.

In the calves of blood transfusion group, there was a significant ($P < 0.01$) increase observed from pre therapeutic mean value of 72.69 ± 0.87 to 101.86 ± 1.76 by day-1. There after, the mean continued to increase (Fig. 5) and reached to 106.37 ± 1.41 and 107.88 ± 1.26 by day-10 and 20 respectively showing a significant ($P < 0.01$) variation.

In animals of group III which received hematinic therapy for anemia, there was a significant ($P < 0.01$) increase from the pre therapeutic mean of 69.66 ± 0.99 to 105.27 ± 2.11 by day-1 which was maintained till day-3. There after, there was a further significant ($P < 0.01$) increase in the mean serum iron value on day-5, 10 and 20 with mean values of 114.75 ± 1.96 , 122.16 ± 1.88 and 130.30 ± 2.19 respectively.

4.3.3.5 SERUM CALCIUM

In calves of healthy control, the mean serum calcium recorded was 8.78 ± 0.05 (Table 3). As per table 8, the pre therapeutic calcium level in untreated control was 8.04 ± 0.08 on zero day of anemia and this revealed a significant ($P < 0.01$) increase only at the end of the study with a mean value of 8.48 ± 0.08 .

The mean serum calcium level in blood transfusion group before treatment was 7.82 ± 0.16 . The calcium level was significantly ($P < 0.01$) increased to 8.42 ± 0.18 by day-1 after transfusion with fresh blood and the level was maintained till the end of the study.

In hematinic group, the pre therapeutic mean serum calcium level was 8.14 ± 0.07 and after hematinic therapy, a significant ($P < 0.01$) improvement in the mean value could be observed only on day-20 with a mean of 8.84 ± 0.16 .

4.3.3.6 PLASMA GLUCOSE

The mean plasma glucose recorded in healthy control was 57.05 ± 0.52 (Table 3). From the table no 8, it is seen that the mean glucose value of untreated control was 48.95 ± 0.82 mg/dl on zero day of anemia and the mean glucose value increased significantly ($P < 0.01$) by day-1 reaching to

52.73±0.61 and was maintained around that level till day-5. The mean value significantly ($P<0.01$) increased further and reached to 56.88±0.66 by the end of the study

Animals of group II showed a mean pre therapeutic value of 46.27±1.33. After transfusion with fresh blood, the mean glucose increased significantly ($P<0.01$) with a value of 58.13±1.08. The mean continued to increase (Fig. 6) gradually reaching 57.90±0.52 by the end of study.

In the calves of hematinic group, the mean pre therapeutic glucose value was 47.88±1.07. After treatment with hematinics, the mean glucose values showed a significant ($P<0.01$) increase by day-1 with a value of 56.74±1.40. The mean glucose continued to increase gradually reaching 59.47±0.74 by day-20.

4.3.4 THERAPEUTIC TRIALS

The anemic calves of group II were treated with fresh blood and that of group III were treated with hematinics. The clinical, hematological and biochemical changes were recorded at regular intervals for 20 days in both the treated as well as untreated control.

4.3.4.1 TRANSFUSION WITH FRESH BLOOD (GROUP II)

Calves of this group during anemic stage (0 day) were dull, depressed, lethargic with mild pale mucous membranes and moderate anorexia. By next day of therapy i.e. on day-1 there was considerable improvement in the general activity of the animal, alertness and marked improvement of the colour of visible mucous membranes with partially improved appetite. However, the appetite became completely normal by 3rd day of study. Both the pulse and respiratory rates significantly ($p < 0.01$) decreased following transfusion with fresh blood (Table 4).

The hematological values *viz.*, Hb, PCV, TEC, Erythrocyte indices (MCV, MCH, MCHC) were found to be significantly ($P < 0.01$) low in these calves during anemia when compared to healthy control. There was a rise in all these parameters by day -1 except for MCHC which slightly decreased and again increased on 10th day of study.

Serum iron, glucose, serum calcium, total plasma protein and plasma albumin which were found to be significantly ($P < 0.01$) low in the anemic calves have significantly ($p < 0.01$) increased by day 1 after blood transfusion, While A/G ratio did not show any significant change through out the experimental period.

4.3.4.2 HEMATINIC THERAPY (GROUP III)

Calves of group III were given iron dextran (imferon) at the rate of 3.4 mg/gram haemoglobin deficient/kg body weight in divided doses intramuscularly on alternate days for three occasions, followed by oral hematinics for a week.

In anemic condition, all 6 calves were moderately anorectic, dull, depressed and lethargic, with slightly pale mucous membranes (conjunctiva). Two calves showed rough hair coat. After completion of the hematinic therapy there was a considerable improvement in appetite, general appearance of the animals, which appeared bright and alert with improved soundness of the animal by day one, pink mucous membrane by day-5 and a considerable improvement in hair coat by day-10.

There was a significant ($p < 0.01$) decrease in temperature by 10th day of study. There was significant ($p < 0.01$) reduction in the pulse and respiratory rates.

The hematological values *viz.*, Hb, PCV, TEC and erythrocytic indices (MCV, MCH, MCHC) which were 6.98 ± 0.22 , 20.24 ± 0.95 , 5.84 ± 0.17 and 33.07 ± 1.10 , 11.78 ± 0.25 , 35.79 ± 0.88 before therapy have significantly

($p < 0.01$) increased by day one after completion of therapy with parenteral and oral hematinics in a period of 12 days. By the end of study period these mean values reached highest i.e. Hb (10.57 ± 0.2), PCV (32.47 ± 0.42), TEC (7.42 ± 0.07), MCV (42.63 ± 0.57) and MCH (14.03 ± 0.23). But the mean MCHC values decreased significantly ($p < 0.01$) and was recorded as 33.02 ± 0.31 .

Serum iron, glucose, serum calcium, total plasma protein and plasma albumin which were significantly ($P < 0.01$) low during anemia were found to have significantly ($p < 0.01$) increased by day 1 after treatment, while serum calcium showed a significant ($P < 0.01$) increase only at the end of day-20. However, A/G ratio did not show any significant change through out the experimental period.

CHAPTER V

5. DISCUSSION

Anemia is a condition characterized physiologically by insufficient circulating haemoglobin (Hb) and clinically by reduced exercise tolerance and pale mucous membranes. It could be due to decreased production, increased destruction, or rapid loss of red blood cells (Fraser et al, 1991). Anemia is more commonly a sign of some other disease (Jain, 1986). Treating the primary disease alone will not make the animal immediately totally normal as anemia affects the well being and performance of the animal.

In the present study an attempt was made to find out the incidence of anemia and its probable etiology in the calves of white cattle. Later after eliminating the etiological factors of anemia an attempt was made to treat the anemic calves by employing blood transfusion in one group of calves and hematinic therapy in another and their efficacy was compared. Six animals which were treated for the etiology of anemia alone thus serving as untreated control were also monitored.

To find out the incidence of anemia, thorough clinical examination was carried out on about 105 calves (which consisted of those presented for treatment at different hospitals and that of organized dairy farms) and their blood samples were analyzed for haemoglobin and packed cell volume. The calves with a haemoglobin of less than 8g/dl and with a PCV of <25% were considered as frank cases of anemia. Jain (1986) stated that anemia is indicated when one or more of the red cell parameters (PCV, haemoglobin and RBC count) is below the normal level for the age, sex and breed of the species concerned. Of the three red cell parameters, the packed cell volume provides a simple, quick and accurate means of detecting anemia and a PCV of <25% indicates anemia in cattle. An animal may be considered anemic. If the haemoglobin level drops less than 8gm% (Schalm, 1986). The incidence of anemia as revealed from the present study was 24.67 %.(26/105) (Table 1) (Fig 1). Analysis of this data with relation to age revealed that the incidence rate was highest i.e. 30% in calves of 0-4months age, followed by 27.91% in 4-8months aged calves and 15.63% in 8-12 months aged calves (Table 1a) (Fig. 1a), while the incidence was 22.73 and 25.64 per cent in HF cross, Jersey cross and non-descriptive breeds of calves respectively. (Table 1b) (Fig. 1b)

Perusal of literature on the incidence of anemia in different parts of the world reveal that in most cases it was linked with the incidence of certain

specific diseases rather than anemia itself. The incidence of anemia associated with kale feeding in cows was found to be 6.4%, 5.1% and 8.1% in different herds (Greenhalgh et al., 1972). An incidence of 30-35% was reported in calves purchased from Sweden (Mollerberg 1975).

Regarding the etiology of anemia in the present study, it was found that coccidiosis, tick infestation, a diet of milk and certain obscure factors were the causes of anemia in 53.85%(14/26), 30.77%(8/26), 7.67%(2/26) and 7.67%(2/26) of cases. All the calves showing coccidial oocysts in their faeces (Plate 1) (oocysts are sporulated due to delay in processing) were from a single herd being fed only with paddy straw. It was noticed that one calf of the same herd (that was not taken for study) was successfully treated for an acute form of coccidiosis just prior to the period of study in the university hospital, while one each died of acute and chronic form of coccidiosis respectively coupled with malnutrition soon after anemia and the associated infestation were diagnosed. Though most animals in a group become infected, only a minority develop clinical disease, thus a high rate of sub clinical infection may occur, where the animals show inferior growth rate and chronic anemia only (Radostits et al., 1994). The most typical syndrome of coccidiosis is chronic or sub clinical disease in groups of growing animals. In light infections the

oocysts are present in normally formed faeces and with a reduced feed efficiency (Aiello, 1998).

Thus in the present study the cause of anemia in the calves with poor weight gain and showing coccidial oocysts was ascribed to chronic coccidiosis.

All the tick infested calves were from an organized dairy farm. Ticks are active blood suckers and may even cause death due to anemia (Radostits et al., 1994). As the tick infestation was very severe and chronic as revealed from the history and faecal and blood smears were negative, the cause of anemia in these calves was ascribed to tick infestation (Plate 2). Milk, being a poor source iron might have caused anemia in two calves aged 35 and 40 days, as they were found out to be fed with milk alone. In two animals no specific cause could be ascertained for anemia. Hence it may be likely that they recovered from any parasitism but left with anemia.

The anemic calves were divided into three groups (each consisting of 6) i.e. group I (untreated control), group II (blood transfusion) and group III (Hematinic therapy). In the animals of untreated control and hematinic group the symptoms observed were weakness, lethargy, dullness, moderate anorexia

and poor physical condition in all the calves and rough hair coat and unsteady gait in two calves of group I. Rough hair coat was observed in two calves of group III also. The conjunctival mucous membrane was only slightly paler in all the animals. In these calves coccidiosis was found to be the cause of anemia. The inferior food conversion associated with coccidiosis (Chapman et al., 1973), prolonged iron deprivation (Underwood and Suttle, 1999) and poor feeding in these animals might have contributed to the poor physical condition of these animals. Symptoms of lethargy and moderate anorexia could be ascribed to prolonged iron deprivation (Underwood and Suttle, 1999). Symptoms of anorexia and depression during anemia were also reported by Radostits *et al.* (1994) and Martinovich and Woodhouse (1971).

The general symptoms of anemia exhibited in calves of group I & III were also seen in the calves of group II. But the physical body condition of these animals was comparatively better than the calves of group I & III.

The calves of group I and III showing coccidial infection were treated with 10mg of amprolium per kg body weight orally for 5 days, after which their faeces was devoid of oocysts. During the period of study in the untreated control i.e in group I there was partial improvement in appetite and general activity by day-3. Gait was normal by 10th day of study with pink conjunctiva,

while the rough hair coat showed only slight improvement by the end of the study. The improvement noticed in this group might be the effect of correction of primary etiology.

A single application of deltamethrin could make the calves free from the tick infestation in two days, after which they were given blood transfusion. Calves following blood transfusion became alert and active with pink mucous membranes by next day. Their appetite improved partially by day one and became completely normal by 3rd day. The immediate improvement noticed in this group could be a reflection of supplementation with fresh healthy blood. Following completion of hematinic therapy, the calves were observed to be bright and alert with normal appetite on day one. However the mucous membranes turned pink by day five. The rough hair coat showed considerable improvement by day-10.

The mean temperature of healthy calves was $101.57 \pm 0.19^\circ\text{F}$ (Table 2), while the mean pre treatment values in the three groups ranged from 101.97 ± 0.14 to 102.8 ± 0.12 (Table 4) which were found to be normal and were in accordance with the reports of Parthasarthy et al (1985). Kelly (1974) mentioned a normal temperature range of 101.5 - 103.5°F with an average being 102.5°F in calves' up to one year age. In anemic calves of control group the

mean temperature was maintained as that of the pre therapeutic stage (101.97 ± 0.14) throughout the course of study. However there was a significant ($p < 0.01$) decrease after treatment in the temperatures of group II and group III calves, from a mean of 102.80 ± 0.12 & 102.60 ± 0.16 to 102.37 ± 0.10 and 101.83 ± 0.04 by day ten respectively. Rane et al., (2003) reported a non significant decrease in the mean temperature following plasma transfusion in dogs.

Animals of healthy control group revealed a pulse rate of 71.33 ± 1.33 per min (Table 2). Which was almost agreeable with Kelly (1974), the pre therapeutic mean pulse rate in all the three groups of animals was found to be significantly ($P < 0.01$) higher i.e. 78.33 ± 1.31 (Group I), 84.00 ± 0.89 (Group II) and 83.83 ± 1.97 (Group III) (Table 4). This might be due to the development of fast and forceful beating of heart in an effort to improve the cardiac output and maintain arterial blood pressure (Schalm et al, 1975).

There was a significant ($p < 0.01$) decrease in pulse rate observed in all the three groups by day one of the treatment (Table 4) and finally (20th day) reached the mean values of 70 ± 0.52 (Group I), 78.67 ± 0.99 (Group II) and 68.83 ± 0.40 (Group III) respectively and the mean pulse rate by the end of the study in groups I and III reached the level of healthy control group. The

reduction in the pulse rate noted by the next day of blood transfusion might be due to immediate correction of anemia. The steady and gradual correction of anemia in the hematinic group might have resulted in gradual improvement of pulse rate in this group. Srivastava and Pandey (1993) reported a significant decreasing trend in the pulse rate which almost reached the normal by day three in anemic calves following blood transfusion.

The calves of healthy group exhibited a mean respiratory rate of 45.67 ± 1.58 /min (Table 2) which is almost in accordance with Kelly (1974).

The mean respiratory rate of all the calves in the three groups were found to be significantly higher ($P < 0.01$) in their anemic status with a mean rate of 51.00 ± 1.24 (Group I), 58.00 ± 1.86 (Group II) and 56.17 ± 1.56 (Group III) (Table 4). This observation of increased Respiratory rate was due to progressive development of hyperventilation facilitating oxygenation and elimination of carbon dioxide in anemic animals (Schalm et al, 1975). Srivastava and Pandey (1993) reported an increased respiratory rate during anemia in calves.

After therapy the mean respiratory rate decreased significantly ($p < 0.01$) in all the three groups by day one itself and finally (20th day) reached mean

values of 44.00 ± 0.52 (Group I), 48.67 ± 1.23 (Group II) and 39.17 ± 0.75 (Group III). Thus the mean respiratory rate by the end of the study in group I and II was nearer with the value of healthy control while in group III was at a lesser level. The decrease in the respiratory rate of group I calves was found to be slightly less when compared to the other two groups which were treated for anemia and this slight lesser decrease in untreated control could be attributed to the persistence of anemic status. Srivastava and Pandey (1993) also reported a significantly decreased respiratory rate in their study of therapeutic evaluation of cattle blood stored in citrate phosphate dextrose at 4°C for safe transfusion.

The mean haemoglobin in healthy control was 11.37 ± 0.2 g/dl (Table 2) which was in accordance with the reports of Shetty et al., (1977), Jain et al., (1982), Srivastava and Pandey (1992), Jain (1993) and Kaneko et al (1997). The mean haemoglobin (g/dl) in anemic calves of all the three groups of the present study was found to be 7.47 ± 0.15 (Group I), 7.02 ± 0.12 (Group II) and 6.98 ± 0.22 (Group III) (Table 5) and these values were significantly ($P < 0.01$) lower than healthy control value. Animals may be considered anemic if their haemoglobin values drop less than 8gm% (Schalm et al, 1986). This decreased haemoglobin observed in the present study in which anemia was confirmed were in accordance with the findings of Dhar and Gautam (1979)

and Sharma et al (1991) where the mean haemoglobin values of 7g/dl and 7 ± 0.1 g/dl were reported during anemia but slightly higher than those reported by Srivastava and Pandey (1993) and Pradhan et al (1993).

The calves of untreated control exhibited a significantly ($P < 0.01$) improved mean haemoglobin values by day 1, however these values were found to be lowest when compared to the other two groups which were treated for anemia. This mild increase in the anemic control group can be attributed to the correction of the primary etiology which might have prevented further blood loss.

In group II calves following blood transfusion the mean haemoglobin was found to have increased significantly ($p < 0.01$) by next day itself (Table 5 and Plate 2) and was maintained at that level up to day 3, which was a reflection of transfusion of blood. But later the haemoglobin decreased significantly ($p < 0.01$) from 5th day to the end of the study which could be attributed to the removal of the infused RBCs from the circulation, as it was mentioned that the survival time of infused red blood cells in the recipients is 72hrs (Radostitis et al, 1994). However following transfusion the mean haemoglobin did not reach the mean values of healthy control even during its peak stage. Parthasarathy et al (1985) reported improvement in the

haemoglobin values of calves after blood transfusion. Srivastava and Pandey (1992) reported a gradual improvement of haemoglobin where the normal levels were attained by day 11.

The calves which were subjected to hematinic therapy, also exhibited a significant ($p < 0.01$) improvement in their haemoglobin level by day 1 itself and the haemoglobin showed a steady increase reaching nearer to the healthy control values by the end of the study. This increase could be attributed for the arrest of blood loss due to the removal of etiology and iron therapy given over a period of 12 days, thus allowing sufficient time for better assimilation of iron and its availability for the synthesis of haemoglobin. Pradhan et al (1993) studied the role of hematinic to maintain the haemoglobin after blood transfusion in cattle and reported that blood transfusion along with oral administration of haematol was superior. Samuel and Srinivasan (2001) studied the effect of hematinics in bovine anemia and reported a significant improvement in the haemoglobin levels of cows treated with both parenteral followed by oral hematinics. Abhiramy et al. (2003) gave parenteral iron in three divided doses for a week in anemic dogs and observed a significant increase in the haemoglobin values after a week though they did not reach the values of healthy control.

The mean packed cell volume in the healthy control group of calves was $32.67 \pm 0.49\%$ (Table 2) which was agreeable with Jain (1986), Aiello (1998) and Samuel and Srinivasan (2001). Significantly ($P < 0.01$) decreased values of mean pre therapeutic packed cell volume were observed in the groups I, II and III as 20.17 ± 0.48 , 19.33 ± 0.42 and 20.24 ± 0.95 (Table 5) respectively. Similar findings were reported by Dhar and Gautam (1979), Verma and Quasimuddin (1981) in anemic animals. Jain (1986) cited that anemia in cattle is indicated by a PCV of less than 25%. Ramin (2000) reported a much less value of 16.8% in anemic cows. Though the post therapeutic packed cell volume increased significantly ($p < 0.01$) in all the three groups, this increase in group I animals was found to be lowest on par with the other two groups. Similar reasons as that for haemoglobin can be attributed for this mild increase of packed cell volume in animals of group I.

Following transfusion, the animals of group II exhibited a significant ($P < 0.01$) increase in their mean packed cell volume with a value of 30.17 ± 0.54 . This increase in mean packed cell volume was around this level for the first three days (Fig. 3), following which there was a gradual decline. This immediate increase and a later decline in the mean PCV might be due to the limited period of survivability of infused red blood cells in the recipients (Radostits et al., 1994). Parthasarathy et al (1985) reported an enhanced

hematocrit by next day of transfusion in cow calves and this increased hematocrit was found to have decreased steadily by 48hrs and 72hrs post transfusion. Contrary to this, Srivastava and Pandey (1992) reported a gradual improvement reaching the normal levels by day 11. Sufiun (2001) reported improvement in PCV following blood transfusion in calves.

The calves of hematinic group also showed a significant ($p < 0.01$) increase in PCV by day 1 itself with a mean of $27.12 \pm 0.71\%$. This increasing trend continued till day 20 ($32.47 \pm 0.42\%$) reaching the level of healthy control. Samuel and Srinivasan (2001) reported the mean packed cell volume of 22.66 ± 0.6 and 26.00 ± 0.52 before and one month after therapy for anemia.

The mean total erythrocyte count (million/cmm) observed in healthy control was 7.45 ± 0.17 (Table 2). Jain (1986) mentioned the normal average of total erythrocyte count in cattle to be $7.00 \times 10^6/\text{ul}$. However Aiello (1998) cited a range of 5.00 to $10.00 \times 10^6/\text{ul}$ in healthy cattle. The three groups of calves exhibited a significantly ($P < 0.01$) lower mean pre therapeutic total erythrocyte count i.e. 5.82 ± 0.15 (group I), 6.01 ± 0.15 (group II) and 5.84 ± 0.17 (group III) during anemia (Table 5). Similar values were reported by Dhar and Gautam (1979) i.e. $5.38 \times 10^6/\text{cmm}$ during the acute phase of theilerial infection and Parthasarathy et al., (1985) i.e. $5.06 \pm 1.26 \times 10^6/\text{ul}$ in anemic calves. Even

lower values were quoted by Srivastava and Pandey (1993) and Pradhan et al (1993) in anemic animals.

Though the post therapeutic TEC increased significantly ($p < 0.01$) in all the three groups (Fig. 4), this increase in group I animals was found to be lowest when compared to the other two groups, while it reached the level of healthy control value i.e. on day two and twenty in the groups II and III respectively. The mild increase of mean TEC noticed even in calves of group I could be due to better erythropoietic activity resulting due to comparatively better availability of iron as its loss was prevented by removal of primary etiology.

In group II, the calves exhibited a significantly ($P < 0.01$) improved mean TEC i.e 7.40 ± 0.20 by next day of blood transfusion. The observation of this improvement persisting up to day three and declining thereafter could be attributed to the short living nature (3 to 4 days) of transfused red blood cells (Peter, 1999). Parthasarathy et al (1985) observed a hike in mean TEC by 24 hrs after blood transfusion, but this increase decreased steadily during 48 hrs and 72 hrs post transfusion. Contrary to this Sharma et al (1991) and Srivastava and Pandey (1992) reported a gradual improvement where the mean TEC was highest on day-12 and 11 respectively.

Calves of group III also exhibited a significantly ($P<0.01$) improved mean TEC (6.53 ± 0.16) by day one after completion of hematinic therapy. Periodical monitoring of these animals revealed that the erythrocytic count continued to increase, reaching almost the level of healthy control by the end of the study. This might be attributable to the gradually improving erythropoietic activity. Samuel and Srinivasan (2001) reported the mean TEC of 4.62 ± 1.12 in cows during anemia which reached to 5.0 ± 0.26 after a month following the administration of parenteral and oral hematinics.

The mean erythrocytic indices like MCV (fl), MCH (pg) and MCHC (g%) in healthy control were found to be 43.9 ± 0.63 , 15.26 ± 0.14 and 34.8 ± 0.25 (Table 2) respectively. These were found to be similar with the findings of Dhar and Gautam (1979), Jain (1986) and Aiello (1998).

The mean pre therapeutic MCV in all the three groups was observed to be decreased significantly ($P<0.01$) i.e. 34.71 ± 0.6 (Group I), 32.22 ± 0.53 (Group II) and 33.07 ± 1.10 (Group III) during anemia (Table 6). In iron deficiency anemia there will be reduction in MCV, (Schalm, 1965). In untreated control no significant ($p<0.01$) changes were observed in the mean MCV through out the period of study, While in group II and group III there was a significant ($p<0.01$) increase observed by day 1 itself.

In blood transfusion group the increase was observed for the first three days. While in hematinic group the increase was continuous up to day 20. Parthasarthy et al (1985) reported no significant changes in the MCV values after blood transfusion.

The mean pre therapeutic MCH in all the three groups was observed to be at a significantly ($P<0.01$) lower level with means of 12.86 ± 0.27 (Group I), 11.70 ± 0.72 (Group II) and 11.78 ± 0.25 (Group III) (Table 6). In iron deficiency anemia, MCH may diminish far more than anticipated for the reduction in MCV. The mean MCH value did not vary significantly ($P<0.01$) in the untreated control during the period of study, while in the other two groups it increased significantly ($P<0.01$). And this significant ($P<0.01$) increase was persistent through out the study period in both the groups. Parthasarathy et al (1985) reported no significant changes in the MCH values after blood transfusion.

The mean pre therapeutic MCHC in all three groups was 37.04 ± 0.53 (Group I), 36.34 ± 0.66 (Group II) and 35.79 ± 0.88 (Group III) (Table 6). The mean MCHC decreased significantly ($p<0.01$) by day I in group II and group

III calves while the decrease was observed by day 3 in group I calves. These values were further maintained till the end of the study in all the three groups.

The healthy calves showed a mean plasma protein value of 7.85 ± 0.1 g/dl (Table 3). These values were almost similar to the findings of Srivastava and Pandey (1992 b), Jain (1993), Kaneko (1997) and Aiello (1998).

The mean total plasma protein was found to range from 5.86 to 6.32 g/dl (Table 7) in the calves of three groups during anemia showing a significant ($P < 0.01$) difference from healthy calves. Hemorrhagic anemia associated with chronic coccidiosis could have resulted in the hypoproteinemia noticed in the untreated control and hematinic group of calves. And the chronic severe tick infestation noticed in the calves of blood transfusion group might have produced hemorrhagic anemia and thus the concomitant hypoproteinemia in these calves. plasma protein concentration becomes reduced in haemorrhage due to loss of plasma from the vascular system, followed by hemodilution as body fluids enter to restore the blood volume (Schalm, 1970).

Ramakrishna (1985) reported that calves fed with concentrate mixture, paddy straw and green fodder exhibited significantly ($P < 0.01$) improved

haemoglobin, packed cell volume, total erythrocyte count and total plasma proteins while being treated for anemia with parenteral and oral hematinics when compared to calves fed with paddy straw alone. Protein in adequate amounts is important for a normal rate of hemoglobin synthesis and erythrocyte production. As a supportive measure protein supplementation may be of benefit to a patient convalescing from anemia particularly if hypoproteinemia is a concurrent problem (Adams, 2001). Protein supplementation has been known to benefit an animal while it has been treated for anemia (Samuel and Srinivasan, 2001).

It was observed that the calves of blood transfusion group were being regularly fed with concentrates apart from paddy straw and green fodder, while the other two groups were being given only paddy straw. So the better availability of nutrition in these animals may contribute to the recovery from anemia apart from blood transfusion. Hence keeping the above factors in view and to eliminate the dietary influence on therapeutic response, and to have parity in all the three groups, even the calves of group I and III were given concentrates and greens ever since they were started therapy for primary etiology till the end of the study. Calves of group I were also given the same diet for the same duration on par with hematinic group. Following this, the mean plasma protein was found to have increased significantly ($P < 0.01$) in

calves of untreated control by day one itself (6.52 ± 0.14) and later on by day ten and twenty. This increase in the protein content could be due to curtailing of blood loss through correction of primary etiology and supplementation of diet with concentrate mixture. The increase in the mean plasma protein of blood transfusion group might be due to supplementation with fresh blood. The improved protein status by day one in the hematinic group could be due to the effect of removal of primary etiology of anemia, supplementation with concentrate mixture and improved appetite exhibited by these animals. However by the end of the study the plasma protein level could not reach to the level of healthy control in any of the groups.

The calves of healthy control group revealed plasma albumin content of 3.78 ± 0.05 g/dl (Table 3). Which is agreeable with Aiello (1998) where a range of serum albumin of 2.8 to 3.9 g/dl was given in healthy bovines. The values noted in the present study were almost similar to Kaneko et al., (1997).

Groups of I, II and III exhibited a significantly ($P < 0.01$) decreased mean plasma albumin levels i.e. 2.94 ± 0.04 , 2.79 ± 0.07 and 2.75 ± 0.03 (Table 7) respectively. Samuel and Srinivasan (2001) also reported the mean serum value of 2.56 ± 0.22 and 2.88 ± 0.34 g/dl in anemic cows. Kerr (1989) stated that chronic hemorrhagic anemia resulted in progressive

hypoproteinemia particularly hypoalbuminemia. Brar and Nauriyal (1993) also reported a significant ($P<0.01$) decrease in plasma albumin after induction of experimental anemia.

Correction of primary etiology of anemia and improved nutritional status might have significantly ($P<0.01$) increased the plasma albumin levels in untreated control. Calves of blood transfusion group also exhibited a significant ($P<0.01$) improvement in the mean plasma albumin levels by next day following transfusion which could be due to supplementation of blood.

The calves of hematinic group revealed a significantly ($P<0.01$) improved mean plasma albumin level by day one itself. And the albumin levels continued to increase in all the three groups reaching the level of healthy control by the end of study. Supplementation of diet through concentrate mixture, correction of primary etiology and improved appetite might be responsible for the improved albumin status noticed in these animals.

The mean A:G ratio exhibited by the healthy animals was 0.93 ± 0.01 (Table 3) which was in accordance with Kaneko et al (1997) and Aiello (1998). During anemic state the mean plasma A/G ratio was found to range from 0.88 ± 0.04 to 1.14 ± 0.08 (Table 7) in the calves of three groups which did

not differ significantly ($P < 0.01$) through out the period of study in any of the groups.

The mean serum iron level was 122.54 ± 2.77 ug/dl in healthy calves (Table 3) which is agreeable with the findings of Saroor (1980) and Kaneko (1997). A significantly ($P < 0.01$) decreased mean pre therapeutic serum iron values were observed in groups I, II and III i.e. 75.5 ± 2.67 , 72.69 ± 0.87 and 69.66 ± 0.99 (Table 8) respectively during anemia. Similar findings were reported by Volker *et al.* (1972) and Gamel (1982). Samuel and Srinivasan (2001) reported the mean serum iron values in anemic calves to be 61.63 ± 2.63 and 63.78 ± 2.52 in their study.

The decreased mean serum iron level observed in calves of group I & III could be due to chronic blood loss associated with coccidiosis. Similarly the chronic heavy tick infestation noticed in calves of group II might have caused this iron deficiency anemia. Many ticks are active blood suckers and may cause even death from anemia (Radostits, 1994). Chronic tick infestation and coccidiosis can cause chronic blood loss with a deficiency of iron (Jain, 1986). The decrease in serum iron observed in chronic blood loss might be due to depletion of body iron stores as it might have been utilized by bone marrow for haemoglobin production in order to be incorporated in the red

blood cells. Untreated control calves exhibited a significantly ($p < 0.01$) improved mean serum iron level by day one of study period which could be due to prevention of loss of blood through the correction of primary etiology.

The improvement in the mean serum iron value in the calves of blood transfusion seen on the next day following blood transfusion was much more significant and persistent through out the period of study with a mild gradual increase (Fig. 5). The serum iron present in the transfused blood coupled with arrest of blood loss arising due to therapy of primary cause of anemia might have contributed to the increase of serum iron level in this group.

From the table no. 8 it can be seen that calves of group III also exhibited a marked increase in the mean serum iron level by day-1 of completion of therapy; It was also observed that the mean serum iron values continued to increase reaching the highest i.e. the values of healthy control group by the end of the study (Fig. 5) which could be attributed to the gradual absorption of administered iron. The mean serum iron reached the level of healthy control only in group III.

The calves of healthy group revealed a mean serum calcium value of 8.78 ± 0.05 (Table 3) which is agreeable with Radostits (1994). Aiello (1998)

mentioned the range of serum calcium to be 8-10.5 mg/dl in healthy ox. The mean serum calcium in the three anemic groups ranged from 7.82 ± 0.08 to 8.14 ± 0.16 (Table 8) showing a significant ($P < 0.01$) decrease compared to the healthy control values. Because approximately one half of blood calcium circulates bound to plasma proteins like albumin, hypoproteinemia can result in a significant decrease in total serum calcium values (Adams, 2001). The decreased calcium levels observed in groups I and III may also partially be due to chronic blood loss and increased excretion of calcium. The calcium excretion was observed to be more, though only for a few days, in a study period in coccidial and nematode infected goats rather than in nematode infections alone (Frandsen, 1982). Srivastava and Pandey (1993) reported a mean serum calcium level of 6.42 ± 0.02 mg/dl and 7.21 ± 0.17 mg/dl after inducing acute hemorrhagic anemia where the mean calcium levels were 10.46 ± 0.2 mg/dl before bleeding. Pradhan et al (1993) reported the calcium levels of 6.1, 6.25 and 6mg/dl in theileria affected calves.

Calves of group I did not exhibit significant variation in mean serum calcium initially but showed a significantly ($P < 0.01$) improved calcium levels by 20th day. The calves of blood transfusion group exhibited a significantly ($P < 0.01$) improved calcium levels by next day of transfusion which could be due to supplementation with fresh blood. Pradhan et al (1993) also reported

significantly ($P < 0.01$) increased calcium levels in theileria affected cattle after blood transfusion. Srivastava and Pandey (1992) reported a significant ($P < 0.01$) improvement of calcium status in the anemic calves following plasma transfusion. The calves of hematinic group exhibited a very mild increase of calcium levels following therapy initially but with a significantly higher values on 20th day which might be due to prevention of excessive excretion of calcium and blood loss through the correction of primary etiology and improved appetite.

The mean plasma glucose value noticed in the healthy animals was 57.05 ± 0.52 mg/dl (Table 3) which was in accordance with Srivastava and Pandey (1992 b) and Kaneko et al.,(1997).

Groups I, II and III exhibited a mean plasma glucose value of 48.95 ± 0.82 , 46.27 ± 1.33 and 47.88 ± 1.07 (Table 8) respectively, during anemic phase which were significantly less when compared to healthy control group. This decreased glucose level could be due to loss of appetite noticed in anemic animals. The slight improvement in appetite in the untreated control might have increased the mean glucose level.

Both the groups treated for anemia also revealed a significantly ($P < 0.01$) improved glucose levels by day one of therapy. However the increase noticed in blood transfusion group could be due to the supplementation of blood, while in hematinic group it could be due to marked improvement in the appetite. In all the three groups, the mean plasma glucose values reached the level of healthy control. (Fig. 6) Pradhan et al., (1993) reported a pre therapeutic blood glucose of 30.4 mg/dl and following transfusion this value was found to have reached 48.5 mg/dl. There was also a gradual increase in the glucose level on the subsequent days of study. Sakai (1980) and Brar and Nauriyal (1994) reported a significant ($P < 0.01$) increase in blood glucose levels in dogs after blood transfusion.

From a therapeutic perspective, anemia can be conveniently attributed to acute or chronic blood loss or chronic inflammation (Peter, 1999). However in domestic animals chronic blood loss arising due to internal or external parasitism is a common occurrence which may result in iron deficiency anemia as each ml of blood contains 0.5mg of iron (Jain, 1986).

In the present study, the anemia was diagnosed as iron deficiency anemia arising due to chronic blood loss based on the etiology and

haematobiochemical findings. Hence therapy was instituted with blood transfusion and parenteral and oral hematinics.

A possible benefit of blood transfusion in chronic blood loss is the provision of rapidly utilizable haemoglobin because these animals may have total depletion of their iron stores (Peter, 1999).

In ancient Indian medicine Lauha Pharma (calcinied-iron) has been used in iron deficiency anemias and in the early nineteenth century Blaud developed his famous Blaud's pill consisting of ferrous sulfate and potassium carbonate for anemia. In the present study parenteral iron dextran followed by the commonly used oral hematinics consisting of ferrous, copper and cobalt sulfates were used in anemic calves and the response was monitored at regular intervals.

Severe iron deficiency paradoxically causes intestinal mal-absorption of iron in dogs and humans thus it is recommended that iron deficiency be treated first by administration of parenteral preparation followed by continued oral supplementation for approximately one month or for as long as the cause of iron imbalance might otherwise continue (Adams, 2001).

Calves of group II were given fresh blood at a flow rate of 60-80 drops/min. Sharma et al (1991) have administered blood at a similar flow rate of 60-80 drops/min, while Nambi (1996) suggested that about 120 drops of blood/min can be given in cattle. Parthasarthy (1985) transfused blood @ 50 drops/min. All the calves were exhibiting symptoms like weakness, lethargy, moderate anorexia and only slightly pale mucous membrane during anemia. Following transfusion the calves became alert and active by next day as blood transfusion improves the oxygen carrying capacity of blood permitting the patient to function more normally (Jain, 1986). The improvement in conjunctival mucous membrane noticed by day one of therapy could be due to significantly ($P < 0.01$) improved haemoglobin and total erythrocyte status of blood as a result of transfusion. Restoration of completely normal appetite by 3rd day of transfusion in these animals could be due to improved iron status as there is loss of appetite in prolonged iron deprivation (Underwood and Suttle, 1999).

The mean temperature decreased by 10th day and pulse rate by next day and respiratory rate by second day in this group. The improved cardiac output, oxygen carrying capacity of blood associated with correction of anemia with blood transfusion might have resulted in the decreased respiratory and pulse rates.

Haematological examination of these animals revealed significantly ($P<0.01$) improved haemoglobin, packed cell volume and total erythrocyte count by next day. However the improved values persisted up to day three and thereafter started declining during the study period. The persistence of a significantly ($P<0.01$) improved haemoglobin, packed cell volume and total erythrocyte values for three days following blood transfusion and later gradual decline might be due to short survival period of infused red blood cells in the recipient (Kalfelz and Whitlock, 1973). Immediate correction of anemia with transfusion of blood might have lead to improved MCV & MCH.

Though the hematological parameters exhibited a declining trend, the clinical improvement in appetite and general activity was seen through out the period of study. The major beneficial affect due to blood transfusion in cattle is derived from transfused plasma present in the blood (Radostits et al., 1994). The calves in the present study also exhibited a significant ($P<0.01$) increase in total plasma protein, albumin, glucose, serum calcium and iron by next day. The improvement noticed in all these parameters by next day of transfusion persisted throughout the study period. This improvement in biochemical parameters could be due to the presence of these substances in the transfused blood (Srivastava and Pandey, 1993) and to a lesser extent due to correction of etiology of anemia.

The calves of group III were treated with parenteral iron and hematinics as per Siegmund (1983) (at a dose rate of 3.4mg/ gram deficient of haemoglobin/ kg. Body weight). Thus parenteral iron was administered in three divided doses on alternate days followed by oral hematinics for a week. The dose rates of parenteral iron for ruminants are empirical and additional treatments with iron may be given when indicated by the MCHC and total haemoglobin concentration (Peter, 1999). Samuel and Srinivasan (2001) treated anemia in cows with parenteral iron sorbitol with folic acid and vitamin B-12 @ 10ml, intra muscularly twice a week and oral hematinics containing 10 grams of ferrous sulfate, 200mg of copper sulfate and 40mg of cobalt sulfate daily for a month.

The improvement in the general activity and appetite exhibited by these calves by day one of completion of hematinic therapy could be a reflection of the efficacy of the treatment. Improvement in the haemoglobin status was reflected by the improved conjunctival mucous membrane. The calves exhibited significantly ($P<0.01$) decreased mean body temperature, pulse and respiratory rates by days ten, one and one respectively.

A significant ($P<0.01$) improvement observed in the haematological parameters by day one might have contributed to the improvement of pulse

and respiratory rates. Though the mean haemoglobin, packed cell volume and total erythrocyte count improved significantly ($P < 0.01$) by day one following therapy in this group, the increase in these values was highest at the end of the therapy. This showed that formation and release of RBCs into circulation was gradual and probably depend upon the availability of iron and protein.

The improvement in the MCV and MCH could be due to pre mature release of macro erythrocytes into the circulation due to stimulation of erythropoiesis with the better availability of the iron (Perman and Schall, 1983). The decrease in MCHC noticed by day one might be due to the presence of macroerythrocytes which do not have a full complement of haemoglobin relative to cell size (Perman and Schall, 1983).

The significant ($P < 0.01$) improvement in plasma protein and albumin by day one in these calves might be due to prevention of stoppage of blood loss by correction of primary etiology and supplementation of the diet with concentrate mixture. A positive nitrogen balance and additional protein diet may produce considerable amounts of new plasma protein (Miller *et al.*, 1947).

The significant ($P < 0.01$) increase in the serum iron noticed might be reflection of absorption of parenterally administered iron and the gradually increased serum iron values could be due to gradual absorption of administered iron. Following iron dextran injection some amount is absorbed within one to three days while a certain portion is absorbed through macrophages in a delayed manner in 4-14 days (Adams, 2001).

The calves showed a significantly ($P < 0.01$) increased glucose and calcium levels by day one and 20 respectively.

From the present study it was evident that the clinical symptomatology like weakness, lethargy and anorexia improved only partially in the anemic control group. These symptoms improved immediately in the blood transfusion group and by day-1 of completion of hematinic therapy in the calves of group III. Similarly, improvement in the pulse and respiratory rate in the treated groups was slightly better compared to animals of untreated control.

Though all the three groups exhibited significantly ($P < 0.01$) improved haemoglobin during the study period, it was noticed that the improvement in the anemic control was lowest, in the blood transfusion group it was at peak

level for three days and in hematinic group it was gradual. Thus by the end of the study, the percent increase of haemoglobin in groups III, II and I were 51, 22.5 and 20 percent respectively. Similar trend was noticed even with the packed cell volume with their percent increase in groups III, II and I as 60.42, 29.33 and 28.90 respectively. Like wise even with TEC the highest percent increase by the end of the study period was noticed in hematinic group (27.05), but followed by anemic control (17.70) and blood transfusion (14.14).

The mean MCV and MCH increased significantly in both the treated groups and the percent increase of MCV in hematinic and blood transfusion groups by the end of the study period was 28.91 and 13.35 respectively, while the percent increase in MCH in these groups was 19.1 and 7.35. The mean MCHC decreased in both the treated groups.

The improvement in mean serum iron level was lowest in the anemic control compared to the two treated groups. The percent increase of mean serum iron in groups III, II and I was 87.05, 48.41 and 31.60 respectively.

All the three groups exhibited significantly increased plasma glucose levels by day-1 and showed little variation by the end of the study as the

percent increase of plasma glucose values were 24.21, 25.14 and 16.20 in the groups III, II and I respectively.

Calves of group I and III revealed a significant increase in serum calcium level only by day-20, while it was by day-1 in calves of group II. All the three groups exhibited a significantly ($P < 0.01$) improved protein and albumin status during the period of study, showing a very little variation by the end of the study.

When the cost of both forms of treatment was taken into consideration it was found in the present study that undertaking blood transfusion in a 70kg calf with 7 g/dl of haemoglobin costed Rs-380, while hematinic therapy in the same calf would cost Rs-55.

It is evident that with the correction of primary etiology responsible for anemia alone, the clinical symptomatology like inappetance and general activity, hematobiology and serum iron did not improve to a desirable level, thus necessitating therapy for anemia.

In the present study it can be seen that when anemic calves with mean haemoglobin of 7.02 ± 0.12 g/dl were treated with blood transfusion, there was

an immediate improvement in hematology but a declining trend in the values was noted after three days. However, there was a persistence of clinical improvement in the appetite and general activity and serum iron status. Therapy with blood transfusion worked out to be expensive with a need for technical expertise.

It is also evident that with the hematinic therapy in calves with a mean haemoglobin content of 6.98 ± 0.22 the improvement of hematological parameters was significantly progressive without any decline during the study period besides the persistency of improved appetite and general activity. Improvement in serum iron was also progressive with a peak level by the end of the study. And hematinic therapy was observed to be far less expensive without requiring any extra technical skills.

Hence from the present study it is concluded that in calves, anemia was mostly associated with external or internal parasitism and in anemia with a haemoglobin of about 7g%, hematinic therapy can be undertaken in order to bring about a desirable response. As blood transfusion provided immediate relief from anemia (as evident from the study) blood transfusions, though expensive may be more useful in critical anemia with a much less haemoglobin.

CHAPTER - VI

SUMMARY

In the present study an investigation was made to find out the incidence of anemia in calves and to study its probable etiology during the study period, clinical, hemato-biochemical changes and therapeutic aspects. A total of 105 calves of different age groups, breeds, nutritional status and clinical condition from among the cases presented at three different hospitals and of organized dairy farms during the study period were screened for anemia by clinical examination and analyzing their haemoglobin and PCV to know the incidence of anemia. Calves having haemoglobin below 8g/dl and a PCV of less than 25% were considered as frankly anemic and the incidence rate of such condition was 24.76% (26/105). Age wise incidence of anemia indicated that highest incidence was noticed in 0-4months age (30%), followed by in 4-8 months (27.91 %) and 8-12 months (15.63 %). Similarly breed wise incidence observed was 25.64%, 25% and 22.73% in non-descriptive calves, Jersey cross and HF cross respectively. To find out the probable etiology of anemia detailed anamnesis was obtained, the diet given was explored and the anemic calves were subjected to thorough clinical examination with their blood and fecal samples examined for any haemoprotozoa and internal parasitism respectively.

It was found that coccidiosis, tick infestation, a diet of milk and certain obscure factors were the causes of anemia in 53.85% (14/26), 30.77 % (8/26), 7 % (2/26) and 7 % (2/26) of anemic calves respectively.

Anemic calves after being treated for primary etiology were divided into three groups each consisting of six, of which the calves of group I served as untreated control, while that of group II and III were treated for anemia with blood transfusion and hematinics respectively. A group of 6 perfectly healthy calves served as healthy control. All the calves were subjected to detailed clinical and hemato-biochemical examination during the period of study i.e. on zero day of anemia and on days 1,2,3,5,10 and 20 following therapy.

The calves of healthy control were bright and alert with normal appetite and pink conjunctival mucous membrane. All the anemic calves of three groups exhibited certain general symptoms viz., dullness, depression, lethargy, moderate anorexia and slightly pale conjunctival mucous membrane. Unsteady gait was seen in 2 calves of group I and rough hair coat in two calves of group I and III. After therapy there was marked improvement of the symptoms of the calves of group II and III. But the improvement in group I was moderate

where general activity and appetite improved only partially and rough hair coat showed only slight improvement by the end of the study.

After therapy there was a significant ($P<0.01$) decrease in pulse and respiratory rates in all the three groups but with the greater reduction in the hematinic group.

The mean hematological values of reference healthy control group were Hb(11.37 ± 0.2), PCV(32.67 ± 0.49), TEC(7.45 ± 0.17), MCV(43.9 ± 0.63), MCH(15.26 ± 0.1) and MCHC(34.8 ± 0.25). the anemic calves of three groups before treatment had Hb, PCV, TEC, MCV, MCH and MCHC as 7.47 ± 0.15 , 20.17 ± 0.48 , 5.82 ± 0.15 , 34.71 ± 0.60 , 12.86 ± 0.27 and 37.04 ± 0.53 (Group I); 7.02 ± 0.12 , 19.33 ± 0.42 , 6.01 ± 0.15 , 32.22 ± 0.53 , 11.70 ± 0.22 & 36.34 ± 0.66 (Group II) and 6.98 ± 0.22 , 20.24 ± 0.95 , 5.84 ± 0.17 , 33.07 ± 1.10 , 11.78 ± 0.25 & 35.79 ± 0.88 (Group III) respectively. All these values were found to be significantly ($P<0.01$) low when compared to healthy control, except for MCHC which did not exhibit any significant ($P<0.01$) difference.

It was found that there was an immediate significant improvement in the hematological parameters including erythrocytic indices (except MCHC) in the calves of group II which persisted for 3 days after which there was a

gradual decline. However, by the end of the study the mean hematological values were still at a higher level when compared to pre therapeutic values. The improvement in hematology in the calves of group III was gradual, reaching the highest level by the end of the study. In calves of group I, though there was significant ($P<0.01$) increase in the Hb, PCV and TEC, the percent increase by the end of the study was markedly less than that of therapeutic groups. The erythrocytic indices in group I did not exhibit any significant ($P<0.01$) difference during the period of study except for MCHC which significantly ($P<0.01$) decreased by day-10.

By the end of the study period (day-20) the increased values in Hb, PCV and TEC in the three groups were 8.97 ± 0.16 , 26.00 ± 0.52 & 6.85 ± 0.10 (Group I); 8.60 ± 0.07 , 25.00 ± 0.37 & 6.86 ± 0.14 (Group II) and 10.57 ± 0.2 , 32.47 ± 0.42 & 7.42 ± 0.07 respectively. Similarly the erythrocytic indices (MCV, MCH and MCHC) by the end of the study period were 37.94 ± 0.56 , 13.07 ± 0.24 & 34.50 ± 0.38 (Group I); 36.52 ± 0.93 , 12.56 ± 0.24 & 34.45 ± 0.75 (Group II) and 42.63 ± 0.51 , 14.03 ± 0.23 & 33.02 ± 0.31 (Group III) respectively.

The mean values of plasma glucose, serum iron, total plasma protein, plasma albumin, plasma albumin-globulin ratio and serum calcium in healthy control calves were 57.05 ± 0.52 mg/dl, 122.54 ± 2.77 ug/dl, 7.85 ± 0.1 g/dl,

3.78±0.05 g/dl, 0.93±0.01 and 8.78±0.05 mg/dl respectively. All these biochemical parameters in the anemic calves of three groups were significantly ($P<0.01$) low when compared to healthy control. The mean albumin globulin ratio did not differ significantly ($P<0.01$) through out the period of study in any group.

The pre therapeutic values of plasma glucose, total protein, albumin, serum iron and serum calcium in the three groups were 48.95±0.82, 75.50±2.67, 5.86±0.10, 2.94±0.04 & 8.04±0.08 (Group I) ; 46.27±1.33, 72.69±0.87, 6.32±0.15, 2.79±0.07 & 7.82±0.16 (Group II) and 47.88±1.07, 69.66±0.99, 5.97±0.17, 2.75±0.03 & 8.14±0.07 (Group III) respectively.

In calves of group I though the biochemical parameters significantly ($P<0.01$) increased by the end of the study, the percent increase when compared to the treated groups was found to be less. After therapeutic trials in group II and III, there was significant ($P<0.01$) improvement in all these biochemical parameters. But the percent increase of serum iron was highest in the calves of group III (87.05) followed by group II (48.41) and group I (31.60).

By the end of the study period i.e. day-20 the mean plasma glucose, total protein, albumin, serum iron and serum calcium values in the three groups were 56.88 ± 0.66 , 99.36 ± 1.53 , 7.24 ± 0.15 , 3.69 ± 0.04 & 8.4 ± 0.08 (Group I); 57.90 ± 0.52 , 107.88 ± 1.26 , 7.20 ± 0.12 , 3.71 ± 0.05 & 8.56 ± 0.15 (Group II) and 59.47 ± 0.74 , 130.30 ± 2.19 , 7.38 ± 0.05 , 3.83 ± 0.05 & 8.84 ± 0.16 (Group III) respectively.

From the above results it is evident that both blood transfusion and hematinic therapy in anemic calves brought improvement in all the values of clinical, hematological and biochemical parameters. But the improvement in hematology was immediate and peak levels were attained for 3 days with blood transfusion, while it was progressive with peak levels noted at the end of the study with hematinic therapy. However even though in untreated control there was significant improvement observed during the period of study the percent improvement by the end of study, when compared to therapeutic groups was markedly low. It was also noticed that in the calves treated with hematinics, haemoglobin, PCV, TEC, plasma albumin and serum iron levels reached almost to that of the healthy control values by day 20, while in calves treated with transfusion, TEC and plasma albumin reached almost to that of healthy control values by day 2 and 20 respectively. However in both the groups the degree of clinical improvement was almost similar.

From the present study it is evident that correction of primary etiology of anemia alone could not bring about clinical and hemato biochemical improvement to a desirable level thus necessitating therapy for anaemia. It can be concluded that anemia in calves was mostly associated with external or internal parasitism and when the mean haemoglobin was around 7g/dl, hematinic therapy which was cheaper and did not require technical skills can be undertaken to bring about an adequate response, while blood transfusions though expensive and require technical expertise may be more useful (as immediate relief from anemia was evident) in criti

**T - TEST PERFORMED TO PRE-THERAPEUTIC
MEANS OF ALL THE GROUPS**

	Healthy control	Untreated control	Blood transfusion group	Hematinic group
Hb	11.37 ± 0.2	7.47 ± 0.15*	7.02 ± 0.12*	6.98 ± 0.22*
PCV	32.67 ± 0.49	20.17 ± 0.48*	19.33 ± 0.42*	20.24 ± 0.95*
TEC	7.45 ± 0.17	5.82 ± 0.15*	6.01 ± 0.15*	5.84 ± 0.17*
MCV	43.9 ± 0.63	34.71 ± 0.60*	32.22 ± 0.53*	33.07 ± 1.10*
MCH	15.26 ± 0.14	12.86 ± 0.27*	11.70 ± 0.22*	11.78 ± 0.25*
MCHC	34.8 ± 0.25	37.04 ± 0.53**	36.34 ± 0.66 ^{NS}	35.79 ± 0.88 ^{NS}
Temperature	101.57	101.97 ± 0.14 ^{NS}	102.80 ± 0.12*	102.60 ± 0.16*
Pulse	71.33	76.33 ± 1.31*	84.00 ± 0.89*	83.83 ± 1.97*
Respiratory	45.67 ±	51.00 ± 1.24*	58.00 ± 1.86*	56.17 ± 1.56*
Protein	7.85 ± 0.1	5.86 ± 0.10*	6.32 ± 0.15*	5.97 ± 0.17*
Albumin	3.78 ± 0.05	2.94 ± 0.04*	2.79 ± 0.07*	2.75 ± 0.03*
A:G	0.93 ± 0.01	0.88 ± 0.04 ^{NS}	1.14 ± 0.00**	1.04 ± 0.05 ^{NS}
Iron	122.54 ± 2.77	75.50 ± 2.67*	72.69 ± 0.87*	69.66 ± 0.99*
Calcium	8.78 ± 0.05	8.04 ± 0.8*	8.21 ± 0.16*	8.14 ± 0.07*
Glucose	57.05 ± 0.52	48.95 ± 0.82*	46.27 ± 1.33*	47.88 ± 1.07*

* (P < 0.01) Means with in a row differ significantly at 1% level

** (P < 0.05) Means with in a row differ significantly at 5% level

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