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HAEMATO BIOCHEMICAL AND THERAPEUTIC STUDIES ON SUBCLINICAL HYPOCALCAEMIA IN CROSSBRED COWS

G. AMBICA[*1] AND D.S.T. RAO[2]

[1]Contract Teaching Faculty, College of Veterinary Science, Korutla – 505 326, Andhra Pradesh, India.
[2]Director of Research, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India.

ABSTRACT

Out of 280 post parturient crossbred cows between 0 to 60 days post calving presented with the history of reduced feed intake and decreased milk yield, 106 (37.86 %) were found suffering from different subclinical production disorders. On detailed clinical examination and urine analysis, 24 cows (22.64%) were positive for subclinical hypocalcaemia and urine samples were low in calcium levels. Blood samples from study group were analyzed for haemoglobin, haematocrit and certain other parameters. Serum samples were analyzed for calcium, glucose, magnesium, phosphorous, AST, ALP, total protein and albumin and compared with that of control cows. Cows with subclinical hypocalcaemia were randomly divided in to two different groups. Group I (n=12) animals were administered Calup Gel at the rate 300g orally for one day and Rumen-FS bolus at the rate 2 boli orally twice in a day for 5 days. Group II (n=12) animals received Inj. 25% Intacal at the rate 450 ml iv for 3 days and Inj. Tribivet at the rate 10-15 ml im for 3 days. There was a significant improvement in haemoglobin and serum calcium and significant decrease in serum glucose, magnesium and AST levels after the treatment. The comparative means of healthy, group I and II cows revealed that the treatment given to group II was more effective.

KEY WORDS: Subclinical hypocalcaemia, Crossbred Cows, Haematological changes, Biochemical changes, Therapy.

G. AMBICA
Contract Teaching Faculty, College of Veterinary Science, Korutla – 505 326, Andhra Pradesh, India

Corresponding author

INTRODUCTION

Subclinical form of a disease means a condition marked by increased or decreased levels of certain metabolites inside the body without exhibiting the clinical signs or in other words aclinical to the farmer except drastic reduction in milk yield[1]. Periparturient dairy cows mostly suffer from immunosuppression and hypocalcemia. Subclinical hypocalcaemia in the postpartum cow is not an emerging disease problem, but it has been an unrecognized problem that may contribute to the development of a host of postpartum production diseases, such as mastitis, retained placenta, displaced abomasum, and ketosis[2]. Subclinical hypocalcaemia is widespread and affect on an average of 50% of all dairy cows at or near calving. In subclinical hypocalcaemia the serum calcium levels are less than 8.0 mg/dL and should be viewed as an impediment to the health of a cow[3].

MATERIALS AND METHODS

280 post parturient crossbred cows between 0 to 60 days post calving presented at Campus Hospital and Dairy experimental station of College of Veterinary Science, Rajendranagar and peri urban dairy farms located in and around Hyderabad, during the study period of 14 months, with the history of anorexia and drop in milk yield were selected and screened for subclinical hypocalcaemia by examination of urine and blood for biochemical profiles.

Urine sample from each cow was collected on day 0 (Day of collection) and on day 5 (post treatment) by stroking her perineum below the vulva, which made her to pass urine. Approximately 5 ml urine was collected from each animal in a sterile glass vial and analyzed for urinary calcium as per the procedure described by[4]; Urine p^H and Glucose by using 'URS-10' strips supplied by M/S Sri Sai Ganesh Agencies, Secunderabad with the help of 'URI-PLUS' urine analyzer supplied by M/S Rapid Diagnostics Pvt. Ltd., New Delhi.

Cows which were low in urine calcium levels were selected for the present study. They were randomly divided into two groups and given oral therapy for first group and parenteral therapy was provided for another group. To study their efficacy group I (n=12) animals were administered Calup Gel at the rate 300g orally for one day and Rumen-FS bolus at the rate 2 boli twice a day for 5 days. Group II (n=12) animals received Inj. 25% Intacal at the rate 450 ml iv for 3 days and Inj. Tribivet at the rate 10-15 ml im for 3 days. Apparently healthy animals within two months of calving formed the control group.

Blood samples were collected from jugular vein on day '0' (day of collection) and day 5 (post treatment) into vacutainers containing heparin as anti coagulant for haematological estimations and also into vaccutainers without anticoagulant for biochemical estimations. Serum vacutainers were kept undisturbed till serum separation and the serum was transferred into another test tube with the help of a sterile Pasteur pipette. The sera were subjected to centrifugation at 5000 rpm for 5 minutes to obtain clear serum. Then the serum was transferred to eppendrof tubes, labeled accordingly and maintained at 4°C. Haematological estimations viz., Haemoglobin (Hb in g %), Packed Cell Volume (PCV in %), Total Erythrocyte Count (TEC in $\times 10^6/\mu L$), Total Leucocyte Count (TLC in $\times 10^3/\mu L$), Differential Leucocyte Count (DLC in %), Mean Corpuscular Haemoglobin (MCH in pg) and Mean Corpuscular Volume (MCV in fL) were estimated on the same day of collection with the help of Humacount in the Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar and biochemical parameters were carried out using 'Star 21 Plus' semi automatic Analyzer supplied by M/S Rapid Diagnostics Pvt. Ltd., New Delhi on the same day of collection with the help of biochemical kits supplied by M/S Sri Sai Ganesh Agencies, Secunderabad. The data was subjected to statistical analysis by one way ANOVA using Statistical Package for

Social Sciences (SPSS) version 10. Differences between means were tested using Duncan's multiple comparison test and significance was set at 5 percent ($p<0.05$) and also at 1 percent ($p<0.01$). The values were represented as mean ± Standard Error.

RESULTS AND DISCUSSION

Out of 280 crossbred cows within 60 days of post calving presented with the history of reduced feed intake and decreased milk yield 106 (37.86 %) were found suffering from different subclinical production disorders. On detailed clinical examination and urine analysis, 24 cows (22.64%) were positive for subclinical hypocalcaemia. Urine samples were low in calcium levels.

The mean haemoglobin concentration in control and group I and II, before and after treatment were 9.97±1.58, 8.13±0.77 v/s 9.85±0.68 and 7.72±0.90 v/s 9.74±0.65 g% respectively. Increased levels were significant ($P<0.05$) in both groups following therapy. Low haemoglobin levels were recorded during postpartum period in hypocalcaemic cows[5]. The mean PCV in control and group I and II, before and after treatment were 45.96±1.74 and 42.63±1.46 v/s 44.52±1.17 and 43.81±0.89 v/s 45.31±0.43 g%, respectively and there was no significant difference in both groups. The mean total erythrocyte count in control and group I and II, before and after treatment were 8.23±1.83 and 8.15±2.61 v/s 8.34±2.29 and 7.85±1.23 v/s 8.02±0.40 ($\times 10^6$/ µL), respectively and no significant difference was observed in both groups. The mean total leukocyte count in control and group Ia and Ib, before and after treatment were 9.97±1.15 and 8.49±1.10 v/s 9.67±1.80 and 6.71±1.53 v/s 9.84±1.20 ($\times 10^3$/ µL), respectively and increased levels were significant ($p<0.05$) in group II. The mean granulocytes percent in control and group I and II, before and after treatment was 79.92±0.75 and 68.25±0.47 v/s 71.52±0.18 and 72.16±0.12 v/s 80.8±0.44 %, respectively. Increased levels was significant ($p<0.05$) in group II. Lymphocytes, monocytes, MCH and MCV levels were low in the affected groups compared to apparently healthy group and there was no significant difference between two groups after therapy. The present findings are in accordance with[6] who also reported moderate decreased levels of Hb, TLC, lymphocytes, MCH and MCV in the post parturient cows. The reason could be development of anaemia following postpartum due to diminished immunologic status[7].

The mean serum glucose in control and group I and II, before and after treatment were 58.10±2.96 and 64.81±0.89 v/s 58.20±0.78 and 72.37±0.73 v/s 55.20±1.14 mg/dL, respectively. The mean serum values were slightly higher in hypocalcaemia and returned to normal after therapy which is in accordance with[8] and the decreased levels were significant in group I ($P<0.05$) and in group II($P<0.01$). In the present study, a decrease in serum calcium concentration below 7.5 mg/dL was considered as subclinical form of hypocalcaemia. The mean serum calcium levels in control and group I and II, before and after treatment were 11.17±0.27 and 7.35±0.92 v/s 10.09±0.29 and 7.26±0.95 v/s 12.39±0.61 mg/dL, respectively. Increased levels were significant ($P<0.01$) in both groups. The observations are in accordance with[8,9and10]. The mean serum magnesium levels in control and group I and II, before and after treatment were 2.691±0.08 and 2.89±0.05 v/s 2.12±0.03 and 3.09±0.04 v/s 2.83±0.05 mg/dL, respectively. Increased levels were significant ($P<0.01$) in both groups. Increased serum magnesium levels in hypocalcaemia was also reported by[8,11,12and13] whereas [14]recorded variable serum magnesium concentrations in cases of hypocalcaemia. The elevated magnesium levels in typical hypocalcaemia cases might be due to increased parathyroid hormone (PTH) concentration which causes increased renal tubular reabsorption of Mg making kidneys to excrete less of the excess dietary magnesium absorbed[15]. The mean serum phosphorous levels in control and group I and II, before and after treatment were 6.27±0.22 and 4.46±0.13 v/s 5.21±0.12 and 4.67±0.15 v/s 5.50±0.08 mg/dL, respectively. Increased levels were significant ($P<0.05$) in both the groups. These findings are in accordance

with[8,13,16and17]. Decrease in serum phosphorus in hypocalcaemia was noticed prior to therapy compared to healthy control group which could be due to substantial decrease in serum inorganic phosphorus (Pi) in the early postpartum due to increased activity of parathyroid hormone (PTH) and a sudden increased excretion of phosphorous into colostrum and milk[15]. Hypophosphataemia in general may accompany hypocalcaemia the reason could be decreased plasma inorganic phosphorus levels can interfere with activation of vitamin D by inhibiting activity of renal 1 hydroxylase enzyme and leading to hypocalcaemia[18]. The mean serum AST levels in control and group I and II, before and after treatment were 98.37±3.43 and 136.40±0.40 v/s 113.89±0.32 and 154.58±0.62 v/s 112.92±0.46 IU/L, respectively. Decreased levels were significant in group I P<0.05) and II ((P<0.01) cows. These findings are in accordance with[8,19,20and21]. Increased serum AST levels can be attributed to ischemic necrosis of muscle resulting in release of contents of the enzyme into the blood[14]. There was no significant difference between two groups in the mean serum ALP levels, which is suggestive that hypocalcaemia has no effect on ALP. The mean serum total protein levels in control and group I and II, before and after treatment were 7.11±0.16 and 6.17±0.11 v/s 7.68±0.08 and 5.83±0.10 v/s 7.72±0.02 g/dL, respectively. Increased serum protein levels were significant (P<0.05) in both groups. The mean serum total protein levels were low prior therapy in both groups and increased after the therapy which is in accordance with[8]. The decreased level of plasma proteins during early lactation might be attributed to their utilization for milk synthesis[23]. The mean serum albumin levels in control and group I and II, before and after treatment were 3.46±0.07 and 2.26±0.04 v/s 2.48±0.05 and 2.13±0.02 v/s 2.85±0.03 g/dL, respectively. Increased serum levels were significant (P<0.05) in group II. In the present study, decreased serum albumin levels were recorded in both groups when compared to apparently healthy animals. However serum albumin levels reached normalcy following therapy. The above findings were in agreement with[8]. Reduced serum albumin levels could be due to the lactation stress. Hypoalbuminaemia occurs when there was excessive loss of albumin or insufficient hepatic production to meet demand. A decreased level of serum albumin is reported as a result of insufficient production or increased consumption in ruminants[24.]

Table 1
Mean haematological findings of control and Cows affected with Subclinical Hypocalcaemia

S. No	Parameter	Apparently healthy cows (n=10)	Group – I (n= 12)		Group – II (n= 12)	
			Before Treatment	After Treatment	Before Treatment	After Treatment
1.	Hb (g %)	9.97±1.58	8.13±0.77	9.85±0.68*	7.72±0.90	9.74±0.65*
2.	PCV (%)	45.96±1.74	42.63±1.46	44.52±1.17	43.81±0.89	45.31±0.43
3.	TEC (× $10^6/\mu L$)	8.23±1.83	8.15±2.61	8.34±2.29	7.85±1.23	8.02±0.40
4.	TLC (× $10^3/\mu L$)	9.97±1.15	8.49±1.10	9.67±1.80	6.71±1.53	9.84±1.20*
5.	Lymphocytes (%)	19.23±0.19	17.32±0.25	18.16±0.98	18.53±0.20	18.62±0.45
6.	Monocytes (%)	0.98±0.26	0.93±0.94	0.97±0.67	1.48±0.56	1.52±0.51
7.	Granulocytes (%)	79.92±0.75	68.25±0.47	71.52±0.18	72.16±0.12	80.8±0.44*
8.	MCH (pg)	15.14±0.52	14.23±0.40	15.14±0.81	14.38±0.62	15.45±0.69
9.	MCV (fL)	52.2±0.13	55.6±0.45	57.3±0.94	50.9±0.39	53.7±0.22

**** Significant at P < 0.05*** ***** Significant at P < 0.01***

Table 2
Mean biochemical findings of control and Cows affected with Subclinical Hypocalcaemia

S.No	Parameter	Control (n=10)	Group – I (n= 12)		Group – II (n=12)	
			Before Treatment	After Treatment	Before Treatment	After Treatment
1.	Glucose (mg/dL)	58.10±2.96	64.81±0.89	58.20±0.78*	72.37±0.73	55.20±1.14**
2.	Calcium (mg/dL)	11.17±0.27	7.35±0.92	10.09±0.29**	7.26±0.95	12.39±0.61**
3.	Magnesium (mg/dL)	2.691±0.08	2.89±0.05	2.12±0.03**	3.09±0.04	2.83±0.05**
4.	Phosphorous (mg/dL)	6.27±0.22	4.46±0.13	5.21±0.12*	4.67±0.15	5.50±0.08*
5.	AST (IU/L)	98.37±3.43	136.40±0.40	113.89±0.32*	154.58±0.62	112.92±0.46**
6.	ALP (IU/L)	68.44±4.26	75.32±0.28	70.93±0.45	78.44±0.40	76.46±0.27
7.	Total Protein (g/dL)	7.11±0.16	6.17±0.11	7.68±0.08*	5.83±0.10	7.72±0.02*
8.	Albumin (g/dL)	3.46±0.07	2.26±0.04	2.48±0.05	2.13±0.02	2.85±0.03*

*** Significant at P < 0.05** **** Significant at P < 0.01**

CONCLUSIONS

It can be concluded that for early detection of subclinical hypocalcaemia urine test was efficacious which helps in the prevention of further progression into clinical form. In the present study, group I animals were administered Calup Gel at the rate 300g orally for one day and Rumen-FS bolus at the rate 2 boli orally twice in a day for 5 days. Group II animals received parenteral therapy with Inj. 25% Intacal at the rate 450 ml iv for 3 days and Inj. Tribivet at the rate 10-15 ml im for 3 days. There was significant improvement in haemoglobin and serum calcium and significant decrease in serum glucose, magnesium and AST levels after the treatment. The comparative means of healthy, group I and II cows revealed that the parenteral therapy given to group II was more efficacious than oral therapy given to group I cows.

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