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A prospective study on haemato-biochemical aspects of subclinical ketosis in dairy cows of early lactation on Thrissur district

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Frequent monitoring of metabolic disorders during early lactation is very essential for the evaluation of successful management strategies during transition period. Major metabolic disorders associated with improper management practices during transition period includes ketosis, post-partum hypocalcaemia, retention of placenta, metritis, displacement of abomasum, and lameness (Oetzel, 2014). A successful adaptation to the existing negative energy balance is essential for the future production performance of the animal.

Subclinical ketosis (SCK) is described as an increase in beta-hydroxy butyrate (BHB) levels in the blood, plasma, or serum over the normal reference range (1.2 mmol/L), or ketonuria in a cow with no clinical indications (Andersson, 1988), is an important metabolic disorder occur due to the failure of adaptation to the existing negative energy balance. Subclinical ketosis causes severe economic impacts on production and profit in farms as reduction in milk production, prolonged calving interval, huge treatment cost, death or culling of animals. Published data regarding scientific researches on haematological and biochemical implications of SCK among cattle population in Kerala are meagre.

With these considerations present study was conducted with the objective of evaluation of haematological and biochemical parameters of subclinical ketosis affected cattle in Thrissur district of Kerala state.

Multiparous heathy cows in early lactation formed the subject of the research work. The selection of animals was based on the risk period for the occurrence of subclinical ketosis (SCK). Post-partum cows with blood BHB value of ≥ 1.2 mmol/L were considered as positive for SCK (Oetzel, 2004). Cows with blood BHB < 1.2 mmol/L were considered as normal animals. Occurrence of SCK was evaluated on 14th and 28th day. Haematology and serum biochemical analysis were conducted for SCK positive (Group I) and normal multiparous animals (Group II) comprising of 8 cows in each group on both test days.

About 2mL of blood in K-EDTA tube was collected via jugular venipuncture and estimated the following parameters viz. total leucocyte count ($\times10^3$/µL), lymphocytes ($\times10^3$/µL), monocytes ($\times10^3$/µL), granulocytes ($\times10^5$/mm^3), total erythrocyte count ($\times10^6$/µL), haemoglobin (g/dL), volume of packed red cells (VPRC) (per cent), and platelets ($\times10^3$/µL) using an automated haematology analyzer (Orphee, Mythic TM 18 Vet) within half an hour of collection of blood samples.

Blood was drawn into 4 mL capacity m-tube vacutainers coated with clot activator from eight SCK positive and eight normal animals on 14th and 28th day post-partum. Serum was separated from the clotted blood. Sera thus separated were stored at -20^{0}C until further analysis. Biochemical analysis was performed with HOSPITEX DIAGNOSTICS, MASTER T machine. Serum parameters were estimated using commercial kits supplied by SPINREACT company, Spain (Table 1).

Statistical analysis was done using SPSS version 24.0. Haematology and biochemical parameters between diseased and normal animals were compared using independent-t-test for day 14 and 28 post-partum separately. Paired t-test was used to compare parameters between 14th and 28th day for diseased and normal animals.

The mean haematological values of SCK positive (Group I) and normal animals (Group II) on day 14 and 28 post-partum are summarized in the Table 2. Statistical analysis did not reveal any significant difference in TLC between groups and within groups in day 14 and 28. A non-significant increase in TLC was noticed in group I on 14th and 28th day. The mean value of lymphocytes, monocytes and granulocytes in animals of group I were 10.6 ± 3.62, 1.04 ± 0.26 and 4.34 ± 0.91 x 10^3/µL, respectively on 14th day and 9.85 ± 3.37, 0.6 ± 0.10 and 4.14 ± 0.62 x 10^3/µL, respectively on 28th day post-partum. Statistical analysis did not reveal any significant difference between two groups during valuation period

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Table 1 List of biochemical parameters and analytical procedures

Sl.NO	Parameter	Kit	Analytical method	Reference
1	Total protein	SPINREACT	Biuret method	Gomall et al. (1949)
2	Albumin	SPINREACT	Bromocresol green method	Doumas et al. (1971)
3	Aspartate amino transferase	SPINREACT	IFCC method without pyridoxal phosphate	Bergmeyer et al. (1986)
4	Total bilirubin	SPINREACT	Diazo method	Jendrassik and Groff (1938)
5	Creatinine	SPINREACT	Jaffe's method	Fabiny and Ertingshausen (1971)

except for the significant ($p \leq 0.05$) monocytosis in group I on 28th day.

There was no significant difference in mean values of TLC, lymphocytes, monocytes, and granulocytes of diseased animals on test days when compared with normal animals in the present study. Similar results were recorded by Marutsova *et al*. (2015) and Paramesh *et al*. (2020). A non-significant leucocytosis in diseased animals might be due to other periparturient infections like mastitis and metritis in the screened population. A significant increase in mean monocyte count was noted in diseased group on 28th day. Ketotic group had a larger predominance of monocytes than normal animals in the research trial by Mezzetti *et al*. (2019) who reported activation of immune system function in all animals before they became sub clinically ketotic. The value was within the normal physiological limit range (Constable *et al*., 2017).

Total erythrocyte count of SCK animals was within their normal physiological limit (Constable *et al*., 2017). No significant difference was detected in total erythrocyte count of diseased and normal animals on 14th and 28th day post-partum. This is in accordance with the results of previous researches (Paramesh *et al*., 2020; Ali and Hassan, 2021). Statistical analysis revealed a significant reduction of haemoglobin in group I on 14th day (($p \leq 0.05$) and 28th ($p \leq 0.01$) day when compared with group II. Significant reduction ($p \leq 0.05$) was observed in mean haemoglobin value of group I animals on 14th day when compared to 28th day. Ali and Hassan (2021) reported a significant reduction in haemoglobin in cows with subclinical ketosis. Yadav *et al*. (2018) observed a significant reduction in haemoglobin concentration of SCK positive goats. The decrease in haemoglobin concentration might be attributed to oxidative stress associated with negative energy balance in peripartum as suggested by Sahoo *et al*. (2009). No significant difference was noticed in the mean value of VPRC in diseased animals when compared with normal animals on 14th day post-partum. Statistical analysis revealed a significant decrease ($p<0.05$) in mean VPRC value in diseased animals 28th day post-partum.

Mean platelet count of animals of group II were 348.63 ± 47.24 and $366.13 \pm 48.37 \times 10^3/\mu L$ on 14th and 28th day post-partum. Corresponding values of group I were 315.38 ± 69.44 and $374.75 \pm 92.41 \times 10^3/\mu L$. Statistical analysis did not reveal any significant difference in platelet count of SCK positive animals and normal animals in the present study. Similar results were recorded by Paramesh *et al*. (2020) and Marutsova *et al*. (2015).

Mean values of serum biochemical parameters of SCK positive animals (group I) and normal animals (group II) on 14th and 28th day post-partum are presented in Table 3. No significant difference in mean total protein and albumin was observed between groups and within groups on both test days. Contrary to this study, Paramesh *et al*. (2020) observed a significant decrease in total protein levels in affected animals. Protein catabolism might lead to decreased protein levels in SCK positive animals due to an increased rate of gluconeogenesis, which acts as an essential source of energy for the synthesis of milk lactose and milk protein while the animals suffer from a state of negative energy balance.

Mean values of AST in animals of group I were 74.2 ± 4.95 and 76.6 ± 4.46 IU/L, respectively on 14th and 28th day. The corresponding values in animals of group II were 89.34 ± 6.01 and 89.29 ± 7.49 IU/L, respectively. No significant difference was observed between AST values of diseased and normal animals on both 14th and 28th day post-partum in present study. Contrary to this, Paramesh *et al*. (2020) and Mohsin *et al*. (2022) recorded higher AST values in cows with SCK than in normal animals. Excess fat metabolism and subsequent deposition of fat globules in the hepatocyte, as well as leakage of enzyme in the blood circulation and fat buildup in the liver, might contributed to excessive

hepatocyte membrane permeability in post-parturient animals and therefore increase in AST values could be used as a tool for diagnosis of metabolic liver diseases. In the SCK group, AST levels spiked in the first week after calving and then remained reasonably steady over the next three weeks. Then it rose to its highest point in the fifth week post-partum, dropped drastically in the sixth week, and then rose rapidly again in the seventh week (Mohsin *et al*., 2022). The variation observed in the present study might be due to the variation in sampling time, as sampling was done only in the 2nd and 4th weeks post-partum. The mean total bilirubin of group I (0.31 ± 0.05) was significantly lower ($p \leq 0.05$) than group II (0.47 ± 0.05 mg/dL) on 14th day post-partum, but both the values were within the normal range. No significant difference noted in mean total bilirubin value of animals in group I (0.37 ± 0.04) and group II (0.32 ± 0.08 mg/dL) on day 28 post-partum. The mean value of total bilirubin of diseased animals was significantly lower ($p \leq 0.05$) than normal animals on 14th day post-partum, but within the

Table 2. Mean values of haematological parameters of animals of group I and group II on day 14 and 28 post-partum

Parameter	Test day	Group I	Group II	t-value	p-value
Total leucocyte count (10^3/µL)	14	16.01 ± 4.71	7.36 ± 0.45	1.829	0.109
	28	15.23 ± 4.26	7.63 ± 0.32	1.78	0.118
	t-value (p-value)	1.003 (0.349)	0.788 (0.457)		
LYM (10^3/µL)	14	10.6 ± 3.62	4.33 ± 0.69	1.701	0.130
	28	9.85 ± 3.37	4.34 ± 0.65	1.606	0.149
	t-value (p-value)	1.431 (0.195)	0.054 (0.959)		
MON (10^3/µL)	14	1.04 ± 0.26	0.54 ± 0.06	1.895	0.096
	28	0.60 ± 0.10	0.33 ± 0.04	2.543*	0.023
	t-value (p-value)	2.088 (0.075)	2.862 (0.024)		
GRA (10^3/µL)	14	4.34 ± 0.91	2.94 ± 0.32	1.451	0.182
	28	4.14 ± 0.62	2.94 ± 0.24	1.802	0.105
	t-value (p-value)	0.382 (0.714)	0^{ns} (1.0)		
RBC (10^6/**µL**)	14	5.91 ± 0.32	5.9 ± 0.35	0.029	0.977
	28	6.18 ± 0.33	6.36 ± 0.33	0.380	0.710
	t-value (p-value)	1.119 (0.300)	3.158 (0.016)		
Hb (g/dL)	14	7.09 ± 0.20	8.18 ± 0.36	2.657*	0.019
	28	7.59 ± 0.13	8.53 ± 0.27	3.075**	0.008
	t-value (p-value)	3.282* (0.013)	2.297 (0.055)		
VPRC (%)	14	27.49 ± 1.04	29.95 ± 1.19	1.558	0.141
	28	28.91 ± 0.73	31.9 ± 0.71	2.927*	0.011
	p-value	1.479 (0.183)	2.867* (0.024)		
PLT (10^3/µL)	14	315.38 ± 69.44	348.63 ± 47.24	0.396	0.698
	28	374.75 ± 92.41	366.13 ± 48.37	0.083	0.935
	t-value (p-value)	1.554 (0.164)	0.606 (0.563)		

Group I – SCK positive, Group II – Normal animals

** significant at 0.01 level, * Significant at 0.05 level

Table 3. Mean serum biochemical parameters of animals of group I and group II on day 14 and 28 post-partum

Parameter	Test day	Group I	Group II	t-value	p-value
Total protein (g/dL)	14	7.88 ± 0.14	7.76 ± 0.19	0.532	0.603
	28	7.88 ± 0.24	7.47 ± 0.14	1.475	0.162
	t-value (p-value)	0.037 (0.972)	1.431 (0.196)		
Albumin (g/dL)	14	3.59 ± 0.09	3.67 ± 0.1	0.597	0.560
	28	3.64 ± 0.07	3.52 ± 0.05	1.396	0.185
	t-value (p-value)	0.697 (0.508)	2.171 (0.067)		
AST (IU/L)	14	74.2 ± 4.95	89.34 ± 6.01	1.945	0.072
	28	76.6 ± 4.46	89.29 ± 7.49	1.456^{ns}	0.167
	p-value	0.420 (0.687)	0.005 (0.996)		
Bilirubin (mg/dL)	14	0.31 ± 0.05	0.47 ± 0.05	2.288*	0.038
	28	0.37 ± 0.04	0.32 ± 0.08	0.655	0.555
	p-value	1.432 (0.195)	1.516 (0.173)		
Creatinine (mg/dL)	14	1.16 ± 0.07	0.98 ± 0.03	2.232*	0.042
	28	1.01 ± 0.07	0.99 ± 0.04	0.537	0.599
	p-value	1.990 (0.087)	0.051 (0.961)		

Group I – SCK positive, Group II – Normal animals

* Significant at 0.05 level

normal physiological range. During the first seven weeks post-partum period, no significant variations were noted in total bilirubin between the SCK and normal healthy groups (Mohsin *et al*., 2022).

Mean creatinine value of diseased animals on 14^{th} day was significantly higher ($p<0.05$) than normal animals, but values were in normal physiological range. No significant difference noticed on 28^{th} day. This is in agreement with findings of Mezzetti *et al*. (2019) and Antanaitis *et al*. (2019). Mann *et al*. (2018) reported a significant decrease in creatinine value of animals positive for SCK after different treatment protocols. The increase in serum creatinine in diseased animals might be due to moderate dehydration status in ketotic animals as opined by Issi *et al*. (2016). Contrary to this finding, a marked reduction was observed in previous studies. The obvious post-partum decline in creatinine in ketotic cows confirmed that these cows were mobilising higher quantities of tissue protein than normal animals, implying that these cows were in negative protein balance post-partum (Rodriguez-Jimenez *et al*., 2017).

Haematological parameters of diseased animals were normal except for a significant reduction in haemoglobin value on both test days. No significant variations were recorded in biochemical parameters of diseased and normal animals except for a significant reduction in total bilirubin value and a significant increase in serum creatinine value in diseased animals. But these values were in normal physiological range.

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