CLINICO-BIOCHEMICAL, BIOMARKERS, HORMONAL AND THERAPEUTIC STUDIES IN KETOTIC COWS[#]

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ABSTRACT

The present study was conducted on ketotic cows in and around Bikaner in the year 2015-2016. The prevalence of the disease was 13.14 per cent. The disease was mainly characterized by hypoglycemia and protienemia with ketonaemia and ketonuria in both A and B groups (A=insulin non-dependent & B=insulin dependent). The major findings of biochemical study were significant increase in serum triglyceride, total serum cholesterol, free cholesterol, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and serum β-hydroxy butyric acid (BHBA) in both groups. The serum insulin level was significantly decreased in group B and serum Cortisol level was significantly decreased in group A. Among Biomarkers parameters, Cholesterol ester and Lecithin Cholesterol Acyl Transferase (LCAT) enzyme activity were significantly lower in both groups. The above parameters almost attained normalcy after treatment in both groups.

***Key words*:** Hypoglycemia, ketonaemia, BHBA, LCAT

Introduction

Major metabolic transitions occur in a cow's body during parturition and transition from a non-lactating to a lactating state (Tanya, 2008). Perhaps the most important physiological change occurring during this period is the decrease in dry matter intake around parturition and the sudden increase in nutrients that cows need for milk production resulting in Negative Energy Balance (NEB) (Ingvartsen and Andersen, 2000). Mobilization of body reserves occurs to compensate this NEB, and probably as a consequence there is a higher susceptibility to diseases and metabolic disorders like ketosis (Lu *et al.* 2013). There is increase in circulating non-esterified fatty acids (NEFA) and β-hydroxy butyric acid (BHBA) and decrease in glucose concentrations (Drackley, 1999).

The predominant ketone in cows is β-hydroxybutyric acid (BHBA). Therefore gold standard for ketone testing is considered to be laboratory measurement of BHBA (Radostits *et al.,* 2007) because BHBA is a more stable ketone body along with its higher serum concentration than acetone or acetoacetate. The release of higher amount of ketone bodies (BHBA) due to incomplete oxidation of fatty acids in liver causes ketosis, which is characterized by depression in feed intake and milk production (Duffield, 2011). Several biochemical alterations such as increased ketone bodies in blood and urine, lowered blood glucose, hyperprotenemia and low level of insulin have been reported in cows showing ketoacidosis.

The regulation of energy metabolism in ruminants is primarily governed by insulin and glucagon. Insulin and Glucagon, both are the counter-regulatory hormones. Their counteracting effects play a central role in the homeostatic control of glucose. Low insulin: glucagon ratio stimulates lipolysis in adipose tissue and ketogenesis in the liver. Cows in early lactation have low insulin: glucagon ratios because of low blood insulin and are in a catabolic state (Radostits *et al.,* 2007).

Control of metabolic diseases like ketosis is crucially important because affected animals even after recovery have high incidences of reproductive and infectious diseases. For treatment of clinical ketosis several drugs including glucose intravenously, corticosteroids, anabolic steroid trenabolone acetate @ 60-120 mg as a single injection, propylene glycol and niacin, glucose orally plus sodium bicarbonate, *Phyllanthus niruri linn* powder plus molasses plus maize (Vaidya*et al.* 2004), glucose + Insulin for Diabetic ketoacidosis (DKA) (Bihani, 2001) and some other combination of drugs depending on biochemical findings have been suggested.

High incidence of clinical and sub-clinical ketosis causes economic loss to the dairy farmers due to loss of milk production as well as sharp drop in the solid not fat (SNF) content of milk (Tufani *et al.,* 2011) including treatment costs, impaired reproductive efficiency, increased involuntary culling and increased susceptibility of suffering animal to other secondary diseases.

Materials and Methods

Sample

Urine samples of 350 post-parturient cows were examined for ketosis during August, 2015 to January, 2016. After clinical examinations and urine Diastick test (Tests for ketone bodies and glucose for 30 and 40 seconds, respectively), the blood samples were collected aseptically in sterilized test tubes for serum separation from jugular vein from forty six ketotic and ten healthy cows (control). The serum was separated in small Pyrex tubes and was kept immediately in the deep freeze at -20°C until analysis.

Biochemical estimation

Serum Glucose, serum total protein, serum cholesterol, serum alanine aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST) were estimated by Idexx Vet Test-Biochemistry analyzer as per the standard protocol using Kit,

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supplied by IDEXX Laboratories, Inc., Westbrook, Maine 04092, USA. Serum triglycerides was estimated spectrophotometerically by enzymatic method as recommended by Fossati and Prencipe (1982) by using kits, supplied by Sowar Pvt. Ltd., CSC, C-9, Vasant kunj, New Delhi. BHBA, serum insulin and serum cortisol were estimated in the ELISA assay plates from the serum samples by kinetic enzymatic method (McMurray *et al.* 1984) using a commercially available kit, supplied by Sigma Diagnostic Distributors, 34, Sudama Nagar, opp. Glass factory, Tonk road, Jaipur**.** Estimation of Free and Ester cholesterol was done by digitonin precipitation (Sperry and Webb, 1950) and estimation of LCAT was done by Glomset (1969) method.

Treatment

The ketotic animals under treatment were divided into two groups:

Group A (n=37)

1st day- Inj. Vetalog (containing triamcinolone acetonide 6 mg/ml) 2ml i/m once with 50% dextrose 500 ml i/v and liquid Enerdyna (Vet-Mankind) 200 ml PO per animal twice.

2nd, 3rd and 4th day - 50% dextrose 500 ml IV (as required, 3 infusions maximum) and liquid Enerdyna (Vet-mankind) 100 ml PO per animal twice daily for 3 days.

Group B (n=9)

Cows showing glycosuria were given Human Actrapid (insulin) containing 40 IU per ml as per the value of serum glucose (Bihani, 2001) as mentioned below:

Serum Glucose	**Dose of Insulin (zinc protamine)**
60-80 mg/dl	2 ml of Insulin subcutaneously
80-100 mg/dl	3 ml of Insulin subcutaneously
100-120 mg/dl	4 ml of Insulin subcutaneously
Above 120 mg/dl	5 ml of Insulin subcutaneously

In group B, The 1st dose of insulin was given with 500 ml of 50% dextrose while the 2nd dose was given without dextrose if the animal under treatment had previously completed treatment with 3 infusions of 50 per cent dextrose (one bottle of 500 ml of 50 % dextrose per day). Liq. Enerdyna was given 200 ml orally bd on 1st day and 100 ml orally bd on 2nd to 4th day.

Statistical analysis

The data were statistically analysed and compared by Snedecor and Cochran (1994). Multiple comparisons of the means were done by using Duncan's new multiple range tests as described by Steel and Torrie (1980), the p values less than 0.05 were considered as significant. The Significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA).

Discussion

Hypoglycemia in clinical cases attributed to the large amount of glucose removed by mammary glands to make lactose coupled with insufficient food intake to replenish the supply of glucose (Baird, 1982) and may be due to the high productive and reproductive status of the animal (Radostits *et al.,* 2007). Decrease of dry matter intake around parturition, increase demand for glucose and insufficient propionate production during the early postpartum period (Drackley and Dann, 2005). According to Bergman (1996) dietary carbohydrates are fermented in the rumen to form volatile fatty acids (VFAs). Acetic acid, propionic acids and butyric acid are the most important VFAs, but propionic acid is the only VFA that can be converted in glucose. In normal condition about 70 per cent glucose formation is from propionic acid but in reduced appetite condition it is about zero so in absence of this there is less formation of glucose resulting in hypoglycemia.

Hyperproteinemia along with increased albumin and globulin accompanied by hypoglycemia might be due to energy deficient and protein rich ration given to the high yielders, though such a ration could provide enough energy and protein requirement to the low yielding cows (Schultz**,** 1968). According to Hibbit (1979) high protein intake exacerbate an energy deficit because of energy losses resulting from its metabolism and excretion. This energy deficit was responsible for development of ketosis.

Ketotic cows developed hepatic damage which was evident through significant increase in Serum AST and ALT activity. Severity of liver damage was associated with the level of serum AST (Forenbacher and Srebocan, 1963). In ketotic animal, permeability of hepatic cells was increased due to which concentration of liver specific enzymes viz. ALT and AST are increased (Agrawal *et al.,* 2002). According to Aiello (1998) in primary ketosis there is acute involution of the pancreas and this might result in decreased production of insulin. Insulin deficiency may be associated with hypocalcaemia, and diminished activity of β-cells of endocrine pancreas to synthesize and release insulin. According to Sartin *et al.* (1988) adrenocorticotropin is the pituitary hormone that regulates adrenal secretion of cortisol and due to the low concentration of ACTH in high milk yielding cows, there is significant low concentration of cortisol. According to Rijnberk *et al.* (1997) glucocorticoids like cortisol, supply glucose to the organism by the transformation of proteins and thus reduction in the concentration of cortisol, may impair glucose metabolism and associate with hypoglycemia.

In response to a negative energy balance and low serum concentrations of glucose, cows will mobilize adipose tissue with consequent increases in serum concentrations of non-esterified fatty acids (NEFA) and subsequently BHBA (Radostits *et al.,* 2010). Lactogenesis is accompanied by alterations of metabolism, which include increased lipolysis and decreased lipogenesis in adipose tissue, decreased glycogenesis and increased gluconeogenesis and glycogenolysis in the liver, decreased use of glucose and increased use of lipid as energy sources by body tissues (Reynold *et al.,* 2003). Higher producers have higher demands of glucose and more mobilization of NEFA with subsequent increase in ketone body synthesis (Duffield, 2000). NEFA is the primary substrate for the hepatic ketogenesis, and thus, if the plasma concentration of NEFA is high, so is the possibility of hepatic production of ketone bodies (Heitmann *et al.,* 1987). This is in accordance with the fact that hypoglycaemia is the driving force in bovine ketosis that ultimately causes release of higher amount of ketone bodies (BHBA) due to incomplete oxidation of fatty acids in liver.

In ketosis when there are disturbances in the lipoprotein synthesis, triglycerides accumulate in the liver cells with fatty liver as a result. Ketosis and liver lipidosis develops

when the hepatic uptake of NEFA exceeds their oxidation and secretion as triacylglycerols (TAG) in very- low- density lipoproteins (VLDL) by the liver. Serum cholesterol increased due to lipolysis occurring in adipose tissue of ketotic animals which in turn was responsible for other lipids (Brockman, 1979). LCAT is a serum enzyme that catalyses esterification of free cholesterol to produce Cholesteryl ester (CE). LCAT is the enzyme responsible for production of cholesteryl esters in plasma. Cholesterol for reaction comes from peripheral tissues and the donor of the acyl group is lecithin from the high density lipoprotein (HDL) particles. LCAT is thus necessary for reverse cholesterol transport from peripheral tissues. The produced CE is transferred to low-density lipoprotein (LDL) and LDL CE which is finally taken up by the liver (Brown *et al.,* 1981). It has been demonstrated that LCAT activity is reduced in fatty liver in cows developed during the nonlactating stage and those with the fatty liver related postparturient diseases such as ketosis (Nakagawa-ueta and katoh, 2000).

Group-wise comparison of treatment

For the purpose of treatment the ketotic cows were divided into two groups. All the forty six ketotic cows in group A and B were cured with the treatment.

Group A

Out of a total of 37 cases in group A, 4 cases showed ketosis "+" and 7 cases showed ketosis "++" which recovered on 2nd day. Out of 22 cases, showing "+++", 15 recovered on 3rd day while 7 case recovered on 4th day. Rest of the 4 cases showing "++++" recovered on 4th day.

Group B

The non-responding nine cases of group A showing glycosuria, were considered as insulin dependent cases and were placed in group B. The Dipstick were positive (+) for glucose when the serum glucose level was between 78.32 and 95.61 mg/dl. Glycosuria has been reported to occur above 74 mg/dl of serum glucose by Bihani (2001) and 77.54 to 94.02 mg/dl Saini (2013). Such cases were immediately switched on to treatment with 50 % dextrose and Insulin or Insulin alone, depending upon the values of serum glucose.

Comparative efficacy of treatment concluded that the cases which were treated with triamcinolone acetonide 12 mg per animal, liquid Enerdyna and 50% dextrose recovered more satisfactory. Insulin was given where the ketotic animals showed glycosuria and was deficient in Insulin. So insulin therapy is advised where there is deficiency of insulin and not merely for enhancing glucose absorption.

Table 1: Mean ± SE value of biochemical parameters in apparently healthy and insulin non-dependent ketotic cows (Before and after treatment) Group A

S. No.	Parameters	Healthy cows (n=10)	Before treatment (n=37)	After treatment (n=37)
1.	Serum Glucose (mg/dl**)	$56.57^{c} \pm 0.990$	$35.73^{a} \pm 0.698$	$51.95^{b} \pm 0.351$
2.	Serum total protien (g/dl**)	$7.88^{a} \pm 0.217$	$10.33^{b} \pm 0.152$	$7.93^{a} \pm 0.111$
3.	AST (IU/L**)	$47.64^{a} \pm 3.283$	$93.93^{c} \pm 0.713$	$72.12^{b} \pm 2.039$
4.	ALT (IU/L**)	$32.13^{a} \pm 0.823$	$57.06^{b} \pm 2.289$	$36.21^{a} \pm 0.384$
5.	Insulin (µIU/ml**)	$11.62^{a} \pm 0.565$	$10.76^{a} \pm 0.243$	$11.31^{a} \pm 0.167$
6.	Cortisol (ng/ml**)	$5.42^{c} \pm 0.336$	$3.73^{a} \pm 0.093$	$4.70^{b} \pm 0.084$
7.	BHBA(mmol/L**)	$0.68^{a} \pm 0.043$	$1.62^{b} \pm 0.018$	$0.76^{a} \pm 0.018$
8.	Triglyceride (mg/dl**)	$51.33^{a} \pm 1.571$	$73.45^{b} \pm 0.747$	$53.11^{a} \pm 0.589$
9.	Total cholesterol (mg/dl**)	$100.97^{a} \pm 0.928$	$102.14^{b} \pm 0.369$	$100.84^{ab} \pm 0.223$
10.	Cholesteryl ester (mg/dl**)	$72.59^{c} \pm 0.716$	$66.39^{a} \pm 0.240$	$70.59^{b} \pm 0.156$
11.	Free cholesterol (mg/dl**)	$29.11^{a} \pm 0.792$	$35.75^{c} \pm 0.129$	$30.25^{b} \pm 0.067$
12.	LCAT activity (nmol/ml/hr**)	$16.11^{c} \pm 0.759$	$10.81^{a} \pm 0.039$	$14.70^{b} \pm 0.033$

* ($p<0.05$) ** ($p<0.01$) Means with different superscripted letters in the same row differ significantly

Table 2: Mean ± SE value of biochemical parameters in apparently healthy and insulin dependent ketotic cows (Before and after treatment with insulin) Group B

S. No.	Parameters	Healthy cows (n=10)	Before treatment (n=9)	After treatment (n=9)
1.	Serum Glucose (mg/dl**)	$56.57^{c} \pm 0.989$	$38.48^{a} \pm 1.110$	$51.26^{b} \pm 1.006$
2.	Serum total protien (g/dl**)	$7.88^{a} \pm 0.217$	$11.36^{b} \pm 0.379$	$8.54^{a} \pm 0.209$
3.	AST (IU/L**)	$47.64^{a} \pm 3.283$	$87.735^{c} \pm 3.538$	$67.67^{b} \pm 2.409$
4.	ALT (IU/L**)	$32.13^{a} \pm 0.823$	$64.26^{b} \pm 5.099$	$37.0^{a} \pm 2.555$
5.	Insulin (µIU/ml**)	$11.62^{b} \pm 0.565$	$5.091^{a} \pm 0.439$	$10.56^{b} \pm 0.561$
6.	Cortisol (ng/ml**)	$5.42^{a} \pm 0.336$	$4.66^{a} \pm 0.206$	$4.98^{a} \pm 0.195$
7.	BHBA (mmol/L**)	$0.68^{a} \pm 0.043$	$1.72^{b} \pm 0.049$	$0.71^{a} \pm 0.027$
8.	Triglyceride (mg/dl**)	$51.33^{a} \pm 1.571$	$80.13^{b} \pm 4.181$	$56.45^{a} \pm 1.825$
9.	Total cholesterol (mg/dl**)	$100.97^{a} \pm 0.928$	$103.71^{b} \pm 0.718$	$101.61^{ab} \pm 0.615$
10.	Cholesteryl ester (mg/dl**)	$72.59^{c} \pm 0.716$	$64.39^{a} \pm 0.360$	$70.11^{b} \pm 0.525$
11.	Free cholesterol (mg/dl**)	$29.11^{a} \pm 0.792$	$37.82^{c} \pm 0.211$	$31.5^{b} \pm 0.236$
12.	LCAT activity (nmol/ml/hr**)	$16.11^{c} \pm 0.759$	$10.21^{a} \pm 0.057$	$14.63^{b} \pm 0.110$

* ($p<0.05$) ** ($p<0.01$) Means with different superscripted letters in the same row differ significantly

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