



Biochemical Changes of Ketosis in Cows at Post Parturient Period

Mohammed Nazeer¹, Sandeep Kumar², Manu Jaiswal^{3*}, Alok Mishra³ and Pratyush Kumar⁴

¹Department of Veterinary Clinical Complex, Post- Graduate Institute of Veterinary Education & Research (PGIVER), Rajasthan, INDIA

²Department of Animal Husbandry, Rajasthan, INDIA

³Faculty of Veterinary and Animal Science, Institute of Agricultural Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur, (U.P.), INDIA

⁴Department of Veterinary Clinical Complex, Post- Graduate Institute of Veterinary Education & Research (PGIVER), Rajasthan, INDIA

*Corresponding author: M Jaiswal; Email: drmjvet20aug@gmail.com

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ABSTRACT

The present study was conducted to determine biochemical changes in apparently healthy control and ketotic cows. Fresh urine samples were collected from 350 post-parturient cows to screened urinary ketone bodies by using Modified Rothera's test and urine Diastix test. Total forty cows showing clinical signs of ketosis were included for biochemical studies (before and after treatment). It was observed that serum glucose was significantly ($P < 0.01$) lower as compared to healthy animals. While, serum ketones and urine ketones levels were significantly ($P < 0.01$) elevated. Similarly, total serum protein, globulin and albumin levels were significantly ($P < 0.05$) higher in ketotic cows. It can be concluded that group treated with Decadurabolin (Nandrolone Decanoate) in association with parenteral dextrose give excellent recovery rate in Ketosis.

Keywords: Ketosis, Cows, Biochemical Changes, Treatment

Ketosis is a common metabolic disorder frequently observed in dairy cows during the early lactation period characterized by increased levels of ketone bodies in the blood, urine, and milk. In buffalo, ketosis remains one of the major diseases that decrease the productivity (Ghanem and Eldeeb, 2010).

A dramatic increase in energy requirements during late pregnancy and early lactation makes dairy cows highly susceptible to negative energy balance (Youssef *et al.*, 2010). At the onset of lactation, the dairy cow must accommodate a tremendous increase in energy demand by the mammary gland for milk production. This is realized partly by increasing feed intake and partly by fat mobilization from adipose tissue. However, excessive fat mobilization can induce an imbalance in hepatic carbohydrate and fat metabolism, characterized by elevated concentrations of ketone bodies called hyperketonemia (Haelst *et al.*, 2008).

Bovine ketosis is of substantial economic significance and has been found to be responsible for decline in milk production even two weeks before its clinical appearance. Major economic losses have been attributed to the loss of milk yield and failure of the animals to return to the peak production potential even after recovery in clear cut cases of ketosis (Teli and Ali, 2007). Keeping in view the study was conducted to observe the biochemical changes in ketotic cows at their post parturient period.

MATERIALS AND METHODS

Study plan and sample collection

The present study was conducted in forty post-parturient cows showing the clinical signs of ketosis and urine samples positive for Modified Rothera's test and urine Diastix. Animals belonging to the college dairy farm,

outdoor patients brought for treatment at medicine clinic of College of Veterinary and Animal Science, Bikaner and individual animals shown by owners at their holdings in and around Bikaner were considered for this study. The ketotic animals under treatment were randomly divided into three groups as described below in the text.

Group A (n=15) received Isoflud (Zydus AHL) containing Isoflupredone Acetate (2 mg/ml) @ dose rate of 20 mg per animal intramuscularly on day-1 and day-3. Moreover, 500 ml of 50% dextrose was given intravenously on day-1 and day-2.

Group B (n=15) received Decadurabolin (Cadilla Health Care Ltd.) containing Nandrolone Decanoate @ 100 mg per animal intramuscularly on day-1 and day-3. Moreover, 500 ml of 50% dextrose was given intravenously on day-1 and day-2. Group C (n=10) showing glycosuria were given Human Actrapid (Insulin) containing 40 IU per ml through SC route depending upon the blood glucose level. The dose of insulin was 80 IU, 120 IU, 160 IU and 200 IU for serum glucose level 60-80 mg/dl, 80-100 mg/dl, 100-120 mg/dl and above 120 mg/dl, respectively. The 1st dose of insulin was given with 500 ml of 50 per cent 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 (500 ml of 50 per cent dextrose per day).

Sample collection

The blood samples were collected from jugular vein in sterilized test tubes from forty ketotic and ten healthy cows for serum. The separated serum samples were harvested and kept in deep freeze (-20°C) till biochemical analysis. Fresh urine samples were collected directly in sterile vials after massaging the perineal region of the cow to test the urine for the presence of ketone bodies.

Assessment of biochemical parameter

Urine samples of all the cows were subjected to urine Diastix and Modified Rothera's test. The Diastix reagent strip provides test for urine glucose and urine ketone (Acetoacetic acid). About half pinch of modified Rothera's reagent was taken in a small test tube and one ml of urine was added from the side of the test tube. Without mixing, the tube was set aside for few minutes to see the

development of colour. Very light to very deep purple colour was considered positive for ketosis.

Serum glucose (Tietz, 1976), total protein and albumin (Doumas *et al.*, 1971 and Doumas *et al.*, 1981) levels were estimated by colorimetry method. Globulin value was determined as total protein minus albumin. Concentrations of serum and urine ketone bodies were determined by the method as described by Henry *et al.* (1974).

STATISTICAL ANALYSIS

The data obtained were statically analysed by the method described by Snedecor and Cochran (1987). Multiple comparisons of the means were done by using Duncan's new multiple range tests as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Blood glucose level of post parturient ketotic cows was significantly ($P < 0.01$) lowered in group A, B and C as compared to healthy cows. After treatment its level increased significantly ($P < 0.01$) in all the groups. The Mean \pm SE value of serum glucose after treatment in group A was significantly lower ($P < 0.01$) as compared to healthy control. While this value in group B and C didn't differ significantly as compared to healthy control, but the value attained in group B was more nearer to the healthy control with respect to other groups indicating better recovery than others. 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 (Radostitis *et al.*, 2007). It may also be due to decrease intake of dry matter around parturition, increase demand for glucose and insufficient propionate production during the early postpartum period (Drackley, 1999; 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 case of reduced appetite it is about zero, so in absence of this there is less formation of glucose and resulting into hypoglycemia.

Table 1: Mean \pm SE value of biochemical parameters in apparently healthy and ketotic cows before and after treatment

Parameters	Group A			Group B			Group C		
	Healthy cow	Before treatment	After treatment	Healthy cow	Before treatment	After treatment	Healthy cow	Before treatment	After treatment
	(n=10)	(n=15)	(n=15)	(n=10)	(n=15)	(n=15)	(n=10)	(n=15)	(n=15)
Serum glucose (mg/dl)	55.47 \pm 1.25 ^c	35.46 \pm 1.22 ^a	51.71 \pm 0.58 ^b	55.47 \pm 1.25 ^b	36.85 \pm 1.05 ^a	52.99 \pm 0.64 ^b	55.47 \pm 1.25 ^b	42.48 \pm 4.48 ^a	50.08 \pm 1.10 ^b
Serum ketones (mg/dl)	1.28 \pm 0.08 ^a	10.42 \pm 0.41 ^b	1.51 \pm 0.06 ^a	1.28 \pm 0.08 ^a	10.87 \pm 0.61 ^b	1.34 \pm 0.07 ^a	1.28 \pm 0.08 ^a	10.65 \pm 0.47 ^b	1.25 \pm 0.06 ^a
Urine ketones (mg/dl)	1.65 \pm 0.14 ^a	25.43 \pm 1.14 ^b	2.37 \pm 0.12 ^a	1.65 \pm 0.14 ^a	26.55 \pm 1.13 ^b	1.88 \pm 0.05 ^a	1.65 \pm 0.14 ^a	26.69 \pm 1.68 ^b	2.04 \pm 0.08 ^a
Total serum protein (g/dl)	7.74 \pm 0.19 ^a	10.35 \pm 0.27 ^b	7.90 \pm 0.17 ^a	7.74 \pm 0.19 ^a	11.56 \pm 0.29 ^b	7.95 \pm 0.19 ^a	7.74 \pm 0.19 ^a	11.34 \pm 0.33 ^c	8.39 \pm 0.14 ^b
Serum albumin (g/dl)	3.68 \pm 0.12 ^a	4.33 \pm 0.18 ^b	3.84 \pm 0.11 ^a	3.68 \pm 0.12 ^a	4.65 \pm 0.23 ^b	3.63 \pm 0.12 ^a	3.68 \pm 0.12 ^a	4.93 \pm 0.27 ^b	3.58 \pm 0.26 ^a
Serum globulin (g/dl)	4.06 \pm 0.25 ^a	6.02 \pm 0.24 ^b	4.05 \pm 0.23 ^a	4.06 \pm 0.25 ^a	6.91 \pm 0.34 ^b	4.33 \pm 0.25 ^a	4.06 \pm 0.25 ^a	6.41 \pm 0.18 ^c	4.81 \pm 0.21 ^b

Means with different superscripts in rows differ significantly.

Except serum albumin in group A (5% level i.e. $P < 0.05$) all the parameters are significant at 1% level ($P < 0.01$).

Serum ketones are also significantly higher ($P < 0.01$) in ketotic cows of group A, B and C as compared to healthy cows (Table 1).

The present study is in agreement with Sharma (2006), Biswal *et al.* (2009) and Youssef *et al.* (2010). After treatment, serum ketone levels decreased significantly ($P < 0.01$) in all the groups as compared to their pre-treatment levels. However, its value after treatment in group A, B and C didn't differ significantly with the healthy control, but its value in group C reached nearer to the healthy control with respect to other groups indicating better recovery than others groups. High concentration of ketone bodies with a concurrent decrease in blood glucose level was also reported by (Dann *et al.*, 2005). A dramatic increase in energy requirement during late pregnancy and early lactation makes dairy cows highly susceptible to NEB (Turk *et al.*, 2008). Acetyl-CoA production from fatty acids exceeds its removal by the citric acid cycle or lipogenesis, and hence Acetyl-CoA tends to accumulate and contributes to enhanced ketone bodies synthesis. At the onset of lactation, the dairy cow must accommodate a tremendous increase in energy demand by the mammary gland for milk production. This is realized partly by increasing feed intake and partly by fat mobilization

from adipose tissue. However, excessive fat mobilization can induce an imbalance in hepatic carbohydrate and fat metabolism, characterized by elevated concentrations of ketone bodies called hyperketonemia (Haelst *et al.*, 2008). The high demands for glucose for the mammary gland coupled with insufficient carbohydrate supply causes blood level of glucose to drop and glucagon levels to increase, resulting in increased lipid breakdown for energy. To be mobilized, lipids are hydrolyzed into non-esterified fatty acid and glycerol which provides energy. Sickness can also elevate cortisol level resulting in release of NEFA from adipose tissue (Drackley, 1999).

Significant increase ($P < 0.01$) in urine ketone levels in ketotic cows of group A, B and C as compared to healthy cows are depicted in Table 1. This is in agreement with Tyler *et al.* (1994), Bihani (2001), Sharma (2006) and Elitok *et al.* 2010. There was significant decrease ($P < 0.01$) in urine ketone as compared to its pre-treatment level in group A, B and C. After treatment group A, B and C didn't show significant difference with the healthy control, but the value attained in group B was very close to the healthy control with respect to other groups indicating better recovery rate. When an overload of ketone is produced in the liver, they begin to spill in to the blood stream

for subsequent mobilization (Baird, 1982). As the blood stream becomes saturated with acidic ketone they appear in the urine. Acetone begins to appear in the urine as soon as it begins to appear in the plasma probably due to great lipid solubility of acetone, which allows it to penetrate cell membrane with relative ease (Kaneko *et al.*, 1991).

Total serum protein, globulin and albumin levels estimated significantly higher in all the groups to ketotic cows as compared to healthy group as shown in Table 1. Similar observations were also reported by Youssef *et al.* (2010), Ghanem *et al.* (2010) and Elitok *et al.* (2010). Whereas Simenove *et al.* (1978) observed lower concentration of total serum protein in ketotic cows. A significant decrease ($P < 0.01$) in serum total protein was noticed as compared to its pre-treatment level in group A, B and C. However, total serum protein after treatment in group A, B and C had no significant difference as compared to healthy control, but the value attained in group A was more nearer to the healthy control with respect to other groups indicating better recovery than others. The value of serum albumin after treatment in group A, B and C had no significant difference as compared to healthy control, but the value attained in group C was maximum with respect to other groups indicating better recovery. 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. According to Hibbit (1979) high protein intake exacerbates an energy deficit because of energy losses resulting from its metabolism and excretion. This energy deficit was responsible for development of ketosis.

CONCLUSION

Comparative efficacy of treatment trial concludes that the cases which were treated with Decadurabolin recovered more satisfactorily than Isoflud group, because the value attained for blood glucose and urine ketone in group B (treated with Decadurabolin) was more nearer to the healthy control with respect to other groups indicating better recovery than others, making Decadurabolin a better drug of choice than Isoflud. Hence, it can be concluded that group treated with Decadurabolin (Nandrolone Decanoate) in association with parenteral dextrose give excellent recovery rate in Ketosis.

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