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Serum Enzymatic and Lipid Profile in Holstein Friesian Cross Bred Cows with Low Solids-Not-Fat Syndrome

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A B S T R A C T

Present study was carried out to compare serum enzymatic, lipid and blood metabolic profile of Holstein Friesian crossbred cows with and without low solids-not-fat syndrome. The California Mastitis Test (CMT) negative Holstein Friesian crossbred cows in their 3rd and 5th lactation were selected for the study. Twenty four HF crossbred cows were divided into four groups *viz*., Group I (3rd lactation HF crossbred cows with normal milk SNF), Group II (3rd lactation HF crossbred cows with low milk SNF), Group III (5th lactation HF crossbred cows with normal milk SNF) and Group IV (5th lactation HF crossbred cows with low milk SNF) with six animals in each group. Blood samples collected from all the animals during 4th and 8th week of 3rd and 5th lactations were utilized for determination of blood glucose, blood β-hydroxybutyric acid (BHBA), serum enzyme activities, lipid profile and total serum protein. At 3rd lactation, significantly ($P<0.05$) higher ALP activity and HDL-Cholesterol level were observed in normal SNF cows compared to low SNF cows. Significantly ($P<0.05$) lower ALT activity was observed in normal SNF cows at both 4th and 8th week of 3rd lactation. At 5th lactation, blood glucose levels was significantly ($P<0.05$) higher in normal SNF cows compared to low SNF cows at both 4th and 8th week of lactation. Significantly higher lactate dehydrogenase (LDH) activity was found at 4th week and cholesterol was significantly higher at 8th week of 3rd lactation. Triglycerides and total serum protein were significantly higher in normal SNF cows at 8th week of both the lactations. From the study it is concluded that the decreased blood glucose level in low SNF groups could be a major reason for low SNF syndrome in HF crossbred cows.

Introduction

India ranks first in milk production, accounting for 18.50 per cent of the total world milk production (Prakash *et al.*, 2017). In India, Holstein Friesian crossbred cows gives 10 to 15 liters of milk per day. The level of solids-not-fat in the milk which is less than 8.50 per cent is treated as low solids-not-fat syndrome.

The low SNF syndrome is common in Holstein Friesian inbred cows which is inherited (Boden, 2005). SNF percentage also varies with the stage of lactation and usually it is high at beginning of lactation which falls to the low level at around 6 to 8 weeks of lactation (Boden, 2005). The altered metabolic profile of the animal that leads to reduction in the quality of the milk produced. During lactation, the mammary gland

secretory cells utilize 80 per cent of the blood metabolites for milk synthesis depending on the infiltration of precursors of milk components like amino acids, glucose and fatty acids (Piccione *et al.,* 2009). Majority of the enzymes that exist in the mammary cells are derived from blood and measurement of activities of such enzymes in milk has been used to monitor udder health in dairy cows (Fox and Kelly, 2006). The aminotransferases like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) act as a catalyst in metabolism of amino acids and carbohydrates. Aminotransferases are responsible for the protein balance which is very important during the periods of intensive metabolism like peak lactation (Whitaker, 1997). Alkaline phosphatase (ALP) is a membranous enzyme particularly present in lipoprotein structures of the golgi complex and is secreted in milk in high concentrations. Lactate dehydrogenase (LDH) is an NAD-dependent enzyme that plays a role in the metabolism of glucose in aerobic and anaerobic conditions. LDH activity is associated with active milk secretion so that it represents a constitutive component (Kocic *et al.,* 2010).

Blood glucose, cholesterol and triglycerides are regarded as important indicators of energy status in ruminants (Hagawane *et al.,* 2009). Beta-hydroxybutyrate (BHBA) is the main indicator of lipomobilization in ruminants (Wittwer, 1995). Serum protein has been used as physiological marker of stress in lactating cow (Giesecke, 1985).

The Bangalore and Chickaballapur districts of Karnataka have huge population of best Holstein Friesian crossbred cattle. There is scarce availability of literature on metabolic profile of Holstein Friesian crossbred cows yielding milk with low solids-not-fat contents. The present study was undertaken in Holstein Friesian crossbred cows with the objectives of evaluating the levels of blood glucose, β-hydroxy butyric acid (BHBA), lipid profile and activities of certain serum enzymes during low solids-not-fat syndrome in Holstein Friesian crossbred cows.

Materials and Methods

The present study was conducted in Holstein Friesian crossbred cows at Bangalore Rural and Chikkaballapur districts of Karnataka. The Holstein Friesian crossbred cows in their third and fifth lactation with average milk yield of 10 to 16 kg/day were selected for the study. The milk samples were initially screened for mastitis using California Mastitis Test (CMT) to rule out the mastitic animals. Milk samples of selected animals were collected in sterile containers and were analyzed for solid not-fat content using automatic electronic milk tester called 'Ksheera' analyzer. The study was carried out in 24 Holstein Friesian crossbred cows that were divided into four groups based on lactation interval and SNF content of milk with each group containing six animals. HF crossbred cows in their 3rd lactation with normal milk SNF content (SNF > 8.5 %) is considered as Group 1. Third lactation HF crossbred cows with low milk SNF (SNF < 8.5 %) is considered as Group II. HF crossbred cows in their 5th lactation with normal milk SNF content (SNF > 8.5 %) and with low milk SNF content (SNF < 8.5 %) is considered as Group III and IV, respectively.

Ten ml of blood samples were collected twice from each of the selected animal, one at 4th week of lactation and another at 8th week of lactation by jugular venipuncture. Serum was separated from the blood samples by centrifugation at 3000 rpm for 20 min and the separated serum was stored at -20 ˚C until it was analyzed for different biochemical parameters. A small portion of the blood collected from each animal was immediately utilized for the determination of blood glucose and blood β-hydroxy butyrate by

glucometer using commercially available strips. The serum samples were analyzed for enzyme profile **(**Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH) and lipid profile (triglycerides, Total cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol) with the help of microlab 300 semi- automated biochemical analyzer (supplied by Merck Pvt. Ltd, Mumbai) using commercially available reagent test kits. The data obtained in the study were analyzed statistically by two way ANOVA with the application of Bonferroni posttest using GraphPad Prism version 5.01 (2007) computerized software.

Results and Discussion

Serum enzyme activities

No significant differences were observed in the activities of serum Aspartate Aminotransferase activity (AST) in cows with normal SNF (Group I and Group III) and in cows with low SNF content (Group II and Group IV), respectively, both at 4th and 8th week of lactation (Table 1). However, the activities of AST were numerically higher in cows with low SNF content when compared to the cows with normal SNF at 4th week of lactation. The results indicated slightly altered metabolism with respect to liver in HF crossbred cows with low SNF syndrome. Nessim (2010) observed increased level of aspartate transaminase in blood plasma while decreased level of alanine aminotransferase during early lactation in cows. The results of the present study were in accordance with the findings of Jozwik *et al.,* (2012) who mentioned that the AST levels were lower in medium yielding cows compared to high yielding cows. The serum Alanine Aminotransferase (ALT) activities in normal SNF group (Group I) were lower compared to low SNF group (Group II) both at 4th week and 8th week of 3rd lactation (Table 1). The increase in ALT activities in low SNF group might be attributed to abnormal liver function as a result of mobilization of fat in early lactation (Kramer ad Hoffmann, 1997). Sharma and Ray (1983) reported that the ALT level was significantly greater throughout two lactation periods and suggested that the liver was in a state of hyperfunction coinciding with mammary gland activity. ALT plays an important role in the process of glucose and amino acid intermediate metabolism (Ray *et al.,* 2008) indicating a higher requirement of glucose and the need for gluconeogenesis during lactation. The increased levels of ALT in low SNF cows at 3rd lactation could be due to demand from the mammary gland for more synthesis of lactose, as ALT could play a role in glucose metabolism.

At 3rd lactation, the serum Alkaline Phosphatase (ALP) activities were significantly lower in low SNF group (Group II) both at 4th week and 8th week of lactation when compared to respective control group (Group I) (Table 1). At 5th lactation, the ALP levels were significantly lower in low SNF group (Group IV) compared to cows with normal SNF content (Group III) at 4th week of lactation. The ALP is mainly involved in calcium and phosphorus metabolism and assists in supplementing calcium to the milk (Sato *et al.,* 2005). In the present study, it is opined that the lowered activity of ALP enzyme could be one of the reason for low SNF syndrome in cows. The increased ALP activity recorded in the present study in normal SNF cows were in accordance with Gorski and Saba (2012) who observed increased alkaline phosphatase and aspartate transaminase activity in the blood of lactating cows than dry cows. Nozad *et al.,* (2012) observed highest concentration of alkaline phosphatase in the fifth month of lactation in Holstein dairy cows. The increased ALP

activity in normal SNF cows could be associated for physiological response of draining the calcium and phosphorus into the milk, as indicated by Sato *et al.,* (2005). The serum Lactate Dehydrogenase (LDH) activities in normal SNF cows (Group I) were significantly higher compared to low SNF cows (Group II and Group IV) at 4th week of lactation (Table 1).

El-Zubeir *et al.,* (2005) correlated the activities of the LDH with the levels of various minerals among which negative correlation was recorded for sodium and iron and positive correction was seen with potassium, calcium, magnesium, copper, zinc and phosphorus in healthy Holstein Friesian cows. The comparatively lower levels of LDH in low SNF cows in the present study could be indicative of low mineral levels in milk that finally result in low SNF values.

Serum lipid profile

The serum total cholesterol values in the cows with normal SNF level (Group I) were comparatively higher than Group IV at 4th week of lactation. At 8th week, the values were higher in Group I compared to other groups such as Group II, Group III and Group IV (Table 2). Chladek *et al.,* (2004a, 2004b) arrived at a positive correlation between blood plasma cholesterol and milk protein content. The increased cholesterol levels observed in the present study in normal SNF cows could be correlated to better protein content of the milk. In the present study, the cows with low SNF syndrome had lower cholesterol content when compared to respective control groups. The lactation period progressed from 4th to 8th week, the cholesterol levels decreased. However, Naser *et al.,* (2014) mentioned substantial increase in cholesterol levels as the lactation advanced. Nazifi *et al.,* (2002) and Roche *et al.,* (2009), reported that lipid reserves are utilized for parturition and initiation of lactation and as the lactation advance the lipid reserves are depleted resulting in decrease in serum cholesterol. The levels of serum triglycerides were higher in the cows with normal SNF content (Group I and Group III) when compared to low SNF cows at 8th week of 3rd and 5th lactation (Table 2). Blum *et al.,* (1983) reported that there is a negative correlation between triglycerides and milk yield throughout lactation. In the present study, the concentration of triglycerides decreased numerically from 4th week to 8th week. Schwalm and Schultz (1976) reported positive correlation between milk yield and plasma triglyceride concentration. Gradinski-Urbanac *et al.,* (1986) reported significant decrease in serum triglycerides during early and mid lactation in sheep.

The serum HDL cholesterol level was significantly higher in Group I (cows of 3rd lactation with normal SNF) when compared to Group II and Group IV at 4th week of lactation. Similar trend was also observed at 8th week of lactation (Table 3). The HDL-C levels being the good cholesterol indicate the nutritional wellbeing of the control group cows when compared to those cows with low SNF syndrome. As the energy and other nutritional requirements during the early stages of lactation are far higher, it is opined that the animals could be supplemented with better nutrients during early lactation. The 8th week values of HDL cholesterol were significantly higher in Group I, II and IV compared to values at 4th week. HDL cholesterol is the major lipid bearing class in bovines during lactation as opined by Raphael *et al.,* (1972). Serum Low Density Lipoprotein Cholesterol (LDL-C) showed no significant differences between different groups both at 4th week and 8th week of lactation. However, serum LDL cholesterol levels were significantly higher at 4th week when compared to 8th week in all the groups

(Table 3). Each LDL-C has a highly hydrophobic core consisting of polyunsaturated fatty acid known as linoleate and hundreds to thousands esterified and unesterified cholesterol molecules. In early lactation HMG-CoA reductase, HMG synthase and other enzymes involved in LDL metabolism are elevated suggesting that the requirements for increased hepatic cholesterol availability are great in the initial period of lactation (Viturro *et al.*, 2009). In poorly fed cows during lactation there is mobilization of fats for utilization of energy and for production of milk fats. The increased LDL level in cows with low SNF syndrome might be an indication of low levels of nutrition of these animals. The serum Very Low Density Lipoprotein Cholesterol (VLDL-C) levels did not differ significantly both at 4th week and 8th week of lactation within the groups and between the groups (Table 3). Ruminants have inherently low capacity for synthesis and secretion of VLDL to export triglycerides from the liver (Pullen *et al.*, 1989) and also a reduced capacity to reconvert NEFA back to triglycerides (Graulet *et al.*, 1998).

Metabolic profile

The blood glucose levels in the cows of normal SNF content (Group I and Group III) were significantly higher compared to low SNF groups (Group II and Group IV), at 4th week of lactation. At 8th week, in Group III the level was higher compared to Group IV (Table 4). Blood glucose is regarded as important indicator of energy status in ruminants (Hagawane *et al.*, 2009).

Low blood glucose levels in high yielding cows indicate utilisation of large amounts of blood glucose by mammary gland for synthesis of lactose (Drackley *et al.*, 2001). Two molecules of glucose are required for the synthesis of one molecule of lactose, the milk sugar, a disaccharide (Nale, 2003). The results of the present study indicate that there is direct relationship between blood glucose levels and SNF levels in the milk.

Table.1 Serum enzyme activities

Groups	AST (IU/L)		ALT (IU/L)		ALP (IU/L)		LDH (IU/L)	
	Weeks of lactation							
	4th	8th	4th	8th	4th	8th	4th	8th
Group I	54.87 ± 2.05	58.77 ± 1.55	17.14 ± 0.72^{a}	20.90 ± 0.45^{a}	46.31 ± 2.56^{a}	52.53 ± 1.23^{a}	1295.67 ± 32.37^{a}	1330.33 ± 19.50^{a}
Group II	62.97 ± 4.34	57.90 ± 3.33	24.30 ± 2.40^{b}	25.79 ± 2.00^{b}	34.19 ± 3.41^{bc}	42.74 ± 3.30^{b}	1176.17 ± 47.83^{b}	1240.33 ± 42.77^{ab}
Group III	52.51 ± 3.44	54.03 ± 3.34	18.97 ± 1.03^{ab}	20.72 ± 2.02^{ab}	40.58 ± 0.69^{ab}	43.05 ± 2.30^{b}	1185.00 ± 31.73^{ab}	1283.17 ± 26.00^{ab}
Group IV	62.09 ± 1.89	59.37 ± 2.78	20.44 ± 0.83^{ab}	20.80 ± 0.75^{ab}	31.48 ± 3.00^{c}	38.39 ± 2.39^{b}	1151.16 ± 37.50^{b}	1189.17 ± 34.87^{b}

Table.2 Serum total cholesterol and triglyceride levels

Groups	TOTAL CHOLESTEROL (mg/dL)		TRIGLYCERIDES(mg/dL)	
	Weeks of lactation			
	4th	8th	4th	8th
Group I	140.53 ± 1.76^{aA}	125.43 ± 2.64^{aB}	18.62 ± 0.56	13.53 ± 1.08^{a}
Group II	131.57 ± 5.29^{abA}	112.85 ± 2.44^{bB}	14.74 ± 1.86	11.62 ± 1.17^{b}
Group III	135.10 ± 4.86^{abA}	110.12 ± 1.87^{bB}	17.90 ± 2.96	13.19 ± 2.45^{a}
Group IV	126.41 ± 2.53^{bA}	106.92 ± 3.17^{bB}	13.67 ± 1.93	10.42 ± 1.52^{b}

Table.3 Serum cholesterol profile

Groups	HDL-C (mg/dL)		LDL-C (mg/dL)		VLDL-C (mg/dL)	
	Weeks of lactation					
	4th	8th	4th	8th	4th	8th
Group I	49.13 ± 2.42^{aA}	59.47 ± 2.55^{aB}	87.66 ± 2.70^{A}	63.26 ± 3.65^{B}	3.72 ± 0.11	2.71 ± 0.22
Group II	39.19 ± 2.96^{bcA}	47.33 ± 2.10^{bB}	89.43 ± 4.86^{A}	63.20 ± 2.11^{B}	2.95 ± 0.37	2.32 ± 0.23
Group III	48.39 ± 1.84^{abA}	54.50 ± 1.42^{abA}	83.14 ± 5.32^{A}	52.99 ± 1.72^{B}	3.58 ± 0.59	2.64 ± 0.49
Group IV	36.28 ± 1.43^{cA}	44.15 ± 1.62^{bB}	87.41 ± 3.15^{A}	60.68 ± 2.61^{B}	2.74 ± 0.39	2.09 ± 0.30

Table.4 Serum metabolic profile

Groups	GLU (mg/dL)		BHBA(mmol/L)		TP(g/dL)	
	Weeks of lactation					
	4th	8th	4th	8th	4th	8th
Group I	45.83 ± 2.29^{a}	42.50 ± 3.97^{ab}	0.82 ± 0.06	0.85 ± 0.07	8.54 ± 0.31	8.28 ± 0.19^{a}
Group II	37.00 ± 1.88^{b}	39.50 ± 0.67^{b}	0.82 ± 0.13	0.93 ± 0.17	8.10 ± 0.37	7.14 ± 0.26^{b}
Group III	47.50 ± 2.20^{a}	52.33 ± 0.16^{a}	0.78 ± 0.07	0.87 ± 0.08	8.56 ± 0.30	8.21 ± 0.18^{a}
Group IV	37.50 ± 1.38^{b}	37.33 ± 1.33^{b}	0.70 ± 0.10	0.71 ± 0.11	8.06 ± 0.42	7.01 ± 0.24^{b}

The lower blood glucose levels in cows with low SNF syndrome might be an important contributor for low SNF in these cows. It is opined that, on improvement of blood glucose levels, the low SNF problem could be addressed to certain extent since the glucose is necessary for synthesis of lactose which forms about 4.42 to 4.97 % of milk. It is opined that the decreased lactose content in the milk could contribute to low SNF syndrome in inbred Holstein Friesian cattle. Propionate is the major gluconeogenic

precursor and is not an important precursor of ketones and propionate inhibits ketogenesis in ruminant liver Propionate is converted to glucose in ruminants and this glucose is one of the sources of lactose in milk (Gorden and Timms, 2015). If the propionate/serum glucose is at optimum levels then the lactose content in milk might be towards a higher range which could be based on the genetic potential of cattle. No significant differences were found in blood β-hydroxybutyric Acid (BHBA) levels among various groups (Table 4). The BHBA levels from 1.00 to 1.40 mmol/L are considered as the indicator of subclinical ketosis as opined by Iwersen *et al.*, (2009) and Rollin *et al.*, (2010). In the present study, the BHBA levels were in the range of 0.70 ± 0.10 to 0.93 ± 0.17 which indicated that there was no subclinical ketosis in any of the groups. Total serum protein did not differ significantly between different groups at 4th week of lactation. However, at 8th week, the total serum protein levels were significantly ($P<0.05$) higher in Group I (cows of 3rd lactation with normal SNF) and Group III (cows of 5th lactation with normal SNF) when compared to Group II (cows of 3rd lactation with low SNF) and Group IV (cows of 5th lactation with low SNF) (Table 4). Total serum protein level was lower in low SNF groups (Group II and Group IV). Krajnicakova *et al.*, (2003) were of the opinion that during lactation in goats, the variations in total serum protein might be attributed to catabolism of protein for milk synthesis. Considering this opinion, in the present study, the lower levels of serum proteins in low SNF cows at 8th week could be attributed to catabolism of proteins in blood that help in protein synthesis in the mammary gland for milk production.

In conclusion, serum enzyme activities, lipid profile and blood metabolic profile were determined and compared in Holstein Friesian crossbred cows with normal milk SNF and low milk SNF contents at 4th and 8th week of 3rd lactation and 5th lactation. At 3rd lactation, significantly higher ALP activity and HDL-C levels were was noted in cows with normal SNF content compared to cows with low SNF syndrome. Whereas, significantly lower activity of ALT was found in cows with normal SNF group at both 4th and 8th week of lactation. At 5th lactation, among all the parameters, only blood glucose was found to be significantly higher in cows with normal SNF group when compared to cows with low SNF syndrome at both 4th and 8th week of lactation. The values obtained in the study can be utilized for further studies in related to low SNF syndrome in Holstein Friesian crossbred cows in particular and dairy cows in general.

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