Dynamics of lipid mobilization and other serum metabolites during transitional period in Frieswal dairy cattle

Sumit Mahajan (sumit22_mahajan@rediffmail.com)
ICAR - Central Institute for Research on Cattle https://orcid.org/0000-0001-6984-0823
Indu Devi
CIRC: ICAR - Central Institute for Research on Cattle
Naimi Chand
CIRC: ICAR - Central Institute for Research on Cattle
Suresh Kumar
CIRC: ICAR - Central Institute for Research on Cattle
Megha Pande
CIRC: ICAR - Central Institute for Research on Cattle
Ajayvir Singh Sirohi
CIRC: ICAR - Central Institute for Research on Cattle
Shrikant Tyagi
CIRC: ICAR - Central Institute for Research on Cattle

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Abstract

The transition period in a dairy cow is defined as a change from a gestational non-lactating to a non-gestational lactating state and usually spans between 3 weeks prior to calving and 3 weeks post-calving. The period is characterized by sudden changes in both metabolic and immune functions associated with an increased incidence of both infectious and metabolic diseases, especially in high-yielding dairy cows. The present study depicts the dynamic change in lipid, protein, mineral, and other serum metabolites during the transitional period in Frieswal dairy cows. Significant ($P \leq 0.05$) changes in lipid profile (total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol and very-low-density lipoprotein) protein profile (total protein, globulin, albumin globulin ratio, blood urea nitrogen, and creatinine)**,** mineral (calcium and Phosphorus) and enzyme activity (aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) were recorded during the transition period. Whereas, non-significant ($P \geq 0.05$) changes were noted in the concentration of albumin, glucose, magnesium during the transitional period. Frieswal dairy cows were in negative energy balance as depicted by a decrease in the total cholesterol, its fractions, and unable to recoup the same even after 21 days post-calving when compared with the animals of the control group. Further, these dynamic changes in the present study will be helpful in deciding and planning ration and other managemental interventions to minimize the metabolic stress during the transitional period.

Introduction

Frieswal cattle is one of the established crossbred milch strain having 5/8 Holstein Friesian and 3/8 Sahiwal blood developed by ICAR-Central Institute for Research on Cattle, Meerut, in collaboration with the Ministry of Defense, Government of India after research of more than 3 decades. The Frieswal yields 4,000 kg of milk with 4% butterfat in a mature lactation of 300 days with pan India presence. (Kumar et al., 2018 and 2020). The transition period (3 weeks before to 3 weeks after calving) is generally accredited as the most critical period in relation to the health status of dairy cows during the whole lactation cycle (Pascottini et al., 2020). During this period dairy cow undergoes severe physiological and metabolic stress due to change from a gestational non-lactating to a non-gestational lactating state (Sundrum, 2015). During this period alone, the normal metabolism and immune response of the cow get so much hampered that it is really a challenge for them to even maintain their normal homeostasis and immune response to an infectious agent (Colakoglu. et al., 2017, Singh et al., 2019). These dynamic changes during the transitional period seem to play a pivotal role in the establishment of various metabolic and infectious diseases such as milk fever, retained placenta, metritis, ketosis, left displacement of the abomasum, lameness, clinical mastitis (Colakoglu, et al., 2017 ; Sordillo and Raphael 2013). During late gestation, tremendous demand of nutrients by the gravid uterus to nurture the fast-growing fetus is coupled with additional demand associated with calving, and early lactation which forces these cows to a state of negative energy balance (NEB) leading to intense metabolic stress (Sundrum, 2015).

During the last few decade, many scientific studies from India and abroad have emerged that demonstrated metabolic and biochemical changes in periparturient exotic and varied grades of crossbred genotypes. Freiswal cattle being one of the established milch crossbred strains with pan India presence and having stabilized blood level (62±5% Holstein Friesians). The current study was planned with an aim to evaluate the serum metabolite alterations and lipid mobilization of elite Freiswal during the transition period for devising effective managemental interventions to overcome physiological and metabolic stress produced during the transitional period.

Materials And Methods

Animal: The study was conducted on multiparous elite Frieswal cows kept at ICAR-National Dairy Research Institute, Karnal. A total of 32 clinically healthy Frieswal cows were used in the present study. Animals were divided into two groups of 16 animals in each group. Group 1 animals were in the last trimester of pregnancy (60 to 90 days prior to calving) and Group 2 had 16 non-pregnant Frieswal cows in mid-lactation (>90 days of lactation). A total of 48 blood samples from group 1 were collected at -21 days pre-calving, on the day of calving (day 0), and +21 days post-calving. In view of the difficulty in predicting the exact date of calving, pre-calving samples at -21 days were taken with a standard deviation of ±3 days. Approximately 10 ml of blood sample was collected each time via jugular vein puncture into vials containing clot activator. Serum from the samples was harvested after centrifugation and stored at −20 °C until analysis. Additionally, 16 blood samples from animals of group 2 were also obtained in a similar manner and were used as the standard base value for the analysis of data.

Estimation of Lipid profile: Total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein (VLDL) in serum samples were estimated using commercially available kits procured from ERBA diagnostics as per the instructions of the manufacturer.

Estimation of Protein profile: Total protein, albumin, globulin, and albumin globulin ratio (A:G ratio) in serum was estimated by modified Biuret and Duma's method (Varley *et al.,* 1980), Blood urea nitrogen (BUN) was estimated by di-acetyl monoxime method (Wybenga et al., 1971), and creatinine concentration in plasma was estimated following alkaline picrate method of (Frankel *et al.,* 1970) using commercial kits procured from span diagnostics.

Estimation of other serum metabolites and minerals: Serum concentration of Calcium (Ca), inorganic phosphorus (P), magnesium (Mg), glucose, and activity of enzymes like alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were were estimated using commercially available kits procured from ERBA diagnostics as per the instructions of the manufacturer.

Statistical analysis: Data was analyzed by One-Way ANOVA using SPSS software and data were expressed as mean ± SE and P values less than 0.05 were considered significant.

Results

At the day of calving significantly ($P \leq 0.05$), lower levels of serum cholesterol, triglyceride, HDL-C, LDL-C, and VLDL were detected in Frieswal cows when compared with -21 days pre-calving values of the same group and standard base value of the controls group. Significant ($P \leq 0.05$) increased level of serum total cholesterol, triglyceride, HDL-C, LDL-C, and VLDL was recorded from day 0 (day of calving) to +21 days post-calving and reached to pre-calving levels (-21 days) except for total cholesterol and triglyceride. However, none of the metabolites reached to standard base values of the control animals even after 21 days of post-calving (table: 1).

At the day of calving significantly ($P \leq 0.05$), lower levels of total protein and globulin were detected in parturient Frieswal cows when compared with both -21 days pre-calving and +21 days post-calving values of the same group and standard base value of controls group. On the other hand, a remarkable increase in the A: G ratio was noted on the day of calving when compared with the values of -21 days pre-calving, +21 days post-calving, and with the standard base value of the control group. Albumin remains unaltered when compared within and between the groups during the whole transitional period

Significantly, ($P \leq 0.05$) higher levels of blood urea nitrogen (BUN) and creatinine were recorded on the day of calving when compared with both -21 days pre-calving, +21 days post-calving in the same group, and standard base values of the control group. Although significant ($P \leq 0.05$) decrease in the level of BUN was noticed +21 days post-calving when compared with the values of the day of calving of the same group, still, these values were significantly higher than -21-day pre-calving of the same group and the standard base value of the control group (table 2).

Serum level of Calcium and inorganic phosphorous decreases significantly ($P \leq 0.05$) on the day of calving compared to -21 day values of the same group and the standard base value of the control group. Further, the significant ($P \leq 0.05$) decrease in the level of calcium remains consistent in all parturient cows +21 days post-calving whereas, the inorganic phosphorus returned to pre-calving level within the same group. (Table 3).

Significant ($P \leq 0.05$) activity of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) remains elevated till +21 days of post-calving when compared to standard base value of the control group and -21 pre-calving values of the same group. Whereas, the serum values of Alkaline phosphatase (ALP) remains constantly higher during the entire study period in the experimental group compared with control group. (Table 3). Non-significant but consistent decrease in serum level glucose was noted on day -21, day 0, and + 21 days in these parturient cows when compared with the standard base value of the control group. Similarly, serum levels of magnesium remained unaltered during the transitional period and values remained statistically identical to the control group.

Discussion

Every physiological stage during the life span of the dairy cow has its metabolic characteristics. In dairy cow transitional period between -21 days prior to calving and +21 days, post-calving is most dynamic in nature. During this period, dairy cows undergo several metabolic and immunological changes due to a shift in physiological status from a gestational non-lactating to a non-gestational lactating state leading to physiological, metabolic and immunological stress (Contreras et al., 2011). In the present study, significant ($P \leq 0.05$) change in lipid profile, serum metabolites, and enzymatic activities were recorded both within and between the groups at various stages of sampling during the transition period (Table 1, 2 & 3.). Lipid mobilization in mammals is an acquired physiological adaption to survive (Contreras and Sordillo, 2011) during reduced nutrient and energy availability and can be defined as in balance between lipogenesis and lipolysis. Mobilization of lipid and protein is a characteristic feature in dairy cows during the transitional period as animals have to cope with production stress and negative energy balance (NEB) initially produced by the high demand of energy by the gravid uterus and then for production of colostrum and lactogenesis for ensuring optimal milk production during early lactation (Sordillo and Raphael, 2013). To cope with the NEB produced during the transitional period the body of the dairy cow will first mobilize its body fat into the bloodstream in the form of non-esterified fatty acids (NEFA) which then enter into the liver. In the liver, these NEFA will be oxidized to generate energy via Kreb's cycle, or either they will be converted to Beta-hydroxybutyrate (BHB) and will be re-synthesized to triglycerides where they can either be exported as VLDL or stored in the liver (Sordillo and Raphael, 2013). This is probably the reason for a lower level of serum total cholesterol, triglyceride, HDL-C, LDL-C, and VLDL during all stages of the transitional period in the present study when compared with the standard base values as most of the lipids would have been directly oxidized through Kreb's cycle to cope up the NEB produced during the transitional period. Further, significantly lower value of triglyceride and VLDL on the day of calving and 21 days post-calving may be attributed to the limited capacity of cattle liver to export triglyceride via VLDL during early lactation. (De Koster and Opsomer 2013) table 1.

The findings of the current study are in agreement with the findings of Quiroz-Rocha et al. (2009) who concluded that there is a constant decrease in the level of total serum cholesterol concentration in dairy cows as parturition approaches but the values begin to increase gradually after calving. Similarly, Guretzky et al. (2006) stated that these changes may be attributed to changing pattern of Dry Matter Intake (DMI) during the periparturient period. Kim and Suh (2003) in their study concluded that a decrease in body score is associated with lower cholesterol and its constituents produced due to NEB suggesting that serum cholesterol and its constituents may prove as a useful predictor for the energy balance status of the cow during the transitional period. This imbalance of energy coupled with alternation in serum lipid profile may add up the risk of many infectious and non-infectious diseases that may establish during the transitional period (Radostits et al. 2007, Sordillo and Mavangira et al., 2014). During the past two decades, various studies have been conducted to establish the association between serum cholesterol level and risk of various infectious and non-infectious diseases with varied and inconsistent results. Quiroz-Rocha et al. (2009) in their study concluded that with an increase in the level of serum cholesterol there is an increased risk in cases of retained placenta. Whereas Kaneene et al. (1997) find contradictory observations and correlate the lower level of prepartum concentration of serum cholesterol with increased risk of retained placenta in cows. Lower levels of cholesterol have also been found associated with other diseases. Sepúlveda-Varas et al. (2015) reported a lower level of cholesterol during the postpartum period associated with severe metritis or more than one clinical disease. In the present study although there was a significant increase in the level of cholesterol and its fraction like HDL-C, LDL-C, and VLDL +21 days post-calving compared with the day of calving but the values were significantly lower than the standard bases values in control animals which shows that the cows are still coping with the NEB produced during the transitional period.

Blood protein profile is a significant indicator of health status and represents an initial screening test to identify animals that require further clinical investigations (Bobbo et al., 2017). In the past, serum proteins have been used to evaluate the infections that could occur during the postpartum period and that contribute to a lengthening of the calving-to-conception interval (Mallard et al. 1998). Similarly lower A:G ratio in dairy cows has been associated with subclinical mastitis (Gain et al., 2015). Most of these problems manifest after calving and, usually, establish as early as at the end of the previous lactation or at the end of the dry-up period (Polakova et al. 2010). In the present study significantly ($P \leq 0.05$), lower levels of total protein and globulin were detected in parturient Frieswal cows on the day of the calving compared to the rest of the transitional period and standard base value in the control group. In agreement with the present study Piccione et al., 2011 reported a lower level of total protein and globulin during the first week of lactation when compared to the pre-partum value in HF dairy cows. Similarly, Grünberg et al. 2011 also reported that the total protein concentrations at calving were lower than concentrations outside the parturient period. In the present study, albumin concentration was similar throughout the transitional period with a non-significant small increase on the day of calving. The slight increase of albumin at calving could be due to higher albumin synthesis by the liver or to a decrease of plasma volume masked by hyperglobulinemia (Piccione et al., 2011). In clinical medicine, serum globulin concentration has been used as an indicator of the animal's immune response (Chorfi et al., 2004) but great importance is given to A:G ration than serum globulin concentration alone as it can be used to identify dysproteinaemia (Eckersall, 2008) and a marker to assess immune status of the cow (Piccinini et al., 2004). During the end of pregnancy, serum globulin concentration is low because γ-globulins are transferred from the blood to the colostrum (Weaver et al., 2000). In the present study significant increase in A: G ratio on the day of calving when compared with the values of -21 days pre-calving, +21 days post-calving, and with the standard base value of the control group may be possibly due to increased excretion of globulin in colostrum on the day of calving.   Piccione et al. (2011) reported that stage of gestation and lactation affected serum total protein and globulins (α1, β, and γ) concentration and A: G of five HF cows, particularly during the transition from late gestation to early lactation, when cows must typically cope with pronounced metabolic stress. On the other hand, Cozzi et al. (2011) did not find any effect of stage of lactation when comparing total protein, albumin, and globulin concentrations of plasma from HF cows in early and mid-lactation.

Blood urea nitrogen (BUN) concentration is commonly used as an indicator of protein status or energy balance in dairy cows. In the present study significantly, ($P \leq 0.05$) higher levels of BUN and creatinine were recorded on the day of calving. This significant elevation in the serum creatinine in the present may be attributed to muscular proteolysis during the course of parturition. The other possible reason for the increased BUN might be dehydration in dairy cows during the act of calving (Ismail et al. 2011) or due to impaired liver function due to increased influx of triglyceride in the Liver and the limited ability of cattle liver to export triglyceride via VLDL during early lactation. (De Koster and Opsomer 2013).

The serum macro-minerals like calcium, inorganic phosphorus, and magnesium play an important role in maintaining the normal health and production during the whole life span of a dairy cow. Deficiency or decreased serum concentration of these minerals below the physiological limit especially during the transitional period will lead to clinical or subclinical diseases in dairy cows (Fadlalla et al., 2020). Clinical or subclinical hypocalcemia in dairy cows usually occurs within 24 to 48 hours of parturition and is due to a sudden increase in demand for calcium in colostrum and during early lactation. Significantly ($P \leq 0.05$) lower level of Calcium and inorganic phosphorous on the day of calving in the present study may be physiological concomitant with the onset of colostrum/milk production. Further decrease of calcium on day +21 may be attributed to decrease calcium absorption from intestines due to decreased numbers of receptors for 1, 25-dihydroxy vitamin D in the intestine (Goff 2000).

Liver function enzymes like ALT, AST, and ALP are being used as a promising tool for diagnosis and correction of management and nutritional problems in dairy farms (Bertoni and Trevisi, 2013). In fact, these enzymes along with other metabolites are used to assess the metabolic and pathological status of dairy cows during the periparturient period (Zhou et al., 2016). Significant ($P \leq 0.05$) increase in the activity of AST, ALT, and ALP was seen on the day of calving when compared with -21 pre-calving values of the same group and the standard base value of the control group. In the present study, increased activity of these enzymes on the day of the calving could be attributed to the intense mobilization of body lipid tissue into the liver for production of additional energy to cope with the NEB. This rapid and intense mobilization of lipid tissue may have caused hepatic tissue damage and consequently result in leakage of these enzymes from the liver tissues (Colakoglu et al., 2017). Further, the concentration of enzymes like AST which also has a muscular origin may be increased due to damage of muscular tissue during the act of calving (Kalaitzakis et al., 2010). A significant and consistent increase in the activity of ALP throughout the transitional period could be attributed to the increased enzyme activity originating from uterine tissue or from bones (Kaneko et al., 2008). The level of glucose remained unaltered during the whole transitional period in the experimental and the control group of Frieswal cows. Keeping in view the findings of the present study it can be concluded that Frieswal dairy cows are predisposed to NEB as depicted by a decrease in the total concentration of cholesterol, its fractions, and unable to recoup the same even after +21 days post-calving when compared with the value in the control group and the values at -21 days prior to calving. The dynamic changes in lipid, protein, mineral profile, and other serum metabolites of Frieswal dairy cows during the transitional period will be helpful in deciding and planning managemental intervention to minimize the metabolic stress during the transitional period.

Declarations

Ethics statements: All animal ethics guidelines were followed for the experiment and due permission for the same was obtained from the Institutional animal ethics for conducting the said study.

Conflict of interest: The authors declare that they have no conflict of interest.

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Tables

Table 1: Lipid profile during the transition period of Frieswal cows (mean±SE)

Parameters	Control	21 days Pre calving	Day of calving	21 Post calving
Total Cholesterol (mmol/l)	1.61 ± 0.027^{a}	1.49 ± 0.028^{b}	0.79 ± 0.015^{c}	1.33 ± 0.022^{d}
Triglyceride (mmol/l)	0.49 ± 0.012^{a}	0.52 ± 0.035^{a}	0.28 ± 0.02^{b}	0.39 ± 0.009^{c}
HDL-C (mmol/l)	2.82 ± 0.094^{b}	1.41 ± 0.135^{a}	1.03 ± 0.026^{c}	1.31 ± 0.028^{a}
LDL-C (mmol/l)	0.44 ± 0.018^{b}	0.23 ± 015^{a}	0.12 ± 0.004^{c}	0.25 ± 0.013^{a}
VLDL (mmol/l)	0.082 ± 0.012^{a}	0.075 ± 0.014^{ab}	0.036 ± 0.016^{c}	0.065 ± 0.011^{b}

Mean having different superscripts in the same row differ significantly (P≤0.05)

Table 2: Protein profile during the transition period of Frieswal cows (mean±SE)

Parameters	Control	21 days Pre calving	Day of calving	21 Post calving
Total Protein (g/l)	77.46 ± 2.97^{a}	71.63 ± 3.56^{a}	66.07 ± 3.58^{b}	75.02 ± 4.83^{a}
Albumin (g/l)	33.88±1.71	32.51±1.39	33.39±1.70	32.96±2.56
Globulin (g/l)	43.58 ± 1.03^{a}	39.13 ± 1.70^{b}	32.67 ± 0.87^{c}	42.06 ± 1.21^{ab}
A:G Ratio	0.787 ± 0.034^{a}	0.875 ± 0.041^{a}	1.041 ± 0.049^{b}	0.797 ± 0.035^{a}
BUN (mmol/l)	2.84 ± 0.043^{a}	1.71 ± 0.037^{b}	4.76 ± 0.121^{c}	3.31 ± 0.084^{d}
Creatinine (µmol/l)	109.86 ± 5.62^{a}	97.81 ± 3.18^{b}	163.38 ± 6.27^{c}	130.10 ± 4.76^{b}

Mean having different superscripts in the same row differ significantly (P≤0.05)

Table 3: Minerals and serum metabolite profile during the transition period of Frieswal cows (mean±SE)				
Parameters	**Control**	**21 days Pre calving**	**Day of calving**	**21 Post calving**
Calcium (mmol/l)	2.53±0.057[a]	2.62±0.054[a]	2.04±0.053[b]	2.31±0.099[c]
Phosphorus (mmol/l)	1.59±0.075[a]	1.50±0.082[a]	1.23±0.020[b]	1.32±0.025[a]
Magnesium (mmol/l)	0.77±0.012	0.74±0.014	0.69±0.019	0.73±0.020
AST (u/l)	51.83±1.45[a]	55.27±2.83[a]	75.98±1.52[b]	70.61±2.68[b]
ALT (u/l)	28.72±1.29 [a]	31.10±1.57[a]	34.79±1.54[b]	35.78±0.74[b]
ALP (u/l)	68.98±2.84[a]	76.81±3.32[b]	84.60±2.53 [c]	105.56±2.98[d]
Glucose (mmol/l)	2.75±0.26	2.43±0.25	2.35±0.29	2.18±0.27
Mean having different superscripts in the same row differ significantly (P≤0.05)				