

## DYNAMICS OF LIPID MOBILIZATION AND OTHER SERUM METABOLITES DURING TRANSITIONAL PERIOD IN FRIESWAL DAIRY CATTLE

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### ABSTRACT

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 ( $p \leq 0.05$ ) 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. The dynamic changes in lipid, protein, mineral profile, and other serum metabolites reported in the present study will be helpful in deciding and planning managerial intervention in Frieswal dairy cows during the transitional period.

**Keywords:** Dynamic, Frieswal cattle, Transition Period, Lipid profile, Protein profile, Serum metabolites

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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 hampering their normal metabolism and immune response (Sundrum, 2015). 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). 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).

The current study was planned with an aim to evaluate the serum metabolite alterations and lipid mobilization of elite Frieswal cows during the transition period for devising effective managerial 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

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Institute, Karnal. A total of 32 clinically healthy Frieswal cows 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  $\pm 3$  days. 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 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  $\pm$  SE and p values less than 0.05 were considered significant.

## RESULTS AND DISCUSSION

Every physiological stage during the life span of the dairy cow has its metabolic characteristics. In dairy cow period between -21 days prior to calving and +21 days post-calving is most dynamic in nature. In the present study, at day 0 (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. Similarly, Significant ( $p \leq 0.05$ ) increase in the level of serum total cholesterol, triglyceride, HDL-C, LDL-C, and VLDL were noticed from day 0 to +21 days post-calving which reached to the pre-calving levels (-21 days) except for total cholesterol and triglyceride (table 1).

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 up with this NEB 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 Krebs'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. 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 as most of the lipids would have been directly oxidized through Krebs's cycle to cope up the NEB. 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. (DeKoster and Opsomer, 2013).

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. 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 (Sordillo and Mavangira, 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 post-partum 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

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

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

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

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

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

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

**Table 3. Minerals and serum metabolite profile of Frieswal cows during the transition period (mean±SE)**

Parameters	Control	21 days Pre calving	Day of calving	21 day Post calving
Calcium (mmol/l)	2.53 ± 0.057 <sup>a</sup>	2.62 ± 0.054 <sup>a</sup>	2.04 ± 0.053 <sup>b</sup>	2.31 ± 0.099 <sup>c</sup>
Phosphorus (mmol/l)	1.59 ± 0.075 <sup>a</sup>	1.50 ± 0.082 <sup>a</sup>	1.23 ± 0.020 <sup>b</sup>	1.32 ± 0.025 <sup>a</sup>
Magnesium (mmol/l)	0.77 ± 0.012 <sup>a</sup>	0.74 ± 0.014 <sup>a</sup>	0.69 ± 0.019 <sup>a</sup>	0.73 ± 0.020 <sup>a</sup>
AST (u/l)	51.83 ± 1.45 <sup>a</sup>	55.27 ± 2.83 <sup>a</sup>	75.98 ± 1.52 <sup>b</sup>	70.61 ± 2.68 <sup>b</sup>
ALT (u/l)	28.72 ± 1.29 <sup>a</sup>	31.10 ± 1.57 <sup>a</sup>	34.79 ± 1.54 <sup>b</sup>	35.78 ± 0.74 <sup>b</sup>
ALP (u/l)	68.98 ± 2.84 <sup>a</sup>	76.81 ± 3.32 <sup>b</sup>	84.60 ± 2.53 <sup>c</sup>	105.56 ± 2.98 <sup>d</sup>
Glucose (mmol/l)	2.75 ± 0.26 <sup>a</sup>	2.43 ± 0.25 <sup>a</sup>	2.35 ± 0.29 <sup>a</sup>	2.18 ± 0.27 <sup>a</sup>

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

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 (table 2). 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 but great importance is given to A:G ratio than serum globulin concentration alone as it can be used to identify dysproteinaemia 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  $\gamma$ -globulins are transferred from the blood to the colostrum. 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 (table 2). Piccione *et al.* (2011) reported that stage of gestation and lactation affected serum total protein and globulins ( $\alpha 1$ ,  $\beta$  and  $\gamma$ ) 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.

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 study 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). In the present study, 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 (table 3). This increased activity on the day of the calving could be attributed to the intense mobilization of body lipid tissue into the liver which may have caused hepatic tissue damage and consequently result in leakage of these enzymes from the liver tissues to blood stream (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).

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 concentration of total cholesterol, its fractions, and unable to recoup the same even after +21 days post-calving when compared with the control group and -21 days values of the same group. The dynamic changes in lipid, protein, mineral profile, and other serum metabolites reported in the present study will be helpful in deciding and planning managerial intervention in Frieswal dairy cows during the transitional period.

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