www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 460-463

www.thepharmajournal.com
Received: 14-02-2019
Accepted: 18-03-2019

**Anisha J Perumbilly**
MVSc Scholar, Department of Veterinary Biochemistry, Kerala Veterinary and Animal Sciences University, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

**Shynu M**
Assistant Professor, Department of Veterinary Biochemistry, Kerala Veterinary and Animal Sciences University, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

**Sunanda C**
Assistant Professor, Department of Statistics, Kerala Veterinary and Animal Sciences University, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India

**Correspondence**
**Shynu M**
Assistant Professor, Department of Veterinary Biochemistry, Kerala Veterinary and Animal Sciences University, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

# Metabolic profile indicates NEB and oxidative stress during transition period in stall fed crossbred dairy cows of Kerala

**Anisha J Perumbilly, Shynu M and Sunanda C**

**Abstract**
The concentrations of glucose, non esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), cholesterol, malondialdehyde (MDA) and total antioxidant status (TAS) of 15 healthy crossbred dairy cows maintained under identical conditions in a farm, and fed on standard ration, during transition period were evaluated and compared with that of pre-transition and post-transition periods. The mean concentration of glucose (47.35±1.32 mg/dL) and cholesterol (95.83± 3.62 mg/dL) during transition period was significantly lower than pre and post-transition period. NEFA (0.576 ± 0.08 mmol/L) reported significant increase during transition. The mean concentration of BHB (0.638 ± 0.05 mmol/L) was significantly higher during transition period and the increase was persistent towards post-transition period. The level of MDA, an indicator of oxidative stress was higher during transition period (7.89 ± 1.16mmol/ml), indicating increased rate of lipid peroxidation during the period, whereas TAS did not show any significant change. The blood parameters assessed, indicated negative energy balance and increased lipid peroxidation product during transition period.

**Keywords:** β-hydroxybutyrate, malondialdehyde, metabolic profile, non-esterified fatty acids

## 1. Introduction
Crossbred dairy cows form the major cattle population in Kerala. Although the state adopted scientific breeding policies which aim at higher production potential, higher milk production is always accompanied by greater incidence of diseases more particularly during the period between 3 weeks before calving till 3 weeks after it. This period which is known as the transition period (Grummer, 1995) [1] is the most critical period in the lactation cycle of all dairy cows which is characterized by rapid alterations in endocrine and immune systems and consequent adaptive changes (Puppel and Kuczyriska, 2016) [2]. These changes occur in preparation for parturition and production of colostrum/milk. It has been reported that 75 percent of diseases in dairy herds are reported to occur within the first month of lactation (Leblanc *et al.*, 2005) [3], the maximum risk being the first ten days following parturition (Ingvartsen *et al.*, 2003) [4]. Several causes and risk factors have been identified for the increased disease susceptibility of transition dairy cattle. The major metabolic changes that occur in the animal's body during transition period also contribute to oxidative stress. Metabolic profiling has been reported to provide a valuable method to monitor animal health during this critical period (Payne *et al.*, 1970) [5] In metabolic profiling, blood biochemical constituents are evaluated and are used to assess metabolic homeostasis of the cow which in turn can be used to predict the occurrence of diseases associated with economically important herd parameters like milk yield and reproductive performance.

## 2. Materials and Methods
The study was conducted in 15 pregnant and clinically healthy crossbred dairy cows in second to fifth parity maintained at University Livestock Farm and Fodder Research Station, College of Veterinary and Animal Sciences, Mannuthy and Cattle Breeding Farm, Thumburmuzhy during the period from November 2016 to May 2017. Blood samples were collected at fortnightly intervals from 8 weeks before the predicted calving date until 8 weeks after calving. Approximately 10 ml of blood was collected aseptically from the jugular vein of the selected animals and transferred to a vial without any anticoagulant. Blood was allowed to clot and serum was separated by centrifugation at 3000 rpm for 15 min. Samples were stored at -40°C until further analyzed.

The energy status and oxidative stress status of the animal during transition period were analyzed respectively by measuring the serum level of glucose, NEFA, BHB, cholesterol and MDA, TAS. Concentrations of glucose, NEFA, BHB, cholesterol were measured directly in the semiautomatic analyzer (Hospitex Master T) using commercially available kits. Malondialdehyde (MDA) was measured following the lipid peroxidation assay procedure by Yagi (1984) [6] using the standard 1, 1, 3, 3-Tetramethoxypropane (TMP) manufactured by HiMedia Laboratories Pvt. Ltd. and Total Antioxidant Status (TAS) by ABTS assay as suggested by Re *et al*.(1998) [7] using standard TROLOX (6-hydroxy-2, 5, 7, 8-tetmethychroman-2-carboxylic acid; Sigma Aldrich Co., USA). Both these parameters were measured on UV/VIS spectrophotometer (Perkin–Elmer).

### 2.1 Statistical analysis

Statistical analysis was carried out by classifying the study period into three groups as pre- transition period (-8 wk, -6 wk, -4 wk), transition (-2wk, 0wk, 2wk) and post- transition period (4wk, 6wk, 8wk). Day of calving was considered as day one of lactation. Data was analysed by repeated measure ANOVA using statistical software SPSS version 24.0.

## 3. Results and Discussion

The concentration of various parameters observed during various points of study is shown in Table 1.

**Table 1:** Concentration (Mean ± SE) of various biochemical parameters

| Periods | Glucose (mg/dL) | NEFA (mmol/L) | BHB (mmol/L) | Cholesterol (mg/dL) | Plasma MDA (mmol/mL) | TAS (mM TEAC) |
|---|---|---|---|---|---|---|
| -8 wk | 56.40±3.42 | 0.351 ± 0.12 | $0.520±0.03^{b}$ | $109.26±5.22^{ab}$ | 6.63 ± 1.15 | 0.89 ± 0.06 |
| -6 wk | 51.00±2.10 | 0.308 ± 0.05 | $0.514±0.07^{b}$ | $112.89±5.00^{a}$ | 5.88 ± 1.00 | 1.02 ± 0.06 |
| -4 wk | 52.87±3.88 | 0.368 ± 0.09 | $0.560±0.06^{b}$ | $112.39±6.72^{a}$ | 5.13 ± 1.00 | 0.89 ± 0.06 |
| -2 wk | 49.00±1.85 | 0.702 ± 0.18 | $0.617±0.06^{ab}$ | $96.86 ± 5.67^{b}$ | 7.30 ± 1.84 | 0.92 ± 0.06 |
| 0 wk | 45.06±2.32 | 0.577 ± 0.14 | $0.594±0.06^{ab}$ | $88.70 ± 6.94^{b}$ | 6.75 ± 1.54 | 0.95 ± 0.07 |
| 2 wk | 48.73±2.67 | 0.382 ± 0.17 | $0.703±0.10^{a}$ | $101.92±6.06^{ab}$ | 9.61 ± 2.54 | 0.95 ± 0.05 |
| 4 wk | 51.06±3.09 | 0.247 ± 0.06 | $0.707±0.07^{a}$ | $117.40±10.48^{ab}$ | 5.38 ± 0.91 | 0.98 ± 0.06 |
| 6 wk | 52.06±3.45 | 0.258 ± 0.08 | $0.722±0.09^{a}$ | $122.67 ± 8.72^{a}$ | 5.10 ± 0.94 | 1.00 ± 0.04 |
| 8 wk | 53.00±3.38 | 0.378 ± 0.12 | $0.762±0.06^{a}$ | $126.26 ± 9.98^{a}$ | 4.99 ± 0.72 | 0.93 ± 0.06 |
| F value | $1.269^{ns}$ | $1.348^{ns}$ | 2.177* | 3.139* | $1.246^{ns}$ | $1.058^{ns}$ |

Means bearing different superscripts within a column differ significantly (p<0.05)
ns- Non significant

In this study the concentration of various parameters observed during transition period was compared with that of pre-transition and post-transition period. Table 2 shows the research results on serum levels of glucose, NEFA, BHB as well as cholesterol, in dairy cows during pre-transition, transition and post- transition periods. The concentration of glucose (p<0.05) and cholesterol (p<0.01) showed a significant decrease during transition period while that of NEFA and BHB showed a significant increase (p<0.01). The level of MDA showed a significant increase (p<0.01) during transition period while there was no significant change in the level of TAS.

**Table 2:** Concentration (Mean ± SE) of various biochemical parameters during pre-transition, transition and post-transition periods

| Parameter | Pre-transition | Transition | Post-transition | F value |
|---|---|---|---|---|
| Glucose (mg/dL) | $53.37 ± 1.18^{a}$ | $47.65 ± 1.32^{b}$ | $52.00 ± 1.86^{ab}$ | $3.266^{*}$ |
| Cholesterol (mg/dL) | $111.52 ± 3.22^{a}$ | $95.83 ± 3.62^{b}$ | $122.11 ± 5.53^{a}$ | $9.866^{**}$ |
| NEFA (mmol/L) | $0.372 ± 0.05^{b}$ | $0.576 ± 0.08^{a}$ | $0.279 ± 0.04^{b}$ | $7.216^{**}$ |
| BHB (mmol/L) | $0.531 ± 0.03^{b}$ | $0.638 ± 0.05^{a}$ | $0.731 ± 0.04^{a}$ | $7.843^{**}$ |
| MDA (mmol/ml) | $5.88 ± 0.60^{ab}$ | $7.89 ± 1.16^{a}$ | $5.16 ± 0.49^{b}$ | $3.421^{**}$ |
| TAS (mM TEAC) | 0.94±0.05 | 0.94±0.05 | 0.97±0.03 | $0.669^{ns}$ |

*p< 0.05; **p< 0.01; ns- non significant
Means bearing different superscripts within a row differ significantly

The concentration of glucose observed was within the reported physiological limit of 45mg/dL to 75mg/dL (Kaneko *et al*. (2008) [8]. The concentration of glucose decreased from -8 wk attaining the lower physiological limit at the day of calving and then increasing thereafter; but the change was statistically non-significant. The concentration during transition period was at the lower physiological limit. This is in agreement with the reports of LeBlanc (2010) [9] and Djokovic *et al*. (2015) [10]. Dairy cows are under a state of high energy demand during transition period which includes the late pregnancy and early lactation stages. Simultaneously, the endocrine alterations associated with the process of calving results in reduced dry matter intake by the animal. This imbalance between the energy uptake and utilization is reflected in the lowered concentration of glucose observed in the present study.

The concentration of NEFA showed an inclination to increase towards transition period and decrease afterwards. The highest value of NEFA observed was at two weeks before calving (0.702 ± 0.18 mmol/L) while that of BHB was 8 weeks after calving (0.762 ± 0.06 mmol/L). A highly significant increase (p<0.01) was observed in the concentration of both NEFA and BHB during transition period when compared to pre and post transition period. During transition period the NEB experienced is compensated by the intense lipid mobilization by which the stored triglycerides in the adipose tissue is catabolized to glycerol and NEFAs by adipose tissue lipases. The increased concentration of NEFA and BHB observed during the transition period in the present study is a consequence of the NEB and reduced glucose concentration.

Ketotic shift helps in the utilization of the small amount of available glucose to be prioritized for foetal growth and lactogenesis (Contreras and Sordillo, 2011) [11]. The concentration of NEFA was found to decrease during post-transition period. This could probably be due to its increased uptake by mammary gland for milk fat synthesis or the improvement in dry matter intake by the animal and consequent improvement in energy balance (McArt *et al.*, 2013) [12]. Post-transition period is marked by progressive increase in milk yield and increased concentration of BHB could be attributed to the increased energy demand of lactation (Li *et al.*, 2016) [13].

The mean concentration of cholesterol falls within the reference limits of 80-120 mg/dL in adult healthy cattle reported by Kaneko *et al.* (2008) [8]. It could be inferred from the parameters assessing energy status that the animals were in a low energy level indicating decreased ATP concentration. The activity of HMG CoA reductase, the key regulatory enzyme in cholesterol synthesis, decreases with decrease in ATP concentration. Thus, the observed decrease in concentration of cholesterol also reflects the lowered energy status of animals during transition. Also, during periods of intense negative energy conditions, acetyl CoA becomes restrictive as it gets directed towards ketone body synthesis. Similar results are reported by Gross *et al.* (2015) and Sepulveda-Varas (2015) [14, 15]. Usually inflammatory events are associated with a decrease in cholesterol level and hence cholesterol is reckoned as a negative acute phase protein (Bertoni *et al.*, 2008; Li *et al.*, 2016) [16, 13]. The decrease in concentration during transition could also be due to the transient inflammatory events associated with parturition. The increasing trend in concentration of cholesterol with progression of lactation could be due to the animal's adaptation towards the requirements of lactation and regaining of energy balance.

In transition dairy cows, the increased rate of metabolism results in production of free radicals at a level greater than that can be counteracted by the body's defense mechanism and these results in oxidative stress (Sordillo, 2005) [17]. The concentration of MDA during transition period (7.89 ± 1.16 mmoles/ml) was significantly higher than that of post-transition period. This might be the result of increased oxidants generated by hypermetabolic (catabolic) response to the changes in homeostasis evoked by parturition and lactation. The results are in agreement with that of Castillo *et al.* (2005) who reported increased lipid peroxidation around parturition but with high variation between individual cows [18]. High variation in MDA between individual animals could be observed in this study also. The decreasing trend in the concentration of MDA that could be noted during the post-transition period might be due to the gradual adaptation of the animal's body to the metabolic alterations leading to the animal regaining homeorhesis (Abuelo *et al.*, 2015) [19]. MDA shows a positive correlation with NEFA suggesting that increased lipolysis is associated with increased rate of lipid peroxidation. Thus, adopting practices to alleviate negative energy balance during transition would contribute to better oxidative status also.

Antioxidants are body's natural defense mechanisms against the generation of free radicals. The changes associated with parturition results in a loss in overall antioxidant potential and this could compromise the animal's immunological defenses also resulting in increased incidence of diseases during the transition period (Sordillo, 2005) [17]. The total antioxidant activity remained relatively constant throughout the study period without any significant difference. According to Castillo *et al.* (2005) [18] and Turk *et al.* (2013) [20] a decreased level of TAS were observed during transition period when compared to that of early pregnancy and late lactation.

The lack of significant difference in total antioxidant status in the present study might be due to the inability of the animal to respond to the increasing demands imposed on it. Castillo *et al.* (2006) [21] reported that it is not necessarily a desirable condition to have an increase in TAS value due to adaptive oxidative stress response and also it is not undesirable to have a decreased value of TAS if the production of reactive oxygen species is less. However, in the present study it appeared that the total antioxidant activity was not optimum as eleven out of the fifteen animals studied suffered from ketosis, mastitis, endometritis or hypocalcaemia during the period and remains to be ascertained if supplementation of antioxidants could help in improving the total antioxidant status. The negative correlation shown by TAS (-0.168) with BHB reiterates the oxidative stress suffered by the animal during periods of negative energy balance and consequent lipolysis.

## 4. Conclusion

The remarkable changes observed in parameters like glucose, non-esterified fatty acids, beta-hydroxybutyrate and cholesterol during transition period suggested that high yielding crossbred cattle even when maintained on standard ration are at the risk of developing negative energy balance. This can affect the production and reproduction performance of these animals directly or indirectly through a negative effect on immune system, which in turn adversely affect the profitability of dairying. Good feeding and management practices to provide sufficient energy and to relieve stress on the animal during transition need to be adopted to ensure that dairying remains profitable.

## 5. References


1. Grummer RR. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Journal of Animal Science. 1995; 73:2820-2833.
2. Puppel K, Kuczynska B. Metabolic profiles of cow's blood; a review. Journal of the Science of Food and Agriculture. 2016; 96:4321-4328.
3. LeBlanc SJ, Leslie KE, Duffield TF. Metabolic predictors of displaced abomasum in dairy cattle. Journal of Dairy Science. 2005; 88:159-170.
4. Ingvartsen KL, Dewhurst RJ, Friggens NC. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livestock Production Science. 2003; 83:277-308.
5. Payne JM, Dew SM, Manston R, Faulks M. The use of a metabolic profile test in dairy herds. Veterinary Record. 1970; 87:150-157.
6. Yagi K. Assay for blood plasma or serum. Methods in Enzymology. 1984; 105:328-31.
7. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1998; 26:1231-1237.
8. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. Edn 6. Academic Press, San Diego, 2008, 916p.
9. LeBlanc SJ. Monitoring metabolic health of dairy cattle in the transition period. Journal of Reproduction and Development. 2010; 56:29-35.
10. Djokovic R, Cincovic M, Belic B, Toholj B, Davidov I,

Hristovska T. Relationship between blood metabolic hormones, metabolites and energy balance in Simmental dairy cows during peripartum period and lactation. Pakistan Veterinary Journal. 2015; 35:163-167.
11. Contreras GA, Sordillo LM. Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comparative Immunology, Microbiology & Infectious Diseases. 2011; 34:281-289.
12. McArt JA, Nydam DV, Oetzel GR, Overton TR, Ospina PA. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance Veterinary Journal. 2013; 198:560-570.
13. Li Y, Ding HY, Wang XC, Feng SB, Li XB, Wang Z *et al*. An association between the level of oxidative stress and the concentrations of NEFA and BHBA in the plasma of ketotic dairy cows. Journal of Animal Physiology and Animal Nutrition. 2016; 100:844-851.
14. Gross JJ, Kessler EC, Albrecht C, Bruckmaie RM. Response of the cholesterol metabolism to a negative energy balance in dairy cows depends on the lactational stage. PLoS One. 2015; 10(6):e0121956.
15. Sepulveda-Varas P, Weary DM, Noro M, von Keyserlingk MA. Transition diseases in grazing dairy cows are related to serum cholesterol and other analytes. PloS One. 2015; 10(3):e0122317.
16. Bertoni G, Trevisi E, Han X, Bionaz M. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. Journal of Dairy Science. 2008; 91:3300-3310.
17. Sordillo LM. Factors affecting mammary gland immunity and mastitis susceptibility. Livestock Production Science. 2005; 98:89-99.
18. Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V, Benedito JL. Oxidative status during late pregnancy and early lactation in dairy cows. Veterinary Journal. 2005; 169:286-292.
19. Abuelo A, Hernandez J, Benedito JL, Castillo C. The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. Journal of Animal Physiology and Animal Nutrition. 2015; 99:1003-1016.
20. Turk R, Podpecan O, Mrkun J, Kosec M, Flegar-Mestric Z, Perkov S *et al*. Lipid mobilisation and oxidative stress as metabolic adaptation processes in dairy heifers during transition period. Animal Reproduction Science. 2013; 141:109-115.
21. Castillo C, Hernandez J, Valverde I, Pereira V, Sotillo J, Alonso ML *et al*. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Research in Veterinary Science. 2006; 80:133-139.