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ASSESSMENT OF SERUM LEVELS OF NUTRIENTS AND LIVER ENZYMES DURING EARLY LACTATION IN CROSSBRED DAIRY CATTLE[#]

Aleesha[1], V. Beena[2]*, Athulya Swaminathan[3], V. S. Parvathy[4], B. Ragupathi[5], T. R. Sreekumar[6], S. Harikumar[7], V. L. Gleeja[8], Surej Joseph Bunglavan[9], G.D Vandana[4] and R. Uma[10]

[1]M.Sc., Scholar, [10]Associate Professor, Department of Veterinary Biochemistry, [2]Professor, [6]Associate Professor, [3]M.V.Sc., Scholar, [5]M.V.Sc., Scholar, Department of Veterinary Physiology, [8]Associate Professor and Head, Department of Statistics, [9]Assistant Professor, Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala 680 651, [4]Research Assistant, Centre for Animal Adaptation to Environment and Climate Change Studies, [7]Associate Professor, Department of Livestock Production Management, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala 673 576, Kerala Veterinary and Animal Sciences University

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*Corresponding author: beenav@kvasu.ac.in

ABSTRACT

The study investigates alterations in nutrient utilisation and serum liver enzyme levels in crossbred dairy cattle during the different phases of the early lactation (zero to five days, 25-30 days and 45-60 days post-calving). Seven healthy early lactating cows of third parity were selected from the University Livestock Farm and Fodder Research and Development Station, Kerala Veterinary and Animal Sciences University, Mannuthy, for the research work. Animals were given standard ration with *ad libitum* water. Meteorological variables such as ambient temperature and relative humidity were recorded and the temperature humidity index (THI) was calculated. The overall mean concentrations of BHB, glucose, total protein, AST and P showed no significant differences in all the three phases of early lactation. The mean concentration of TAG showed a significant difference (P>0.05) between zero to five days with 25-30 days and also with 45-60 days. The levels of GGT showed a significant difference (P>0.05) between 25-30 days and 45-60 days. The mean concentrations of Ca showed a significant difference (P>0.05) between zero to five days and 25-30 days also with 45-60 days. Hence it could be inferred that in a season when the THI was below 82 and standard feeding protocols were followed, the animals would be in

positive energy balance even during peak lactation.

Keywords: Early lactation, Temperature Humidity Index, Nutrients, Liver enzymes

INTRODUCTION

In early lactation, dairy cows require more energy than they could obtain from dietary sources to maintain body tissue and milk production. The dairy cow has a massive demand for glucose for milk production and this huge demand for glucose is met by the process of gluconeogenesis by the liver. Most dairy cows experience a state of physiological hypoglycaemia during early lactation due to this sudden increased demand for glucose. High-producing dairy cows during early lactation should rely heavily on energy and protein stores of the body besides the dietary source to meet the high demand for milk production (Botts *et al*, 1979). Since amino acids can provide about 12 per cent` of the lactose in milk, protein reserves are also used for lactose synthesis (Hunter and Millson, 1964). Transitioning from a pregnant, non-lactating to a lactating state causes considerable stress on the dairy cow, which could affect dry matter intake (DMI), milk production and animal health (Grant and Albright, 1995). The reduced feed intake in and around calving, the increased energy demands for the growing foetus in late gestation and milk production during early lactation lead the animal to a negative energy balance (NEB) (Drackley, 2011). During this stage, the body fat of the cow stored in the adipose tissue will be mobilised and utilised as a fuel source. Blood triacylglycerol (TAG) contributes approximately 50 percent of fatty acids used in milk fat synthesis (Emery, 1973).

However, the quantity of fatty acid that could be oxidised by the tricarboxylic acid cycle or exported from the liver as very low-density lipoprotein is limited. When this limit is reached, triglycerides accumulate in the hepatocytes (Goff and Horst, 1997) ultimately impairing hepatic function. Ketone bodies are produced by excessive fat breakdown. Acetone, acetoacetate and β-hydroxybutyrate (BHB) are ketone molecules produced by the incomplete oxidation of fatty acids to acetyl CoA. β-hydroxy butyrate is the most stable ketone body and is suggested as the golden marker for the diagnosis of ketosis (Kaneko *et al*., 2008; Moghaddam and Hassanpour, 2008). Subclinical ketosis was indicated by a level of BHB greater than 1.2 m*M* during the first postpartum week (Ospina, 2010). An increase in the level of blood BHB to 1.4 m*M* was reported to be associated with an increased risk of metabolic disturbances, whereas a level greater than 2000 μ*M* was associated with reduced milk yield (Duffield, 2000).

Minerals like calcium (Ca) and phosphorus are required to balance the complete lactation cycle. Minerals mobilised during early lactation must be replenished before the next calving to maintain health and production. Calcium needs for colostrum production, foetal maturation and incipient lactation are at their peak during the end of gestation. So, in this stage, cows need to mobilise substantial amounts of Ca from bone and increase the efficiency of gastrointestinal tract absorption. Phosphorous (P) is involved in metabolic reactions and energy transfers within the body and is required for normal milk production and growth.

Severe NEB leads to metabolic disorders like ketosis, milk fever, and infectious diseases (Fiore *et al*., 2018). These conditions become severe when the animals are also subjected to thermal stress. Garcia *et al*. (2015) conducted research by examining the correlation between heat stress and various metabolic and milk-related parameters in a cohort of 50 multiparous dairy cows. The findings of the study indicated that in clinical terms, the impact of elevated heat stress on cows was evident when the THI surpassed 80, resulting in a discernible elevation of rectal temperature beyond the threshold of 39.2 °C. So, the analysis of the meteorological parameters should also be done along with the assessment of health and production status for an accurate conclusion.

Metabolic parameters of lipolysis and ketogenesis are useful indicators for measuring early lactation stress in cows. The period from three weeks before to three weeks following parturition is referred to as the transition period. The majority of health issues manifest at this time (Grummer, 1995). Thus, metabolic profiling has been reported as a valuable method for detecting animal health during the transition period. Blood biochemical parameters during the transition period could predict the occurrence of diseases and are also used in the prediction of herd parameters like milk yield and reproductive performance (Huzzey and Overton, 2013). So, this study was formulated to understand the alterations in various physio-biochemical parameters during early post-partum, concerning serum energy status, lipid, protein, Ca and P levels in crossbred cows in their early lactation period. The functional capacity of the liver was also assessed by estimating the liver enzymes aspartate transaminases and γ-Glutamyl transferase.

MATERIALS AND METHODS

The study was conducted at the University Livestock Farm and Fodder Research and Development Scheme, KVASU, Mannuthy, Thrissur, Kerala during the period July 2022 to November

2022. The farm is located at 10°56" N and 76° 26" E at an altitude of 10 m. Multiparous (3rd parity) healthy cows in early lactation formed the subject of this research work. The study period started from zero to sixty days after calving. Meteorological variables such as average minimum temperature, average maximum temperature, average ambient temperature and relative humidity of the farm premises for each month were collected from July to October, 2022, from the Centre for Animal Adaptation to Environment and Climate Change Studies (CAADECCS, KVASU). The average temperature humidity index (THI) was calculated for each month by the formula designed by (Mader *et al*., 2006), i.e., THI= ((0.8*Ambient temperature) + ((percent of Relative Humidity/100) * (Ambient Temperature-14.4) + 46.4), (Jisha, 2020).

Animals were fed with standard ration (ICAR, 2013) with *ad libitum* water (Table 1 and 2). The ration included 1.5 kg maintenance ration + 400 g ration for every one kg milk produced + an additional one kg ration as early lactation allowance for all the animals. The animals were also provided with 35kg of roughage per day. Blood samples were collected at three different intervals from 0-5 days, 25-30 days and 45-60 days after calving. Blood was allowed to clot and serum was separated by centrifugation at 3000 rpm for 15 min. Serum samples were examined using a fully automatic biochemical analyser (Selectra Pro S Lite, Netherlands). β-hydroxybutyrate and glucose were analysed from blood samples collected by puncturing the ear using an electronic handheld device FreeStyle Optium Neo H blood glucose and ketone monitoring system (Abbott Diabetic Care Ltd. Range Road, Witney, Oxon, UK).

The concentration of TAG was based on the method glycerol phosphate oxidase-peroxidase (GPO-POD) method using the colorimetric system (Euro Diagnostic System Ltd., Chennai). Colorimetric determination of total protein based on the principle of the biuret reaction (Agappe Diagnostic Ltd., India).

Aspartate transaminase concentration was estimated based on kinetic assay by mod IFCC method using a commercially available Aspartate transaminase (AST) kit (Coral Clinical Systems, Spain). The estimation of γ-glutamyl (GGT) was based on the carboxy substrate method using γ- glutamyl kit (Coral Clinical System, Spain. Serum Ca estimation was based on the O-Cresolphthalein (OCPC) method (Coral Clinical System, Spain). Phosphorus was estimated using an inorganic phosphorous kit (LABKIT, Barcelona).

The data obtained on various parameters were statistically analysed as per the method of Snedecor and Cochran (1994) using one-way analysis of variance (ANOVA) and repeated measures of ANOVA using computerised software program SPSS V.24.0

RESULTS AND DISCUSSION

The early lactation period imposes metabolic stress on animals because of the altered metabolic demand and reduced dry matter intake due to inflammatory changes associated with parturition (Yuan *et al*., 2013). A thorough analysis of the metabolic profile of the animal during this period would be of great use for adopting better nutritional and management strategies for improving production (Perumbilly, 2019). The blood/serum levels of glucose, BHB, TAG, AST, GGT, Ca, P and total protein were evaluated to assess the nutrient utilisation and hepatic function of seven crossbred healthy lactating dairy cows in early postpartum. Dairy cows in early lactation experience NEB, which is characterised by lipid mobilisation from adipose tissue (Herdt, 2000; Ingvartsen and Andersen, 2000). So, assessment of serum nutrient levels would be of use in assessing the energy status of animals and hence in this study serum levels of nutrients were assessed to understand whether the animals were experiencing energy deficiency during the early stages of their lactation (zero to five days, 25-30 days and 45-60 days). Since the thermal stress imposed on the lactating animals could also have an added effect on the metabolic stress, the meteorological parameters were also analysed during the study period, to understand whether the animals were affected by thermal stress. The THI values in the present study ranged from 77.62 ± 2.05 (in July) to 79.45 ± 0.98 (in October) (Table 3). The maximum average temperature was 28.23 ± 0.73°C in October and the minimum average temperature was 26.28 ±1.47°C in July. But July had the maximum relative humidity (86.25 ± 5.58 %) and October had the lowest relative humidity (75.75 ± 4.05 %).

Jisha (2020) suggested that the cross-bred dairy heifers became stressed only when the THI crossed 82. In the present study, the observed THI was below 82 throughout the study period and the animals did not showed any apparent symptoms of thermal stress during the period of study. So, it could be inferred that the animals were not affected by thermal stress during the study period.

In the current study, the mean concentration of BHB during the whole experimental period of zero to 60 days ranged from 0.61 ± 0.02 m*M*. The concentrations of BHB during zero to five days, 25-30 days and 45-60 days were found to be 0.57 ± 0.09

m*M*, 0.60 ± 0.05 m*M* and 0.65 ± 0.05 m*M* respectively and these values did not show statistically significant differences at five percent level (Table 4). In dairy cattle, the normal level of BHB concentration is less than 1 m*M* (Li *et al*., 2016) and BHB is considered the most stable and easily measurable ketone body for detecting the energy levels and subclinical ketosis in dairy cattle (Duffield, 2000; Geishauser, 2000). Subclinical ketosis is indicated by a BHB concentration greater than 1.2 m*M* (Ospina, 2010). So, in the present study, since BHB concentrations were in the normal range it could be concluded that the animals did not have1 subclinical ketosis throughout the study period. An increase in blood BHB levels during an energy crisis is due to excessive lipid mobilisation from adipose tissue and incomplete oxidation of lipids in the liver (Djoković *et al*., 2015). In the present study, under the existing feeding regime, the animals were not subjected to NEB and they could manage the increased demand for serum lipids for milk production by increased mobilisation of fat reserves.

Glucose is a useful biochemical parameter defining energy status. It is of utmost importance in dairy cows for foetal growth and milk production. In the present study, the mean concentration of glucose during the period of study was observed to be 44.23 ± 0.76 mg/dL. The mean values of glucose for zero to five days, 25-30 days and 45-60 days were 44.42 ± 1.73, 44.85 ± 2.13 and 43.42 ± 0.64 mg/dL respectively (Table 4). As per Kaneko *et al*. (2008) the normal blood glucose level in dairy cattle ranges between 40 to 60 mg/dL. There was a high demand for glucose for lactose synthesis during milk production (Herdt, 2000). In cattle, hepatic glycogen reserve would not be sufficient to support increased glucose demand through glycogenolysis. Gluconeogenesis is the process by which animals adapt to the immediate demand for glucose. There was no significant difference in glucose concentration in three different periods of this study indicating that the animals did not have energy deficiency.

Postpartum increases in AST values were observed in several studies (Tainturier *et al*., 1984; Ingvartsen, 2006; Liu *et al*., 2012 and Mohsin *et al*., 2022). However, in the present study, no increase in AST level could be observed (Table 4); hence, it could be stated that the animals under study had intact hepatic function.

In this study, the overall mean concentration of GGT was found to be 16.97 ± 1.01U/L. The observed GGT values from zero to five days, 25-30 days and 45-60 days were 16.43 ± 1.70 U/L, 16.43 ± 1.02 U/L and 18.06 ± 0.99 U/L respectively (Table 4). There was also a significant difference between 25-30 days

and 45-60 days. The normal levels of GGT in the serum of cattle were reported to be 6.1 to 17.4 U/L (Radostits, 2000). Although the first two values were in the normal range, the last value (45-60 days) was slightly higher than normal. This might be due to the reason that the animals were experiencing oxidative stress due to elevated production. According to Mohsin *et al*. (2022), the GGT levels of ketosis cows were higher than that of healthy cows. Since the animals under study did not have ketosis and also had a normal hepatic function as indicated by GGT levels, the reason that could be attributed to the elevated GGT level would be oxidative stress. As per Koenig and Seneff (2015), GGT is also considered a biomarker for oxidative stress. Though the THI value was below 82, a comparatively higher value of THI observed (79.44) in the last phase of the study might have also been contributed to the elevated oxidative stress. This points to the fact that if at all the animals are provided with the optimum nutritional backup, during periods of peak metabolic stress supplementation of antioxidants would be beneficial to the animal.

In the present study, the overall mean concentration of blood calcium was found to be 8.39 ± 0.16 mg/dL. The concentration of calcium on zero to five days, 25-30 days and 45-60 days were found to be 7.95 ± 0.25 mg/dL, 8.81 ± 021mg/dL and 8.40 ± 0.31 mg/dL respectively (Table 4). The normal concentration of blood Ca was reported to be 8.5 to 10 mg/dL (Goff, 2008). The serum Ca levels showed a significant difference (P>0.05) between zero to five days and 25-30 days also with 45-60 days of lactation but did not show any difference between 25-30 and 45-60 days. A slight hypocalcaemia was noticed during the initial phase of early lactation. Penner *et al*. (2008) also noticed hypocalcaemia during the early phase of lactation. They opined that the sudden demand for Ca for colostrum and milk production would be the reason for the decreased level of blood Ca. In this study, though the animals were slightly hypocalcaemic in the beginning, there was a significant increase of blood Ca in the second phase of early lactation (P>0.05). But this was only slightly higher than the lower value of the normal range. So, in the last phase of the study, the Ca level has fallen slightly below the normal value probably due to the added demand for milk production. So, from this study, it is recommended that an additional Ca supplementation would be beneficial to the animal from the production perspective.

The overall mean concentration of P during the level study period was 5.87 ± 0.18 ranging from 5.400 ± 0.32 mg/dL to 6.143 ± 0.44 mg/dL. The concentrations of P in zero to five days, 25-30 days and 45-60

days were reported to be 5.40± 0.32, 6.08 ± 0.35 and 6.14 ± 044 mg/dL respectively (Table 4). There was no statistically significant difference at the five per cent level in P concentration between the three different points. The average value of serum P was reported to be 5.2 mg/dL (Haag and Jones, 1935). All the serum P levels observed in the present study were in the normal range. So, from the analysis of the metabolic profile, it could be concluded that the animals were provided with a well-balanced diet so that they could maintain optimum nutrient levels during periods of peak energy demand and physiological stress.

The mean concentration of TAG was found to be 18.48 ± 1.38 mg/dL. Triacylglycerol concentrations from zero to five days, 25-30 days and 45-60 days were 14.71 ±2.12, 19.28 ±1.99 and 21.44 ± 0.96 mg/dL respectively (Table 4). A significant difference in TAG concentration was observed (P>0.05) between the mean value of zero-five days with the values of 25-30 and 45-60 days. The observed values of TAG during 45-60 days were not significantly varying from the value of 25-30 days. The normal level of TAG in dairy cattle was reported to be 7-30 mg/dL (Turk *et al*., 2004).So the observed overall mean value and the mean concentrations of TAG at three different stages of lactation were in the reported normal range. Since the animals were in a positive energy balance, the significant increase in TAG levels observed during 25-30 and 45-60 days of lactation might be due to the increased mobilisation of fatty acids for meeting the increased demand associated with milk production. Widayati *et al*. (2019) observed a positive correlation between the blood TAG level and the reproductive performance of the animal. According to them those cattle who maintained the blood TAG levels in the normal range even during the periods of increased demand, showed better reproductive performance postpartum.

The mean concentrations of GGT during the study periods ranged from 16.43 ± 1.706 mg/dL to 18.06 ± 0.99 mg/dL. GGT levels showed significant differences (P>0.05) between zero to five days and 45-60 days but there was no difference between zero to five days and 25-30 days. There was also a significant difference between 25-30 days and 45-60 days (Table 4). The mean concentrations of Ca during the study period ranged from 7.95 ± 0.25 mg/dL to 8.40 ± 0.31 mg/dL (Table 4). The serum Ca levels showed a significant difference (P>0.05) between zero to five days and 25-30 days also with 45-60 days of lactation but did not show any difference between 25-30 and 45-60 days.

SUMMARY

When dairy cattle are reared under standard management and feeding strategies, even during peak periods of metabolic demand and if the animals are not experiencing thermal stress, the animals would not be in a negative energy balance. However, animals undergoing the highest levels of metabolic activity associated with peak lactation are always prone to oxidative stress. Hence, supplementation of antioxidants is a must for sustainable production. At the time of peak lactation, Ca supplementation would also be beneficial to meet the increased blood Ca requirements associated with milk production

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