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Biochemical Indicators of Energy Balance in Blood and Other Secretions of Dairy Cattle: A Review

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ABSTRACT

Negative energy balance condition during transition period is a gateway for production and infectious diseases during periparturient period. Energy balance is calculated directly through difference in energy input and energy output or indirect methods like estimation of energy metabolites (NEFA, BHBA and glucose level), Body condition scoring (BCS), back fat thickness, body weight and milk components (*e.g.*, fat protein ratio), *etc*., at herd level. All the indirect energy balance indicators were studied in relation to postpartum health and performances in dairy animals. Blood biochemical parameters are routinely used for evaluation of general health status of animals. But, their relationship with energy balance in early lactating cows is not clear. In this review, effects of energy balance, critical period to assess the energy balance, and blood biochemical parameters in relation to energy balance, milk yield and health status of dairy animals are discussed. We also discussed energy balance indicators in other biological fluids in dairy cattle.

Key words: Blood biochemical parameters, Dairy cattle, Glucose, Milk, Negative energy balance, Saliva biochemistry.

Energy balance (EB) is defined as the difference between energy consumed from feed and energy required for body maintenance and production in dairy cows. Negative energy balance (NEB) is a frequently experienced by most of the high yielding dairy cows, around calving period due to imbalance between dietary energy supply and production requirements. In fact, NEB is a normal adaptation phenomenon to ensure required quantity of milk synthesis in dairy animals, but the duration and severity of NEB is varying with individual animals. The duration of NEB varies from 2 to 11 weeks and cows normally takes about 6-7 weeks to return positive energy balance. The most severe NEB usually occurs within 1-2 week after calving. The duration and severity of NEB of individual animal is an important factor to decide the postpartum performances and it is influenced by parity (*e.g.*, multiparous cows experience more severe NEB than primiparous cows due to higher milk yield) and transition cow management practices. Occurrence of more severe NEB during early postpartum period is due to calving-associated decreased appetite, feed intake and copious milk secretion. Hormonal and feeding behavioural changes during early lactation period also contribute for lesser dry matter intake (DMI). Therefore, in order to balance the nutrient loss through milk production, cows often mobilize their lipid reserves from visceral or subcutaneous tissue and use them as energy source. The relative contribution of visceral and subcutaneous fats for mobilization of fatty acids are not clear, but few studies reported that visceral fats are more readily mobilized in high yielding cows (Weber *et al*., 2013). Lactating cows will replenish the mobilized fats during mid to late lactation period. The released fatty acids (mainly Non esterified fatty acids: NEFA) are metabolized to Acetyl CoA *via* β-oxidation and enters into Krebs cycle for energy production (Nelson and Cox, 2000). The accumulated Acetyl CoA cannot be completely utilized due to lack of oxaloacetate resulting from glucose depletion. Therefore, excessive acetyl CoA will be converted into ketone bodies, mainly in the form of β-hydroxy butyric acid (BHBA). Therefore, NEFA and BHBA are commonly used as major energy indicators in individual cows and herd levels. This review discusses about blood biochemical parameters in relation to EB and performances and EB indicators in other biological fluids in dairy cattle.

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Effects of negative energy balance in dairy animals

NEB often leads to occurrence of metabolic health disorders, immune suppression, reduced milk yield and fertility during postpartum period. NEB also significantly associated with occurrence of digestive and locomotive disorders (Collard *et al*., 2000). NEB-mediated immune-suppression is associated with various health disorders while, reduction of reproductive efficiency occurs through alteration of pituitary-hypothalamic axis hormones level, alterations of ovarian activities, reduced uterine functionality and uterine immunity (van Knegsel *et al*., 2007; Wathes *et al*., 2007; Fernandez-Novo *et al*., 2020).

EB of dairy cows is measured by several methods. Direct measurement of difference between energy input (feed intake) and energy output (for maintenance, milk yield, growth, and pregnancy) is a traditional method to estimate EB. The indirect methods like estimation of energy metabolites (NEFA BHBA and glucose), Body condition scoring (BCS), back fat thickness, body weight and milk components (*e.g*., fat protein ratio) are also commonly used at herd level. All these EB indicators were studied in relation to postpartum health and performances of dairy animals. For instances, Chapinal *et al*. (2012) found that higher concentration of NEFA, BHBA and lesser calcium during pre-partum and postpartum period were associated with milk loss. However, serum concentrations of NEFA and BHBA were not associated with pregnancy at first AI while lesser calcium during postpartum period was associated with reduced pregnancy at first artificial insemination (AI). Ospina *et al*. (2010a) established critical threshold level for serum NEFA (0.3 mEq/L during pre-partum and 0.7 mEq/L during postpartum) and BHBA (10 mg/dL during postpartum period) and found that the animals with above threshold level had a decreased risk of pregnancy during voluntary-waiting period. Milk yield was decreased in multiparous cows while, increased in heifer cows with above threshold values (Ospina *et al*., 2010b).

Critical period to assess the negative energy balance in early lactating cows

Fenwick *et al*. (2008) reported that second week of postpartum is a physiologically more relevant time to investigate the effect of NEB as the EB goes to lowest point during that period. van Knegsel *et al*. (2007) also reported that NEB was its peak during initial two weeks of postpartum and NEB severity reduce from third week onwards and became positive during 7-9 weeks of postpartum. Studies based on liver fat content during early postpartum period (day 1, 10 and 21) through biopsies and plasma energy indicators during peri-partum period (-20, -7, 0, 7, 14, 28 and 56 days) revealed that EB was more negative in high fat liver (HFL) group with increased NEFA and BHBA levels and decreased plasma glucose levels than low fat liver (LFL) group (Hammon *et al*., 2009). Gross *et al*. (2011) found that plasma glucose levels were lowest at second week of postpartum and increased afterwards, while plasma NEFA and BHBA concentrations were at its peak on second and third week of postpartum, respectively and decreased afterwards. They also observed that homeostatic mechanism and effects of NEB in dairy cow are different during early and mid-lactation period. Metabolic disorders are not exclusively due to NEB and it is also due to homeostatic mechanism during early lactation period. Ilves *et al*. (2012) reported that most of the animals reached nadir of NEB during 10-11 days in milk (DIM; range 10-24). Milk and plasma metabolites were little correlated during early postpartum period and metabolites were found to be more individual variability in plasma than milk. They suggested that phosphorylated saccharide, citrate, and lactose in milk and phosphatidyl choline in plasma as possible energy indicators during early lactation.

Relationship of glucose with energy balance indicators, production and reproduction performance of dairy animals

Glucose is the universal source of energy for the mammalian cells including for energy metabolism and biological synthesis pathways. The blood glucose level is considered as one of the best indicators of EB and development of metritis and clinical endometritis in ruminants. It is negatively correlated with milk yield, NEFA and BHBA levels during first two weeks of postpartum (Bicalho *et al*., 2017; Weber *et al*., 2013). Hagawane *et al*. (2009) found significantly higher level of blood glucose level in dry than the early lactating buffaloes due to heavy drain of glucose for lactose synthesis during early lactation. Knob *et al*. (2021) reported that glucose, NEFA and BHBA were best indicator of EB during transition period and DIM is positively correlated with blood glucose level in dairy cows. Several researchers also reported lower levels of glucose during early lactation and higher level during late lactation (Gonzalez *et al*., 2011; Djokovic *et al*., 2017).

Gonzalez *et al*. (2011) observed that serum concentrations of glucose, total proteins, albumin, and blood urea nitrogen (BUN) were significantly lesser in early than mid lactation period. BHBA and glucose, NEFA and total proteins, NEFA and AST (aspartate transaminase), glucose and creatinine, total protein and AST and globulins and AST were negatively correlated. They indicated that serum glucose, proteins and BUN levels are indicators of hepatic functionality and decreases in their concentration reflects fat infiltration with high lipomobilization. Ruoff *et al*. (2017) stated that plasma glucose concentration is the best indicator of ketosis during first two weeks of lactation. Canfield and Butler (1991) reported that glucose and NEFA plays an integral role in EB estimation during early postpartum and peak NEB occurs at 14th day of postpartum in lactating cows. Bremmer *et al*. (2000) also reported that glucose act as an energy indicator during prepartum period and animals fed with propylene glycol before calving resulted in increased plasma glucose concentration with decreased plasma NEFA and liver triglycerides (TG) concentrations. But, Smith *et al*. (1997) found no effect of propylene glycol on EB indicators such as NEFA, BHBA and glucose.

Glucose is the primary precursor for lactose synthesis, which determines the volume of milk synthesis by mammary epithelial cells. Hence, glucose uptake in the mammary gland plays a key role in milk production and it is required in large amounts for high yielding lactating dairy cows (Zhao and Keating, 2007; Hagawane *et al*., 2009). The mammary gland utilizes 50 to 85% of the whole-body glucose consumption and early lactating cows demands 2.5-fold more glucose than the dry cows (de Koster and Opsomer, 2013). In dairy cows, a higher demand for glucose at the onset of lactation is fulfilled by gluconeogenesis using volatile fatty acids (de Koster and Opsomer, 2013). About 72 g of glucose is required for production of 1 kg milk and 3 kg of glucose is required for the production of 41 kg milk per day (Zhao and Keating, 2007). But, the exact relationship between blood glucose and milk production is not very clear. For instances, Bicalho *et al*. (2017) stated that plasma glucose concentration is negatively correlated with first two weeks of milk yield and Ruoff *et al*. (2017) reported that hypoglycaemia was associated with higher milk production during early lactation period. They found that multiparous cows had a lower glucose concentration than primiparous cows during early lactation period. Gordon (2013) suggested blood glucose concentration ≤2.2 mmol/L had more chance of ketosis development and the animals recovered after treatment with glucose produced more milk than non-ketotic cow. Xu *et al*. (2020) reported that EB was positively correlated with glucose level in plasma, but not in milk and observed lower levels of plasma glucose level during NEB conditions during early lactation and indicated that it could be due to the high priority of mammary gland for glucose for milk synthesis.

Butler (2003) stated that NEB leads to decrease the levels of blood glucose, insulin, and IGF-1, which cause delayed ovulation and anovulation during early lactation period. Glucose and IGF-1 levels act as a metabolic modulator of postpartum ovarian activity in cows (Beam and Butler, 1999). Fonseca *et al*. (2004) observed that feeding with propylene glycol leads to accelerated postpartum cyclicity resumption and also reduced the acyclic cow percentage with higher milk yield. Canfield and Butler (1991) reported that NEB delayed first postpartum ovulation to extent of 10 to 13 days and cows with higher glucose levels showed early ovulation.

Blood biochemical parameters in relation to energy balance, milk yield and health status of dairy animals

Civelek *et al*. (2011) reported that the levels of serum glucose, cholesterol, TG, total proteins, albumin and BUN concentrations were not altered, but the levels of NEFA and BHBA were higher in cows with retained placenta (RP). Our study revealed that cows with RP had significantly lower concentrations of glucose, total protein, cholesterol and higher concentrations of NEFA, BHBA and BUN than cows that normally expelled the foetal membranes (Kumari *et al*., 2016). Djokovic *et al*. (2017) found significantly higher levels of NEFA, BHBA and AST activities in early lactating cows. They found that EB was positively correlated with glucose and negatively correlated with NEFA, BHBA and AST. They also found negative correlation between dry matter intake (DMI) and NEFA, BHBA and AST and positive correlation of DMI with glucose and TG. They suggested that blood biochemical parameters are useful indicators of the nutritional and metabolic status of dairy cows. Wu *et al*. (2019) reported that serum biochemical parameters (BUN, creatinine, glucose, total protein, total cholesterol, total bilirubin, albumin, triglycerides, NEFA and BHBA), oxidative stress indicators (*e.g*., SOD) and hormones (leptin, glucagon, IGF-1, insulin, cortisol) levels are affected by DIM and parity, of which DIM had more effects than parity on biochemical parameters. Stojevic *et al*. (2005) reported that blood biochemical parameters like AST, ALT and GGT levels are influenced by DIM. Seifi *et al*. (2007) observed that NEFA concentration was positively correlated with triglyceride, BHBA and AST and NEFA was negatively correlated with albumin. BHBA had significant correlation with triglyceride and AST. Mohammed *et al*. (2021) reported that serum BHBA level in periparturient diseases affected cows were significantly higher than non-affected cows but, had no significant correlation with milk yield. Blood glucose level had no significant correlation with periparturient diseases, parity, milk yield and days open. Coroian *et al*. (2017) reported that the total protein, cholesterol, albumin, ALT, AST, GGT activities were low in first lactation and increased with parity. Elshahawy and Abdullaziz (2017) reported that total proteins, albumin, globulin, glucose, BUN, total cholesterol, triglycerides, calcium levels were significantly lower in first week of postpartum and then gradually increased during subsequent weeks. Collectively it indicated that serum biochemical parameters are altered with EB, DIM, parity and metabolic disorders.

Once mobilized fatty acids from peripheral tissues is accumulated in liver tissue during early lactation period, the structural and functional integrity of liver cells will be altered and thus hepatocytes will undergo necrosis and release of liver enzymes and bile contents in circulation. For example, mild to moderate association of ALT or SGPT, AST and GGT levels with fatty liver condition was reported by Bobe *et al*. (2004). They also reported moderate increase of circulatory concentration of bile constituents such as bilirubin, bile acids and cholic acid in cows with fatty liver conditions due to obstruction of bile flow. Li *et al*. (2016) reported that plasma glucose levels decreased, and NEFA and BHBA levels increased in clinical ketosis and subclinical ketosis affected cows than healthy cow. Further, total cholesterol was significantly decreased while; AST, ALT and LDH were significantly increased in ketosis cows. They found positive correlations of plasma NEFA with ALT, AST, LDH and negative correlations between the plasma NEFA and total cholesterol (TC). In addition, a positive correlation of BHBA was observed with ALT, AST and LDH and negative correlations was observed between plasma BHBA and TC levels. Calamari *et al*. (2015) studied the relationship of GGT activity in milk with EB and milk yield in healthy cows and found that GGT activity was in decreasing trend during early lactation period until second month. They found negative correlation between milk yield and BUN and positive correlation between glucose and GGT activity. They found no relationship between plasma and milk GGT activities.

Higher blood NEFA levels and lower blood glucose levels are indicative of the normal process of nutrient partitioning that occurs in early postpartum cows. Garverick *et al*. (2013) reported that serum NEFA levels were less and plasma glucose concentrations were greater in cows that conceived at first AI than cows that remained non-pregnant. Kessel *et al*. (2008) estimated blood biochemical parameters, energy metabolites, hormones and milk yield. They classified animals retrospectively based on their plasma BHBA concentration (*i.e*., >1 mM at least once during study period and <1 mM) and found differences in NEFA, glucose, IGF-1 and leptin concentrations but no difference was observed in calculated EB, milk yield and fertility parameters. They found similar trends of acetone level in blood and milk and revealed that the considerable individual variation of the metabolic adaptation in study animals.

Higher levels of plasma NEFA and BHBA and lower glucose level are associated with lower reproductive performance in dairy animals. The NEB cause increase of NEFA, BHBA and liver TG, and decrease the blood glucose and insulin levels in dairy cattle. Significantly more milk SCC and incidence of mastitis treatment was observed in cows fed with lipogenic diets than glucogenic diet. The cyclicity resumption was also tended to be earlier in glucogenic fed group (van Knegsel *et al*., 2007). The blood glucose levels increase once the animal moves to positive EB (Jorritsma *et al*., 2003). Chamberlin *et al*. (2013) found that hypocalcaemia cows had significantly higher NEFA level and more lipid in hepatocytes. However, plasma AST and GGT activities are similar in both the groups. They found no difference in milk yield, milk SCC milk composition and occurrence of clinical mastitis, metabolic diseases, or fertility indicators such as postpartum cyclicity, number of services per conception, and days open. Patel *et al*. (2022) reported that biochemical parameters like NEFA, BHBA, AST, GGT and ALP were significantly increased while triglycerides, TC and glucose levels were decreased significantly in buffaloes affected with hepatic liposis and indicated that these biochemical parameters can be useful for screening the hepatic lipidosis at farm level. Spaans *et al*. (2022) reported that moderate-yielding dairy cows maintained under pasture-based system undergo similar homeorhetic changes to high-yielding cows maintained under total mixed ration. But BHBA concentration was more in moderate yielding cows when compared to their milk yield and only a small percentage of these cows undergoes severe hepatic lipidosis or ketosis conditions. During the transition from gestation to lactation period metabolic functions of liver is increased with more activation of the immune system even in absence of clinical signs of metabolic stress along with changes in mineral concentrations and imbalances in some minerals levels.

Energy balance indicators in milk, saliva and other biological fluids

The existing methods of energy metabolites based early prediction of peripartum complications are laboratory based and thus mostly it is feasible in organized dairy farming conditions. Direct methods of EB calculation are also not practically possible under small holder dairy farming system where any cow-side testing would be ideal. For example, keto-test (M/s Elanco, USA), a cow side milk dip-stick based test kit identify the milk levels of BHBA ≥ 100 µmol/L which is equivalent to blood BHBA levels of 1200 µmol/L and indicative of ketosis. Since, short and medium chain fatty acids of milk are mainly synthesised in mammary gland and long chain fatty acids are mobilized from body fat, milk contains more of long chain fatty acids and lesser quantity of short chain fatty acids in cows with severe NEB (Churakov *et al*., 2021). They also reported that milk biomarkers are more useful for prediction of severe NEB than blood plasma biomarkers. Pires *et al*. (2022) reported that metabolites and fatty acids secreted in milk can serve as non-invasive markers of EB in early lactating cows. They found NEFA concentration in plasma and milk isocitrate and fatty acid (Σ C6:0 to C15:0. and cis-9 C18:1) had the best correlation with EB. Further, milk BHBA, galactose, glutamate, and creatinine were also correlated with energy status and lipomobilization in early lactating dairy cows. Infrared spectroscopy is routinely used for analysis of individual fatty acids concentration and they can be used for prediction of EB (Mantysaari *et al*., 2019). Milk fat to protein ratio and EB have good genetic correlation and thus it is a valid indicator of EB during early lactation period than DMI or single milk components. Reduction of fat to protein ratio during early lactation period would have slight negative impact on milk yield in dairy cows (Buttchereit *et al*., 2011). Milk fat to protein ratio and other milk variables can predict the plasma NEFA level with moderate accuracy and inclusion of body trait such as BCS and body weight along with milk variables models can further increase the accuracy of EB prediction in dairy cows. Xu *et al*. (2020) also identified nine metabolites (glycine, BHBA, creatinine, hydroxyproline, carnitine, aspartate, acetone, *etc*.,) in both milk and plasma and its correlation with EB, particularly in relation to ketosis and β-oxidation of fatty acids. They also reported glycine, choline and carnitine as important metabolites in milk to estimate EB in early lactating dairy cows (Xu *et al*., 2018). Klein *et al*. (2013) observed significant direct relationships between milk acetone levels with plasma BHBA concentration. In contrast, few researchers reported that most of the milk metabolites were not correlated with their plasma concentrations (Maher *et al*., 2013). Identification such relationship of metabolic indicators such as NEFA and BHBA or any biochemical indicators of EB in blood and any secretion such as milk, urine or saliva would be very useful to develop non-invasive cow-side tests for early identification of the animals under NEB.

Saliva is an alternative biological fluid to serum and it is being used for diagnostic purposes in human and veterinary medicine. For instances, salivary NEFA concentrations were estimated in human being in relation to oral health (Kulkarni *et al*., 2012). Several other researchers were also estimated NEFA in human saliva in relation to salivary lipolytic activity (Kulkarni and Mattes, 2014; Neyraud *et al*., 2017) and as a potential gustatory signalling molecule (Kulkarni and Mattes, 2013). In veterinary medicine, Ellah *et al*. (2015) compared serum and salivary biochemical constituents in dairy cows during lactation and dry period and found that the most of the biochemical constituents in saliva changes in different way from serum during lactation and dry period and the variation of biochemical parameters between serum and saliva are not clear in dairy cattle. The relationship among EB, metabolic indicators in blood and saliva has not been studied well in dairy cattle.

Saliva is a watery fluid that lubricates the entire oral cavity and helps for mastication and swallowing of feed in ruminants. It also facilitates for rumen bacteria, protects the upper alimentary tract mucosa, mineralization of teeth, taste perception and acts as buffer for digestion (Pedersen *et al*., 2002). Cattle secrete about 56-220 litters of saliva per day, which contains about 300 to 350 g of sodium carbonate and 86 mg of ascorbic acid with average pH of 8.5 (range of 7.9 to 8.9) (Mallikarjunappa *et al*., 2019). They also reported significant portion of solid part of saliva as proteins, particularly as mucins. Saliva is not only the secretions of salivary glands, it also contains the serum and blood derivatives through various mechanisms such as transcellular or paracellular routes, passive diffusion and active transport. Therefore, saliva is used for early diagnosis, prognosis and monitoring of treatment outcome. The advantages of saliva over serum and tissues samples includes non-invasive method of sample collection, easy method of collection with good cooperation with patients, cost effectiveness, easy for storage and transportation, greater sensitivity due to its good correlation with blood levels. Collectively it makes salivary biomarkers as a promising tool for diagnosis of different disease conditions like cancer, autoimmune, viral, bacterial, cardiovascular, and metabolic diseases like diabetes conditions (Malathi *et al*., 2014). Saliva is also used as a diagnostic tool of COVID-19 (Fernandes *et al*., 2020). Traditionally, it has been recognized that saliva is one of the drug excretion routes in human, particularly for unionized lipid soluble drugs, though it will be again swallowed and reabsorbed (*i.e*., salivary recycling). In most cases, salivary drug concentration represents the free drug concentration in plasma (Babu *et al*., 2014) and has been suggested as alternatives to blood samples in drug monitoring program and used as diagnostic medium for many years (Kaufman and Lamster, 2002). Saliva contains various compounds that can change during local and systemic pathological conditions including inflammation. For example, changes in acute phase proteins and oxidative stress markers during inflammation were reported in saliva of humans and animals. Kulkarni *et al*. (2012) analysed salivary NEFA through gas chromatography-mass spectrometry and found four major species of NEFA such as palmitic, linoleic, oleic and stearic acids with concentrations ranged from 2 to 9 µmol in humans. Neyraud *et al*. (2017) estimated total fatty acid (TFA) and free fatty acid (FFA) concentrations about 9 and 3.6 µg/mL respectively, in human saliva. Similar kind of studies in animals in relation to EB will be useful.

Ellah *et al*. (2015) compared serum and salivary biochemical constituents in healthy dairy cows during lactation and dry period and found that the significantly highest levels of serum FFA, BHBA and aceto-acetic acid during first month of lactation. The serum glucose level was highest and serum FFA, BHBA and AST levels were lowest during the dry period. They also found that the both serum and salivary FFA showed the highest value during the first month of lactation. Saliva contains a high level of GGT. The level of ammonia in saliva was higher than its serum level during all months of lactation and dry period. They suggested that most of the biochemical constituents in saliva changes in different way from serum during lactation and dry period. They estimated the biochemical parameters at monthly interval and not done any correlation between any parameters. Since NEB is more prominent during first month of lactation, more frequent sampling during that time is required to analyse the correlation. Abdulah *et al*. (2017) reported that progesterone concentration was positively associated with glucose levels in plasma and urine in pregnant and non-pregnant zebu cattle, within 24 days of pregnancy. In non-pregnant crossbred cows, the progesterone concentration was positively associated with glucose level only in urine.

Contreras-Aguilar *et al*. (2019) evaluated the alterations of stress, inflammatory and oxidative stress indicators in saliva of mastitis cows. They detected significant increase of cortisol, alpha-amylase activity, uric acid and lactate levels and decreases of cholinesterase activity in saliva of mastitic cows. They reported that significant positive correlations of milk SCC with haptoglobin and uric acid in serum and saliva indicated that salivary biomarkers can be useful for understanding the severity of the mastitis in dairy animals. Contreras-Aguilar *et al*. (2020) studied the alterations in salivary analytes before and after treatment (*i.e*., hoof trimming) of lameness in cows and found that total esterase activity in saliva was higher in lameness cows than healthy cows and these activities was decreased after treatment. The total esterase activity was positively correlated with lameness severity score, but no correlation was observed between salivary total esterase or lipase activity and plasma cortisol level. They indicated that total esterase activity can be a potential marker for lameness in cows.

Dzviti *et al*. (2019) observed non-significant correlation between plasma and salivary cortisol level due to repeated handing and restraining in beef cattle. Singh *et al*. (2018) investigated the cortisol level in saliva and blood of goats that were experimentally induced an endotoxemia and observed significant increase of cortisol concentration in both saliva and blood plasma up to 8 hrs and also found significant positive relationship of salivary cortisol with plasma cortisol concentration. Greenwood and Shutt (1992) estimated stress index through estimation of plasma and salivary cortisol level in goats and found significant increase of both total cortisol and free cortisol in plasma, and only free cortisol in saliva. Schmidt *et al*. (2016) found positive correlation of leptin level with body weight in pigs. Merlot *et al*. (2011) found that the salivary cortisol concentrations increased during stress and also found significant correlation between salivary and plasma cortisol level in pigs. Escribano *et al*. (2014) observed significantly higher salivary testosterone concentrations when pigs were subjected to various stress. Salivary adenosine deaminase (ADA) activity was found to be significantly increased pyometra affected dogs compared to healthy dogs. ADA also had a positive correlation with leucocyte counts and haptoglobin level indicated its diagnostic potential in pyometra (Tecles *et al*., 2018). Caixeta *et al* (2020) reported the relationship of salivary glucose level with diabetes in rats. Shehab-El-Deen *et al*. (2010) indicated alteration of biochemical concentrations in the follicular fluid of dairy cows with NEB and characteristic metabolic changes occur in serum of high yielding cows are also imitated in follicular fluids (Leroy *et al*., 2004).

CONCLUSION

Glucose plays a key role in milk synthesis and altered with EB. Despite of significant correlation with EB indicators, it is not routinely used as a major EB indicator in large ruminants like NEFA or BHBA, due to very tight regulation of glucose balance in high yielding cows such as gluconeogenesis, increased capacity to glucose absorption, *etc*. More studies are required to understand the relationship of glucose with EB and metabolic health status. Alterations of various blood biochemical parameters during early lactation period in relation to EB and health status are scanty and differential. On the other hand, understanding the relationship the blood biochemistry with EB will be useful for improving the management of dairy cows. The changes in milk and salivary biochemical parameters during early lactation period in relation to EB is not clear and such information will be useful to discover the milk constituents and salivary parameters as possible indicators of EB in dairy cows.

Conflict of interest: None.

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