ASSOCIATION OF BLOOD METABOLITES WITH REPRODUCTIVE DISORDERS IN POSTPARTUM MURRAH BUFFALOES

Bhabesh Mili*, Sujata Pandita and Bharath Kumar B.S.

ABSTRACT

The present study was aimed to identify association of selected blood metabolites with postpartum disorders in buffaloes. For this, 25 Murrah buffaloes were selected from National Dairy Research Institute (NDRI) livestock herd. Through clinical examination 5 buffaloes were found to be infected with metritis, 6 with endometritis and 8 exhibited Mastitis. Remaining 6 were apparently healthy. Blood samples were drawn by jugular venipuncture in sterile heparinised vacutainer tubes from each healthy buffalo at 6.00 AM in the morning on days +7, +14, +21, +28, +35, +42, +49, +56 postpartum.

The blood samples were also collected from unhealthy buffaloes twice in sterile heparinised vacutainers on alternate day as and when the symptoms of reproductive abnormalities were noticed. Plasma NEFA and β-HBA level was significantly elevated ($P<0.05$) in buffaloes suffering from endometritis, metritis and mastitis whereas glucose level was significantly low ($P<0.05$). Calcium levels were low ($P<0.05$) in metritis and mastitis cases. The results indicated that deviations in plasma metabolites as against the normal range could be used to predict the health status and postpartum disorders in buffaloes.

Keywords: NEFA, β-HBA, postpartum, endometritis, mastitis, metritis

INTRODUCTION

Buffalo is one of the main sources of milk and meat production in India. Therefore, early identification of both infectious as well as productive diseases during transition time period to early lactation in this species is crucial requirement to prevent production losses. It has been reported in dairy cows that the chances of acquiring infections due to adaptive changes from pregnancy to early lactation are tremendous (Grummer, 1995). The mammary gland of dairy cow requires a tremendous increase in energy demand for milk production at the onset of lactation. This is realized partly by increasing feed intake and partly by fat mobilization from adipose tissue that eventually results in characteristic elevation of circulatory NEFA and β-HBA levels.

However, this homeostatic pathway may not always meet energy requirement; as a result, most of the high yielding cows suffer from metabolic imbalances during early lactation. This metabolic imbalance coupled with impaired immune functions make transition cow more susceptible to postpartum infections. The immunosuppression during the transition period as a result of the change in physiological state was one

Dairy Cattle Physiology Division, National Dairy Research Institute, Haryana, India, *Email: dr.bhabesh87@gmail.com

of the primary factors associated with increased incidence of mastitis during early lactation in dairy cows (Mehrzad *et al*., 2001). The incidence of mastitis was reported to be highest during early lactation in sahiwal cows and buffaloes and during late lactation in case of cross bred cows (Prem *et al*., 1994) as well as high incidence of metabolic and infectious diseases occurring during early lactation (Stabel *et al*., 2003). The blood calcium concentration less than 2.0 mmol/L immediately post-calving might be an indicator of subsequent peri-parturient metabolic diseases (Oetzel, 2004).

Despite significant advances in knowledge of nutrition, reproduction and animal husbandry (Olson, 1992) the incidences of peripartum metabolic and uterine infections/ disorders have not decreased. Incidence of metabolic disorders (milk fever, displacement of abomasum and ketosis), mammary gland infections (mastitis, and mammary gland edema) and reproductive disorders (Dystocia, retained placenta and metritis) have been reported to range from 7.8 to 16.8%, 2.8 to 12.6% and 6.7 to 19.2% respectively in high producing herds (Correa *et al*., 1990). Annual incidence of postpartum uterine infections in dairy buffaloes was recorded to the tune of 20 to 75% (Usmani *et al*., 2001). Thus, early identification of these diseases may especially be useful at this time to overcome future production losses (Huzzey *et al*., 2006). Singh and Singh (1994) reported 50% reduction in milk yield in cow suffering from clinical mastitis and in extreme cases, septicemia or endotoxemia resulted that could cause death (Bradley, 2002). The endometritis in particular increased mean days open by 15, decreased the relative risk of pregnancy by 31% and reduced the rate at which cows became pregnant by 16% where as metritis was associated with 7 more days to first service, 20% lower conception rate at first service, resulting in 19 more days to conception (Fourichon *et al*., 2000).

Although qualitative changes in metabolism that occur during the transition from pregnancy to lactation are well known in dairy cows (Bauman *et al*., 1980) but no sufficient scientific investigation is available with respect to the blood metabolites as predictors of postpartum disorders in dairy Murrah buffaloes-the major milk producing animal in India. Hence, the present study was aimed to investigate quantitative evaluation of selected energy metabolites (non-esterified fatty acid, β-HBA, glucose and Ca) and their association with postpartum disorders in buffaloes.

MATERIALS AND METHODS

The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of National Dairy Research Institute, Karnal, constituted as per the article number 13 of the CPCSEA-rules, laid down by Government of India.

Experimental design

National Dairy Research Institute, karnal, is situated on an altitude of 250 m above mean sea level, latitude and longitude position being 29^{o} 42N and 79^{o} 54E respectively. The maximum ambient temperature in summer goes up to 45^{o}C, and minimum temperature in winter comes down to 0^{o}C with a diurnal variation in the order of 15 to 20^{o}C. The average annual rainfall is 700 mm, most of which is received from early July to mid September. The present experiment was conducted on 25 Murrah buffaloes postpartum that were selected from NDRI livestock herd between September 2011 till May 2012. Through routine clinical examination 5 buffaloes were found to be

infected with metritis, 6 with endometritis and 8 exhibited mastitis. Remaining 6 were apparently healthy. The diagnosis of metritis and endometritis was based on the presence of characteristic symptoms as described by Sheldon *et al*., (2006) and was confirmed by institute herd veterinary officer. Clinical mastitis was diagnosed by an elevated somatic cell counts in milk and visual signs of inflammation such as clumpy, watery, bloody, or yellowish milk. All these buffaloes were maintained under general managemental practices as followed at the institute.

Blood collection and processing

Blood sample (15 ml) was drawn in sterile heparinised vacutainer tubes by jugular venipuncture from each healthy buffalo at 6.00 A.M. on day +7, +14, +21, +28, +35, +42, +49, +56 relative to parturition. But from infected buffaloes, blood was drawn upon diagnosis of metritis, endometritis and mastitis, followed by one more sample on alternate day. The heparinised samples were centrifuged at 3000 rpm for 15 minutes; plasma was analyzed for selected energy metabolites NEFA, β-HBA, glucose and Ca.

Laboratory analysis

The NEFA was estimated by Copper soap solvent extraction method (Shipe *et al*., 1980). β-hydroxy butyrate was estimated by ELISA kit that was purchased from Cayman Chemical Company, Ann Arbor, USA. Plasma glucose and Ca were quantified by kits purchased from Span Diagnostic Ltd., India.

Statistical analysis

The data for healthy buffaloes was analyzed by one way analysis of variance through graph prism version 5 to quantify postpartum variations for peripheral levels of NEFA, β-HBA, glucose and Ca. Since post partum variations were not statistically significant between days, the data for each parameter was clubbed and expressed as mean ±standard error (SE). This served as the reference value for healthy buffaloes. Similarly for unhealthy buffaloes all the values were expressed as mean ± standard error (SE). $P<0.05$ was considered as statistically significant level. The unpaired student "t" test using graph prism version 5 was applied to compare the data of healthy and unhealthy buffaloes. Association of metabolic variables with occurrence of post partum disorders was evaluated by binary logistic regression in SAS software (7.0 versions) which produced odds ratio as an estimate of the strength of association of the two.

RESULTS AND DISCUSSION

The changes in metabolism that occur during the transition from pregnancy to lactation are well known and represent the concept of homeorhesis (Bauman *et al*., 1980) in dairy cows. Rapid changes key metabolites NEFA, β-HBA, glucose and Ca have been blamed for production and could be used as predictors for diagnosis of metabolic and reproductive diseases in veterinary practice in dairy cow but, limited information is available in buffaloes. So, our aimed to find out association of key metabolites NEFA, β-HBA, glucose and Ca with postpartum infection in buffaloes.

We revealed significantly elevated levels ($P<0.05$) of NEFA in buffaloes with metritis, endometritis and mastitis as compared to healthy buffaloes with level averaging 405.47±16.87, 495.89±55.48, 420.28±30.77 and 343.36±19.58 μmol/L respectively in four groups (Figure 1).

The data tallies with previous reports of different authors for cattle. Upon testing the association of metabolic variables with occurrence of post partum disorders by binary logistic regression, the odds ratio was found to be 1.0075 ($P<0.05$) for endometritis and 1.005 ($P<0.05$) for mastitis in 95% confidence limit.

This indicated a positive association of NEFA with endometritis and mastitis but there was no significant association of NEFA with metritis. Leblance *et al*. (2005) reported the high concentrations of serum NEFAs were associated with an increased incidence of periparturient diseases viz retained fetal membranes, ketosis, and mastitis; displacement of the abomasum and immune-suppression in dairy cattle. NEFA concentration >0.4 mmol/L in the last 7 to 10 days before expected date of calving was associated with 2 times increases risk of retained placenta (Leblance *et al*., 2004; Quirtoz-Rocha *et al*., 2009). Bremmer *et al*. (1999) reported that cows with NEFAs<1.2 mEq/L were one-half to one-third as likely to develop clinical mastitis and milk fever, respectively, as compared with cows with ≥1.2 mEq/L. Dyk *et al*. (1995) reported that cows with ≥1.0 mEq/L NEFAs had a higher incidence of retention of fatal membrane, ketosis, and clinical mastitis than the cows with <1.0 mEq/L serum NEFAs. The cut off value of 1.2 mEq/L offered more remarkable significance in odds ratio for mastitis and milk fever than cows with <1.2 mEq/L.

It is well known that the free fatty acids are either oxidized to β-HB by β-HB dehydrogenase or spontaneously decarboxylized to acetone and acetoacetate (Herdt, 2000). However, during the period of negative energy balance increased β-HBA concentration reveals incomplete oxidation of NEFA in tricarboxylic acid cycle (Grummer, 1993). Besides, there are several reports in cattle (Surlyasathapon *et al*., 2000; Hammon *et al*., 2006; Duffield *et al*., 2009) which have revealed high levels under different infections during postpartum period.In our present investigation, registered significantly elevated level ($P<0.05$) of β-HBA in buffaloes infected with endometritis (644.78±68.00 µmol/L) and mastitis (542.74±27.50 µmol/L) and as compared to 322.16±8.10 µmol/L for healthy buffaloes, but non-significant elevation of β-HBA in buffaloes infected with metritis (497.26±22.77 µmol/L) as depicted in figure 2. The odds ratio of 1.02 in all cases indicated a positive association of β-HBA with postpartum infections in buffaloes. Markusfeld (1987) demonstrated an increased risk of metritis in cows with subclinical elevation of ketone bodies in blood. Duffield *et al*. (2009) demonstrated that serum β-BHB concentration >1200 µmol/L at week 1 of calving was associated with 3 times greater risk of metritis and 4 to 6 times increased risk of clinical ketosis; increased probability of subclinical endometritis at week 4 postpartum (Hammon *et al*., 2006) and increased severity of mastitis (Surlyasathapon *et al*., 2000). Our data also indicated a positive association of β-BHB with postpartum health status with levels elevating significantly ($P<0.05$) among infected buffaloes. In contrast Jackson *et al*. (2011) did not find association of β-BHB with metritis or any other post partum infectious disease owing to small statistical power of analysis.

Glucose is the primary metabolic fuel and is required for vital organ function, fetal growth and milk production (LeBlanc, 2010). A minimum level of 40-60 mg/ml is required to maintain the physiological processes of the body (Duke, 1970). It is also a short term energy metabolite. We registered significantly lowered ($P<0.05$) in infected buffaloes with levels averaging 53.28±3, 53.28±3.15 and 45.52±2.26 mg/dl during metritis, endometritis

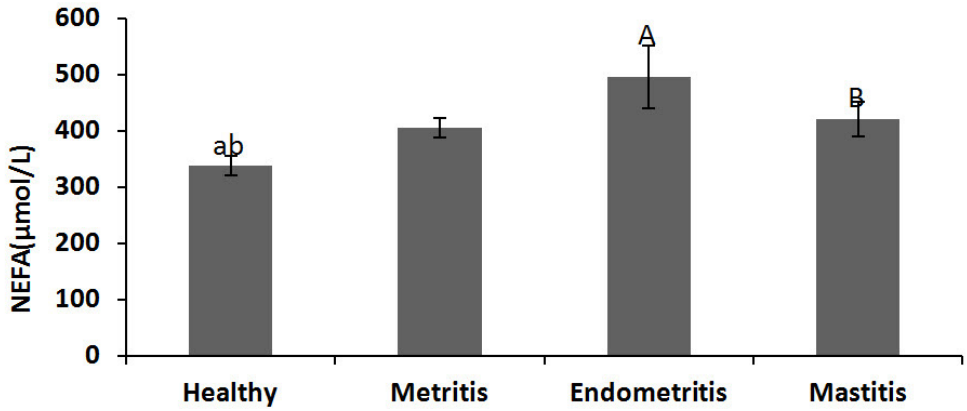

Figure 1. Plasma NEFA concentration in buffaloes exhibiting postpartum disorders.
Bar ab with correspondending A and B superscripts differ significantly (P<0.05) from each other.

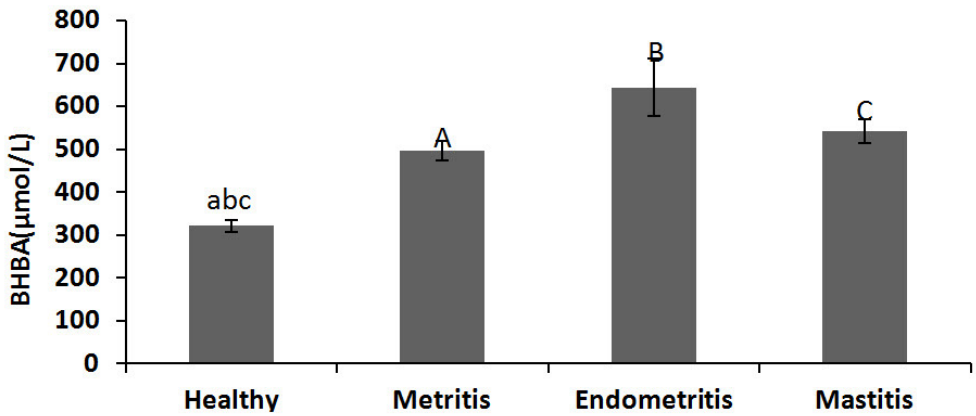

Figure 2. Plasma β-HBA concentration in buffaloes exhibiting postpartum infections.
Bars with superscripts aA, bB and cC differ significant (P<0.05) from each other.

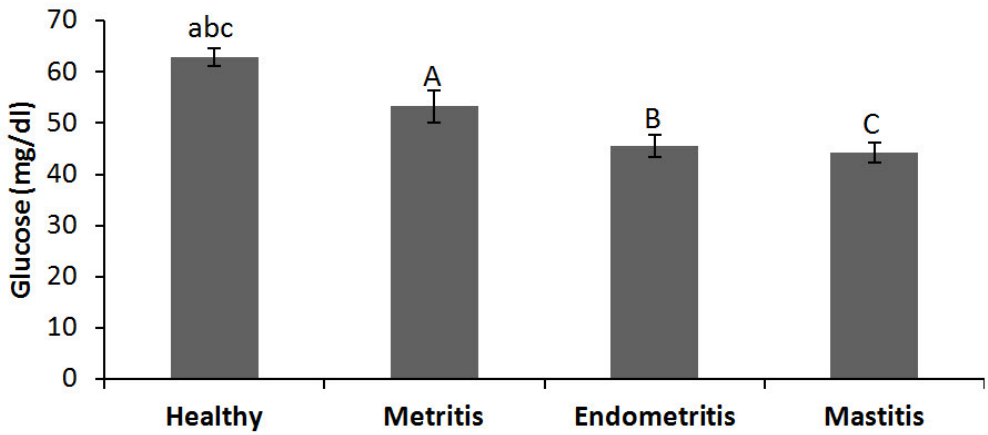

Figure 3. Plasma glucose concentration in buffaloes exhibiting postpartum infections.
Bars with superscript aA, bB and cC differ significantly (P<0.05) from each other.

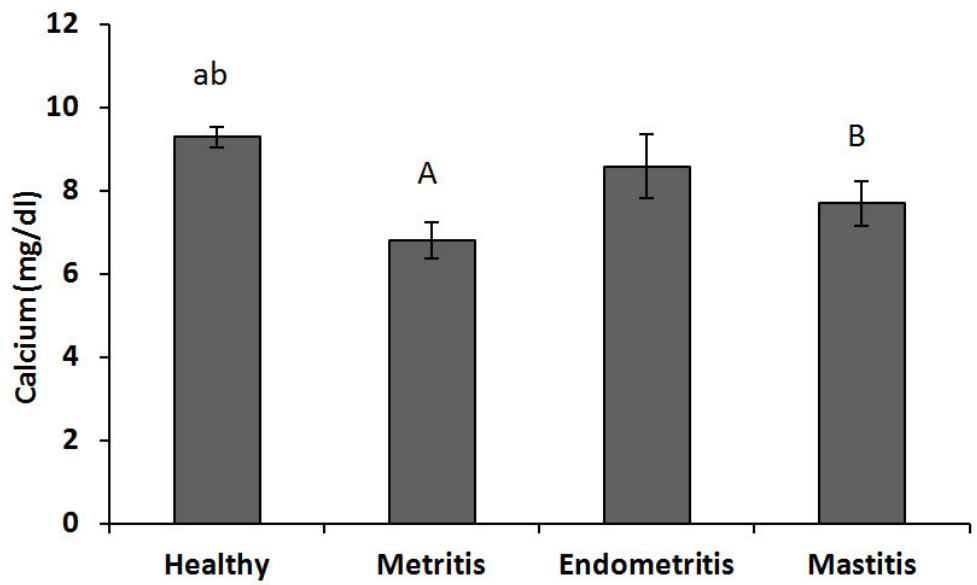

Figure 4. Plasma calcium concentration in buffaloes exhibiting postpartum infections.
Bars with superscripts aA and bB differ significantly (P<0.05) from each other.

and mastitis respectively as against 62.84±1.80 62.84±1.33 mg/dl (Figure 3) healthy group.Magnus and Lali (2009) also reported average glucose level of all the metritic cattle below the reference values of 45 to 75 mg/dl as documented by Radostitis *et al*. (2000). But Majeed *et al*. (1999) recorded (P<0.01) higher serum glucose level in endometritis cows than healthy ones. In contrast, our data indicated a significantly low plasma glucose level (P<0.05) in buffaloes exhibiting endometritis. In binary logistic regression, odds ratio amounting to 0.96 for metritis and 0.86 for endometritis and mastitis respectively in 95% confidence limit indicated a negative association of glucose with metritis, endometritis and mastitis. A negative association of plasma glucose with postpartum infections in buffaloes might be due to negative energy balance during early parts of lactation coupled with immune suppression (Mehrzad *et al*., 2001) making favorable conditions for development of postpartum infections. The reduction in plasma glucose levels might occur in response to energy restriction in the diet (Bremmer *et al*., 1999), especially at the early stage of lactation when rate of glucose utilization is high in the mammary gland (Nazifi *et al*., 2008).

Calcium sensitizes female tubular genitalia for the action of hormone like oxytocin. So, calcium deficiency could act as a predisposing factor for uterine inertia leading to dystocia, retention of fetal membranes and metritis (Dabas *et al*., 1987; Mohanty *et al*., 1994). We observed significantly lower (P<0.05) levels of Ca in buffaloes suffering from metritis (6.80±0.44 mg/dl) and mastitis (7.70±0.53 mg/dl) as shown in Figure 4. The levels in these buffaloes were below the reference range thus indicating hypocalcaemia. Our data is supported by previous researches linking hypocalcaemia to suppressed immune response and hence greater incidence of infectious disorders such as retained placenta, metritis and mastitis (Wilson and Stevenson, 37). The odds ratio for calcium was 0.23 with metritis and 0.65 with mastitis in 95% confidence limit and indicated a negative association of calcium

CONCLUSION

Our data clearly suggested that elevated levels of plasma NEFA and β-BHB coupled with low Ca and glucose was associated with the development of postpartum metritis, endometritis and mastitis. Detection of these selected metabolites could be used for early identification of postpartum infectious diseases to overcome future productive losses. However, large scale studies are required to find out the crucial threshold levels of these attributes to trace out the onset of metritis, endometritis and mastitis.

REFERENCES

Bauman, D.E. and W.B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci*., **63**: 1514-1529.

Bradley, A. 2002. Bovine mastitis: An evolving disease. *Vet. J.*, **164**: 116-128.

Bremmer, D.R., J.O. Christensen, R.R. Grummer, F.E. Rasmussen and M.C. Witbank. 1999. Effects of induced parturition and estradiol on feed intake, liver triglyceride concentrations and plasma metabolites of transition dairy cows. *J. Dairy Sci*., **82**: 1440-1448.

Correa, M.T., C.R. Curtis, H.N. Erb, J.M. Scarlett and R.D. Smith. 1990. An ecological analysis of risk factors for postpartum disorders of Holsteian Frisian cow from thirty two New York farms. *J. Dairy Sci*., **73**: 1515-1524.

Dabas, Y.P.S., S.P. Singh and O.P. Saxena. 1987. Semen level of minerals in crossbred cows and buffaloes during retained placenta and post partum vaginal prolapse. *Indian J. Anim. Rep.*, **8**: 145-147.

Duffield, T.F., K.D. Lisemore and B.W. McBride. 2009. Impact of hyperketonemia in early lactation in dairy cows on health and production. *J. Dairy Sci*., **92**: 571.

Duke, H.H. 1970. *Physiology of Domestic Animals*, 8th ed. Comstock Publishing Associates. Ithaca and London.

Dyk, P.B., R.S. Emery, J.L. Liesman, H.F. Bucholtz and M.J. VandeHaar. 1995. Prepartum non-esterified fatty acids in plasma are higher in cows developing periparturient health problems. *J. Dairy Sci*., **78**: 264.

Fourichon, C., H. Seegers and X. Malher. 2000. Effect of disease on reproduction in the dairy cow: a meta-analysis. *Theriogenology*, **53**: 1729-1759.

Grummer, R.R.1995. Impact of changes in organic nutrient metabolism on feeding the transition cow. *J. Anim. Sci*., **73**: 2820-2833.

Grummer, R.R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J. Dairy Sci*., **76**: 3882-3896.

Hammon, D.S., I.M. Evjen, T.R. Dhiman, J.P. Goff and J.L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. *Vet. Immunol. Immunop.*, **113**(1-2): 21-29.

Herdt, T.H. 2000. Variability characteristics and test selection in herd level nutritional and metabolic profile testing: metabolic disorders of ruminants. *Vet. Clin. N. AM-Food A.*, **16**: 387-403.

Huzzey, J.M., J.J. DeVries, P. Valois and M.A.G. Keyserlingk. 2006. Stocking density and feed barrier design affect the feeding and social behaviour of dairy cattle. *J. Dairy Sci*., **89**: 126-133.

Jackson, R.A., J.R. Wills, N.R. Kendall, J.M. Green, R.D. Murray and H. Dobson. 2011. Energy metabolites in pre- and postpartum dairy cattle as predictors of reproductive disorders. *Vet. Rec.*, **168**(21): 562.

LeBlanc, S.J. 2010. Assessing the association of the level of milk production with reproductive performance in dairy cattle. *J. Rep. Dev.*, **56**: S1-S7.

LeBlanc, S.J., T.H. Herdt, W.M. Seymour, T.D. Duffieid and K.E. Leslie. 2004. Peripartum serum vitamin E, retinol, and beta carotene in dairy cattle and their associations with disease. *J. Dairy Sci*., **87**: 609-619.

Leblance, S.J., K.E. Leslie and T.D. Duffieid. 2005. Metabolic predictors of displaced abomasum in dairy cattle. *J. Dairy Sci.*, **88**: 159-170.

Magnus, P. and K.F. Lali. 2009. Serum biochemical profile of post-partum metritic cow. *Vet. World*, **2**(1): 27-28.

Majeed, M.A., J. Iqbal and M.N. Chaudhry. Blood chemistry of clinical merits in Nili- 1990. Ravi buffaloes of two age groups and at two stages of lactation. *Pak. Vet. J*., **10**(2): 55-59.

Markusfeld, O. 1987. Periparturent traits in serum high dairy herds, incidence rate, association with parity, and interrelationships among traits. *J. Dairy Sci*., **70**: 158-164.

Mehrzad, J., H. Dosogne, E. Meyer, R. Heyneman and C. Burvenich. 2001. Respiratory burst activity of blood and milk neutrophils in dairy cows during different stages of lactation. *J. Dairy Res*., **68**: 399-415.

Mohanty, K.C., B.N. Mohanty, S.K.H. Ray and D.N. Mohanty. 1994. Levels of glucose, calcium and alkaline phosphatase in blood with relation to retention of placenta in bovines. *Indian J. Anim. Rep*., **15**: 21-23.

Nazifi, S., F.M. Mohebbi, E. Rowghani and M.R. Behbood. 2008. Studies on the relationship between subclinical ketosis and liver injuries with first two months of lactation period in high producing Iranian holstein cows. *J. Dairy Sci*., **3**(1): 29-35.

Oetzel, G.R. 2004. Monitoring and testing dairy herds for metabolic disease. *Vet. Clin. N. AM-Food A*., **20**: 651-674.

Olson, J. 1992. Health and reproductive aspects of the peripartum cow. *In Proceeding A Seminar for the Dairy Industry on Dairy Cattle Feeding and Management*. University of Minnesota, St. Paul, MN.

Prem, C., G.D. Behra and A.K. Chakarvarty. 1994. Comparative incidence of mastitis in relation to certain factors in cattle and buffaloes. *Indian J. Anim. Sci.*, **65**(1): 12-14.

Quirtoz-Rocha, G.F., S.J. Leblanc, K.E. Leslie, T.F. Duffieid, D. wood, K. Leslie and R. Jacobs. 2009. Evaluation of peripartum serum cholesterol and fatty acids concentrations as postpartum retained of placenta in dairy cows. *J. Am. Vet. Med. Assoc*., **234**: 790-793.

Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff. 2000. *Veterinary Medicine,* 9th ed. W.B. Sauders Co., London, p. 1820-1821.

Sheldon, I.M., S.L. Lewis, S. LeBlanc and R.O. Gilbert. 2006. Defining postpartum uterine disease in cattle. *Theriogenology*, **65**: 1516-1530.

Shipe, W., G.F. Senyk and K.B. Fountain. 1980. Modified copper soap solvent extraction method for measuring free fatty acid in milk. *J. Dairy Sci*., **63**: 193-198.

Singh, P.J. and K.B. Singh. 1994. A study on economic losses due to mastitis in India. *Indian J. Dairy Sci*., **47**: 265-272.

Stabel, J.R., J.P. Goff and K. Kimura. 2003. Effect of supplemental energy on metabolic and immune measurement in periparturients dairy cows johne's diseases. *J. Dairy Sci.*, **86**: 3527-3535.

Surlyasathapon, W., C. Hever, E.N. Noordhuizen-Stasen and Y.H. Schukken. 2000. Hyperketonemia and udder defiance review. *Vet. Res*., **31**: 397-412.

Usmani, R.H., N. Ahmad, P. Shafiq and M.A. Mirza. 2001. Effect of subclinical uterin infections on cervical and uterine involution, oestrus activity and fertility in postpartum buffaloes. *Theriogenology*, **55**: 563-571.

Wilson, G. and M. Stevenson. 1998. *Calcium Crucial Both Sides of Calving*. New Zealand Dairy Exporter. 32p.