The Indian Journal of Veterinary Sciences & Biotechnology *(2017) Volume 13, Issue 1, 1-8*

ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT

http://dx.doi.org/10.21887/ijvsbt.v13i01.8725

Submitted : 07-03-2017 *Accepted : 18-04-2017* *Published : 16-08-2017*

Effect of Bypass Fat and Minerals Supplementation during Transitional Period on Plasma Levels of Thyroid Hormones, Metabolites and Postpartum Fertility in Crossbred Cows

V.K. Theodore, M.T. Panchal, A.J. Dhami*, S.C. Parmar, B.B. Bhanderi and Sandhya Chaudhary

Department of Animal Reproduction Gynaecology and Obstetrics

College of Veterinary Science and Animal Husbandry, AAU, Anand - 388 001, India

Corresponding Author: ajdhami@aau.in

Abstract

Twenty healthy advanced pregnant crossbred (HF x K) cows of 2-4 parity were included in the study from 2 weeks prepartum to 8 weeks postpartum. They were equally divided in to control (routine farm feeding-RFF) and treatment (RFF plus area specific multi-minerals @ 50 g/h/d and bypass fat @ 100-200 g/h/d) groups to evaluate the effect of bypass fat and minerals supplementation on plasma metabolites and hormonal profile and postpartum fertility. The plasma levels of T_3 increased (P<0.01) on the day of calving and then abruptly dropped till day 14 postpartum as compared to levels at other periods, while T_4 decreased (P<0.05) consistently throughout the study period particularly in control group. However, the influence of nutritional supplementation was found to be non-significant on both these hormones. The blood glucose levels were at peak concurrent to highest plasma cortisol on the day of calving. The cows under supplemented group had significantly (p<0.01) higher mean blood glucose (64.51±3.10 vs 59.13±3.06 mg/dl) and cortisol (16.19±2.51vs 11.23±1.34 ng/ml)than the control cows. The mean plasma levels of NEFA increased from day 14 prepartum to the highest (p<0.01) on the day of calving and thereafter reduced in postpartum days in both the groups with significantly higher mean value in supplemented than control group (0.58±0.09 vs 0.47±0.07 mmol/l). The prepartum plasma BHBA also increased as parturition approached, continued to increase further in the early postpartum period to reach a peak (1.21±0.09 and 0.87±0.04 mmol/l for control and treatment group) (p<0.01) on day 14, and thereafter decreased. In general, the plasma NEFA, BHBA, cortisol and glucose levels were found to be higher in the cows of nutrient supplemented group which had lesser time intervals for expressing first postpartum estrus and service period with better pregnancy rate. Thus the nutrient supplementation during transition period was beneficial in maintaining energy status of the cows and improved reproductive performance postpartum.

Key words: Crossbred cow, Transition period, Nutritional management, metabolic profile, postpartum fertility.

Introduction

The transitional period, also called peripartum period, is characterized by a decrease in dry matter intake particularly with onset of lactation leading to sharp decrease in glucose and an increase in body fat mobilization in the form of NEFA and results in the accumulation of products of incomplete oxidation of NEFA such as BHBA (Vazquez-Anon *et al.*, 1994). The circulating NEFA and BHBA are the commonly used indices of negative energy balance (NEB) or ketosis in transition animals. Although some elevation of these metabolites is normal as these animals balance energy intake and energy demands in early lactation, excessive elevation of NEFA or BHBA can indicate poor adaptation to NEB (Herdt, 2000). At the time of parturition, progesterone and estradiol concentrations cascade to basal levels facilitating the almost immediate resumption of recurrent transient increase in FSH within 3–5 days of parturition that occurs in 7- to 10-days interval and produces a dominant follicle by days 7-10 postpartum (Crowe *et al.*, 1993).

Fats in the diet can influence reproduction positively by altering both ovarian follicle and corpus luteum function via improved energy status and by increasing precursors for the synthesis of reproductive hormones such as steroids and prostaglandins (Rahbar *et al.,* 2014). The dietary fat supplementation has been reported to hasten uterine involution and postpartum first ovulation with greater follicular population, larger preovulatory follicles and elevated plasma progesterone levels in cattle (Lammoglia *et al.,* 1997; Khalil *et al.,* 2012) and buffaloes (Hussein *et al.*, 2013). Therefore, this study was planned to assess the effect of incorporating minerals as well as bypass fat in the ration of transitional crossbred cows on their plasma metabolic and hormonal profile and postpartum fertility.

Materials and Methods

Selection and Management of Animals

This investigation was carried out at University Farm in Anand during November 2014 to May 2015 on 20 advanced pregnant HF x Kankrej crossbred cows of 2nd to 4th parity. The experiment was initiated at two weeks prepartum by randomly dividing the animals equally into control and treatment groups. All the pregnant cows were maintained in well ventilated hygienic sheds and were stall fed as per routine farm feeding schedule and had free access to drinking water. The cows approaching parturition were segregated in calving pen, and calving events were monitored closely to follow weaning system and machine milking.

Experimental Groups

Control Group (n=10): The cows of control group were maintained on routine farm feeding schedule (green fodder, hay and compounded concentrate mixture @ 18-20, 4-5 and 3.0-3.5 kg, respectively, with 50 g of mineral mixture, Amul brand) during last two months of pregnancy and early postpartum. After calving the level of concentrate fed was @ 40 per cent of milk produced.

Treatment Group (n=10): These cows, in addition to routine farm feeding, were supplemented daily with extra 50 g of area-specific chelated multi-minerals (developed by AAU) and 100 g of bypass fat (Sunegry, Polchem) with compounded concentrate mixture (Amul brand) for 2 weeks each before and after calving. The level of bypass fat was then increased as per the milk production @ 15 g per litre of milk produced until 60 days postpartum limiting to maximum of 200 g/day.

Blood Sampling, Lab Assay and Reproductive Events

Blood samples were collected from jugular vein in heparinised vacutainers on days -14, -3, 0, 3, 14, 28 and 42 of calving from all the cows. The level of blood glucose was estimated immediately in fresh blood by direct strip technique using Accu-Chec Integra Kit (Roche Diagnostics India Pvt Ltd).Then the plasma was separated out by centrifugation of blood samples and stored at -20°C with a drop of merthiolate until analyzed. The levels are plasma non-esterified fatty acids (NEFA) were estimated by colorimetric method (DeVries *et al.,* 1976) using diagnostic kits (Randox, India), and those of plasma betahydroxybutyrate (BHBA) using ELISA assay kits (Cayman Chemicals, USA) (Galan *et al.,* 2001). The plasma levels of hormones cortisol, T_3 and T_4 were estimated by employing standard RIA techniques. Labelled antigen (with I^{125}), antibody coated tubes and standards were procured from Immunotech, France. The sensitivities of the assays were 0.10, 0.24 and 0.50 ng/ml for three hormones, respectively.The occurrence of first estrus and fertile estrus postpartum and conception rates were also recorded. Cows showing estrus 60 days after calving were inseminated and pregnancy was confirmed 45 days after last AI.

Statistical Analyses

The periods for first estrus postpartum and fertile estrus were compared between two groups using 't' test. The data on plasma hormones and metabolites within group were analyzed using ANOVA and DNMRT, and between groups by 't' test for each trait employing SPSS software version 20.00 (Snedecor and Cochran, 1994).

Results and Discussion

Plasma Tri-iodothyronine (T_3) and Thyroxine (T_4) Profile

The mean plasma concentrations of tri-iodothyronine (T_3) among both nutrient treated and control cows increased significantly ($p<0.01$) on the day of calving as compared to values on day 14 prepartum, and dropped suddenly on days 3 to 14/28 postpartum with gradual recovery in subsequent periods. However, no significant difference was noted between control and treatment groups at any of the intervals including overall values (Table 1). As regards thyroxine (T_4), among control group a gradual and significant decrease was observed in the values throughout the study period while in treatment group the decline was not significant and no significant difference was noted between groups at any of the intervals. These observations with respect to trend and levels of thyroid hormones (T_3& T_4) agreed well with the reports of Lohan *et al*. (1989) and Garg *et al*. (1997),who showed that serum T_3 and T_4 levels were low from day 1 to 28 after calving compared with advance pregnancy, and again increased on day 35 and 42 postpartum indicating a role of thyroid hormones in the resumption of postpartum ovarian activity. Aruga *et al.* (2001) observed a decreasing trend of serum T_4 and T_3 levels 2 weeks before calving reaching the minimum values by 35 days after calving and thereafter both recovered by about 2 months postpartum in HF cows. The serum thyroid hormone levels are also shown to be influenced significantly by the stage of estrous cycle (Borady *et al*., 1985) and stage of lactation (Garg *et al.,* 1997). The relatively low

Table 1: Mean plasma thyroxine and tri-iodothyronine levels during peripartum periods in crossbred cows under control and nutrient supplemented (treatment) groups

Days pre- and postpartum	Thyroxine (ng/ml)		Tri-iodothyronine (ng/ml)	
	Control group (n=10)	Treatment group (n=10)	Control group (n=10)	Treatment group (n=10)
-14	35.84±3.22^{b}	31.58±3.33	1.15±0.10^{b}	1.12±0.08^{b}
-3	35.07±3.10^{b}	32.17±3.35	1.10±0.11^{b}	1.08±0.13^{ab}
0	33.53±3.18^{ab}	29.98±2.94	1.48±0.13^{c}	1.40±0.15^{c}
3	30.68±2.27^{ab}	27.88±1.72	1.03±0.13^{ab}	0.94±0.18^{a}
14	30.58±1.96^{ab}	27.59±2.00	0.87±0.11^{a}	0.91±0.09^{a}
28	29.31±1.81^{ab}	28.23±2.17	0.96±0.10^{a}	1.16±0.13^{b}
42	26.83±1.39^{a}	27.38±1.41	1.21±0.14^{b}	1.33±0.12^{bc}
Overall	31.69±0.98	29.26±0.94	1.11±0.05	1.12±0.05

The means bearing different superscripts within columns differ significantly (p<0.01).

levels of T_3 and T_4 observed around 3-5 weeks postpartum followed by a rise in the present study might be related with resumption of ovarian follicular activity and first estrus postpartum as observed by Lohan *et al.* (1989) and Garg *et al.* (1997).

Blood Glucose and Plasma Cortisol Profile

The mean blood glucose levels of animals varied significantly between different intervals peripartum in both control and treatment groups, but not between groups at any of the intervals studied (Table 2).The mean blood glucose value spiked up (p<0.01) to reach an apex of 70.20±5.24 and 80.20±6.35 mg/dl on the day of calving in control and treatment groups, respectively, and reduced back to normal levels within the third day postpartum. The values, thereafter, fluctuated non-significantly between other postpartum intervals. The overall mean blood glucose in treatment group was higher (p<0.01) than the control group (64.51±3.10 vs. 59.13±3.06 mg/dl), which can be attributed to the effect of bypass fat supplementation in the diet of treatment group. The mean blood glucose levels observed in cows during pre- and postpartum periods corroborated with the findings of Arieli *et al.* (2008), Cerri *et al.* (2009) and Garverick *et al.* (2013). The increased mean blood glucose concentration obtained on the day of parturition also corroborated with the observations of Hadiya (2006), who reported higher blood glucose concentrations at the time of parturition, which might be due to sudden increase in demand for rapid influx of energy for lactation to start with multiple physiological demands and increased cortisol levels noted at calving leading to gluconeogenesis. In contrast to this, Setia *et al.* (1992) recorded gradual and significant rise in blood glucose level with advancing lactation from calving till 12^{th} week postpartum in cows due to increased cortisol which leads to the production of glucose by gluconeogenesis. The mean blood glucose levels found in the cows under both the groups were within the normal limit (42-75 mg/dl) and concurred with the findings of Kumar (2000), Sivaraman *et al.* (2002) and Arieli *et al.* (2008). The blood glucose level gives an indication of the energy status of an animal. Oxenreider and Wagner (1971) found that both energy intake and lactation had a significant effect on plasma glucose levels during the first 8 weeks postpartum in cows, with negative correlation between plasma glucose and postpartum interval to ovulation.

Table 2: Mean blood glucose and plasma cortisol levels during peripartum periods in crossbred cows under control and nutrient supplemented (treatment) groups

Days pre- and postpartum	Glucose concentration (mg/dl)		Cortisol concentration (ng/ml)	
	Control group (n=10)	Treatment group (n=10)	Control group (n=10)	Treatment group (n=10)
-14	59.60 ± 2.08^{a}	63.60 ± 1.98^{a}	9.92 ± 1.76^{a}	$6.68 \pm 1.02^{a*}$
-3	58.70 ± 3.00^{a}	64.20 ± 2.46^{a}	8.87 ± 1.96^{a}	10.06 ± 2.07^{ab}
0	70.20 ± 5.24^{b}	80.20 ± 6.35^{b}	30.66 ± 1.44^{b}	28.60 ± 2.09^{d}
3	58.30 ± 2.73^{ab}	58.90 ± 2.24^{a}	7.10 ± 0.45^{a}	$19.89 \pm 4.59^{bcd*}$
14	56.50 ± 2.93^{a}	60.10 ± 2.50^{a}	8.49 ± 1.42^{a}	13.90 ± 3.75^{abc}
28	54.20 ± 3.51^{a}	62.60 ± 3.06^{a}	6.90 ± 1.21^{a}	$21.05 \pm 3.52^{cd*}$
42	56.40 ± 1.92^{a}	62.00 ± 3.10^{a}	6.46 ± 0.71^{a}	$13.19 \pm 2.91^{abc*}$
Overall	59.13 ± 3.06^{p}	$64.51 \pm 3.10^{q**}$	11.23±1.34	16.19±2.51**

*The means bearing different superscripts within columns differ significantly (p<0.01) between the time intervals; ** (P<0.01) between groups.*

The mean plasma cortisol concentrations (ng/ml) in cows of both the groups were at the basal level between day 14 and 3 prepartum, but increased rapidly to nearly 3 times ($p<0.01$) on the day of parturition to reach a peak of 30.66±1.44 and 28.60±2.09 ng/ml for control and treatment groups, respectively. Thereafter, the levels decreased significantly on day 3 postpartum in control cows and non-significantly fluctuated in treatment group for all the subsequent days postpartum (Table 2). The nutrients supplemented cows tended to have significantly ($p<0.01$) higher cortisol values than the control cows for most of the days, both pre- and postpartum, with an exception on day of parturition. The observed levels and trend of changes in cortisol values were in accordance with the findings of Chaiyabutr *et al.* (2000) and Vannucchi *et al.* (2015). Ahmed *et al.* (2013) opined that the levels of cortisol postpartum had effects on luteal activity. It is suggested that cortisol may play a role in the corpus luteum as an anti-apoptotic factor in the bovine luteal cells (Rueda *et al.,* 2000) and that the CLs have the potential to respond to a locally generated cortisol (Michael *et al.,* 2003). The higher blood glucose noted on the day of calving was positively associated with significant rise in plasma cortisol concentration, a stress/gluconeogenic hormone.

Plasma Non-Esterified Fatty Acids and Beta-Hydroxy Butyrate Profile

The data on mean plasma NEFA concentration in cows under control group (Table 3) evinced that there was an increase in the value from 0.38±0.06 mmol/l on day 14 prepartum to the highest 0.72±0.09 mmol/l on the day of calving and thereafter the values declined subsequently from days 3 to 42 postpartum to reach 0.28±0.03 mmol/l. Similar trend was also observed in the cows under treatment group. The mean plasma concentration of NEFA in fat supplemented group was higher than in control at both pre- and postpartum periods with significant ($p<0.01$) difference in overall mean values (0.58±0.09 vs. 0.47±0.07 mmol/l). This was also substantiated by the findings of Staples *et al.* (2005). Peripartum supplementation of bypass fat probably reduces the process of lipogenesis by adipose tissues which caused increased lipolysis resulting into the higher NEFA values (Staples *et al.,* 2005). The present findings corroborated with the observations of Cerri *et al.* (2009), Garverick *et al.* (2013), Benzaquen *et al.* (2015) and Shin *et al.* (2015). All these workers observed NEFA values to be higher at parturition and the early postpartum days.

Table 3: Mean plasma non-esterified fatty acids and beta-hydroxybutyrate levels during peripartum periods in crossbred cows under control and treatment groups

Days pre- and post-partum	Non-esterified Fatty Acids (mmol/l)		Beta-hydroxybutyrate (mmol/l)	
	Control group (n=10)	Treatment group (n=10)	Control group (n=10)	Treatment group (n=10)
-14	0.38±0.06^{abc}	0.53±0.06	0.51±0.05^{ab}	0.46±0.05^{a}
-3	0.43±0.05^{abc}	0.61±0.10	0.60±0.06^{ab}	0.60±0.06^{ab}
0	0.72±0.09^{c}	0.70±0.08	0.70±0.05^{bc}	0.67±0.14^{ab}
3	0.62±0.11^{c}	0.63±0.08	0.90±0.05^{c}	0.63±0.05^{ab}
14	0.57±0.08^{bc}	0.62±0.07	1.21±0.09^{d**}	0.87±0.04^{b}
28	0.29±0.03^{a}	0.51±0.11	0.36±0.04^{a}	0.51±0.08^{a}
42	0.28±0.03^{ab}	0.46±0.10	0.42±0.06^{ab}	0.54±0.12^{a}
Overall	0.47±0.07	0.58±0.09**	0.67±0.06	0.61±0.08

*The means bearing different superscripts in columns differ significantly ($p<0.01$) between the time intervals; ** ($p<0.01$) between groups.*

In the present study, the mean plasma BHBA values in both the groups increased as parturition approached. The values tended to increase further in the early postpartum period to reach a peak of 1.21±0.09 and 0.87±0.04 mmol/l for control and treatment group, respectively ($p<0.01$) on day 14 postpartum, and thereafter decreased (Table 3). The BHBA values tended to be higher in control group than in treatment group particularly during early postpartum indicating negative energy balance; however the difference was non-significant and none of the animals evinced signs of ketosis. The increasing trend of mean BHBA levels observed corroborated with the findings of Wathes *et al.* (2007), Cerri *et al.* (2009) and Benzaquen *et al.* (2015). The transition period is characterized by a decrease in dry matter intake leading to a sharp decrease in glucose and an increase in body fat mobilization in the form of NEFA and results in the accumulation of products of incomplete oxidation of NEFA such as BHBA (Vazquez-Anon *et al*., 1994). The circulating NEFA and BHBA are the commonly used indices of NEB or ketosis in transition animals. Although some elevation of these metabolites is normal as these animals balance energy intake and energy demands in early lactation, excessive elevation of NEFA or BHBA can indicate poor adaptation to NEB (Herdt, 2000).

The present findings in general concurred with Rahbar *et al.* (2014), who opined that the supplemental fat may increase glucose production, which in turn may have a positive effect on LH release. Supplemental fat stimulated programmed growth of preovulatory follicle, increased total number of follicles and increased the size of preovulatory follicle.NEB delayed the time of first ovulation postpartum through inhibition of LH pulse frequency and low level of blood glucose (Butler *et al.,* 2000). In the present study, the nutrients treated cows had higher blood glucose, cortisol, NEFA and lower BHBA profile and had initiated postpartum ovarian activity comparatively earlier than the control cows (38.00±1.95 vs. 42.32±4.14 days), with shorter service period (85.22±7.17 vs. 100.67±5.60 days) and improved conception rate (80 vs 60 %).Therefore, it would be worth to infer that peripartum nutrients supplementation might have played its positive contributory role in view of early exhibition of postpartum first and fertile estrus through preventing NEB.

Acknowledgements

The authors thank Dean of the Faculty and Research Scientist Livestock and Research Station for the facilities and cooperation extended. The financial support received from ICAR, New Delhi under the project “AICRP on Nutritional & Physiological Interventions for Enhancing Reproductive Performance of Dairy Animals” is also gratefully acknowledged.

Conflict of Interest: All authors declare no conflict of interest.

References:

Ahmed, A.E., Ismail, M.N., Aref, M.S., El-Abedin, A.Z. and Kasab, A.Y. (2013). Cortisol and postpartum luteal function in cattle. *Iranian J. Applied Anim. Sci.* **3**(3):465-470.

Arieli, A., Dicken, U., Dagoni, I., Spirer, Y. and Zamwel, S., (2008). Production and health of cows given monensin prepartum and a high-energy diet postpartum. *J. Dairy Sci*. **91**:1845-1851.

Aruga, T., Uzuka, Y., Tanabe, S. and Sarshina, T. (2001). Changes of thyroid hormones level in periparturient Holstein cows. *J. Japanese Vet. Med. Assoc.***54**:172-176.

Benzaquen, N., Galvao, K.N., Coleman, A.E., Santos, J.E.P., Goff, J.P. and Risco, C.A. (2015). Effect of oral mineral and energy supplementation on blood mineral concentrations, energetic and inûammatory proûle, and milk yield in dairy cows affected with dystocia. *The Vet. J.* http://doi: 10.1016/j.tvjl.2015.03.001.

Borady, A.M.A., Abdelaal, A.E. and Farghaly, H.A.M. (1985). Seasonal variation in thyroid hormones and reproduction in Egyptian water buffalo. *Egyptian J. Anim. Prod*. **25**:83-92.

Butler, W.R., Forsenberg, M., Greve, T., Gustafsson, H., Kalita, T., Kindahl, H. and Ropstad, E. (2000).

Nutritional interactions with reproductive performance in dairy cattle. *Anim. Reprod. Sci.* **60**:449-457.

Cerri, R.L.A., Juchem, S.O., Chebel, R.C., Rutigliano, H.M., Bruno, R.G.S., Galvão, K.N., Thatcher, W.W. and Santos, J.E.P. (2009). Effect of fat source differing in fatty acid profile on metabolic parameters, fertilization, and embryo quality in high-producing dairy cows. *J. Dairy Sci.* **92**:1520-1531.

Chaiyabutr, N., Prueksagorn, S., Komolvanich, S. and Chanpongsang, S. (2000). Plasma levels of hormones and metabolites as affected by the forages type in two different types of crossbred Holstein cattle. *Asian-Aus. J. Anim. Sci.* **13**(10):1359-1366.

Crowe, M.A., Goulding, D., Baguisi, A., Boland, M.P. and Roche, J.F. (1993). Induced ovulation of the ûrst postpartum dominant follicle in beef suckler cows using a GnRH analogue. *J. Reprod. Fertil.* **99**:551-555.

DeVries, G.H., Mammunes, P., Miller, C.D. and Hayward, D.M. (1976). *Analytical Biochem.* **70**:156-166.

Galan, A., Hernandez, J.M., and Jimenez, O. (2001). Measurement of blood acetoacetate and β-hydroxybutyrate in an automatic analyser. *J. Automated Methods and Management in Chemistry.* **23**(3):67-76.

Garg, S.L., Suresh Chader, Bugalia, N.S. and Rao, A.R. (1997). Thyroid function from late pregnancy till the onset of postpartum ovarian activity in buffaloes (*Bubalus babalis*). *Buffalo J.,***13**: 375-378.

Garverick, H.A., Harris, M.N., Vogel-Bluel, R., Sampson, J.D., Bader, J., Lamberson, W.R., Spain, J.N., Lucy, M.C. and Youngquist, R.S. (2013). Concentrations of non-esterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. *J. Dairy Sci.* **96**:181-188.

Hadiya, K.K. (2006). Effect of supplementation of minerals and enzymes on postpartum fertility in triple crossbred cows. M.V.Sc. Thesis, A.A.U., Anand, Gujarat, India.

Herdt, T.H. (2000). Ruminant adaptation to negative energy balance influences the etiology of ketosis and fatty liver. *Vet. Clin. North Am. Food Anim. Pract.* **16**:215-230.

Hussein, H.A., Senosy, W. and Abdellah, M.R. (2013). Relationship among uterine involution, ovarian activity, blood metabolites and subsequent reproductive performance in Egyptian buffaloes. *Open J. Anim. Sci.* **3**(1):59-69.

Khalil, W.A., El-Harairy, M.A. and Abul-Atta, A.A. (2012). Impact of dietary protected fat *(Magnapac)* on productive and reproductive performances of lactating Holstein cows. *J. Anim. and Poult. Prod.,* **3**(10):437-450.

Kumar, R., Sharma, I.J. and Agrawal, R.G. (2000). Periparturient levels of certain organic and inorganic components in the blood of cows and buffaloes. *Indian J. Anim. Reprod.* **21**(2):117-120.

Lammoglia, M.A., Willard, S.T., Oldham, J.R. and Randel, R.D. (1997). Effects of dietary fat and season on steroid hormonal profiles before parturition and on hormonal, cholesterol, triglycerides, follicular patterns, and postpartum reproduction in Brahman cows. *J. Anim. Sci.* **74**:2253-62.

Lohan, I.S., Maker, M.L., Singal, S.P. and Razdan, M.N. (1989). Thyroid activities of prepartum and postpartum Murrah buffaloes *(Bubalus bubalis). Indian Vet. J.,***66**: 115-118.

Michael, A.E., Thuston, L.M. and Rae, M.T. (2003). Glucocorticoid metabolism and reproduction: a tale of two enzymes. *Reproduction* **126**:425-441.

Oxenreider, S.L. and Wagner, W.C. (1971). Effect of lactation and energy intake on the postpartum activity in the cows. *J. Anim. Sci.* **33**:1026-1031.

Rahbar, B., Safdar, A.H.A. and Kor, N.M. (2014). Mechanisms through which fat supplementation could enhance reproduction in farm animal. *European J. Expt. Biol.* **4**(1):340-348.

Rueda, B.R., Hendry, I.R., Hendry, I.W., Stormshak, F., Slayden, O.D. and Davis, J.S. (2000). Decreased progesterone levels and progesterone receptor antagonists promote apoptotic cell death in bovine luteal cells. *Biol. Reprod*. **62**:269-276.

Setia, M. S., Duggal, R. S. and Singh, R. (1992). Biochemical constituents of blood in buffaloes and cows during late pregnancy and different stages of lactation - A longitudinal study. *Buffalo J.* **8**(2):123-29.

Shin, E.K., Jeong, J.K., Choi, I.S., Kang, H.G., Hur, T.Y., Jung, Y.H. and Kim, I.H. (2015). Relationships among ketosis, serum metabolites, body condition, and reproductive outcomes in dairy cows. *Theriogenology* 30: 1-9.

Sivaraman, T., Shanmugasundaram, S., Arunachalam, S. and Sivakumar, T. (2002). Blood profile of Jersey crossbred cows under different physiological stages. *Ind. J. Anim. Res.* **36**(2):114-117.

Snedecor, G.W. and Cochran, W.G. (1994). *Statistical Methods*. 8^{th} edn. Iowa State University Press, Ames, Iowa, USA.

Staples, C.R. and Thatcher, W.W. (2005). Effects of fatty acids on reproduction of dairy cows. In: Garnsworthy PC, Wiseman, J. (Eds.), Recent Advances in Animal Nutrition. Nottingham Univ. Press, Nottingham, UK, pp.229-256.

Vannucchi, C.I., Rodrigues, J.A., Silva, L.C.G., Lúcio, C.F., Veiga, G.A.L., Furtado, P.V., Oliveira, C.A. and Nichi, M. (2015). Association between birth conditions and glucose and cortisol proûles of periparturient dairy cows and neonatal calves. *Vet. Rec.* February 25, 2015. http://doi: 10.1136/vr.102862.

Vazquez-Añon, M., Bertics, S., Luck, M., Grummer, R.R. and Pinheiro, J. (1994). Peripartum liver triglyceride and plasma metabolites in dairy cows. *J. Dairy Sci.* **77**:1521-28.

Wathes, D.C., Bourne,N., Cheng, Z., Mann, G.E., Taylor,V.J. and Coffey, M.P. (2007). Multiple correlation analyses of metabolic and endocrine proûles with fertility in primiparous and multiparous cows*. J. Dairy Sci*. **90**:1310-25.

❏