

Impact of Peripartum Nutritional Supplementation on Thyroid Hormones, Metabolites and Reproductive Peridata in Jafarabadi Buffaloes

KB Vala¹, AJ Dhami², FS Kavani², BB Bhanderi², SC Parmar²

ABSTRACT

Forty advanced pregnant Jafarabadi buffaloes of 2-4 parity were divided into two equal groups, viz., control and treatment (n = 20 each). The animals of control group were maintained on routine farm feeding schedule and those under treatment group were subjected to additional oral supplements daily with 50 g of chelated mineral mixture and 150 g of bypass fat along with concentrates for 6 weeks prepartum till 2 weeks postpartum, and then bypass fat was given @ 15 g/litre of milk produced till 60 days postpartum. All the buffaloes were subjected to blood collection on day -45, -30, -7, 0, 7, 15, 30, 45 and 60 peripartum. Postpartum animals were followed at weekly interval by per rectal palpation and ultrasonographically for uterine involution, first postpartum estrus and conception. The overall mean blood glucose of prepartum period increased significantly ($p < 0.01$) on the day of calving and reduced back to prepartum levels within next 7 days postpartum in both the groups. The buffaloes supplemented with peripartum nutrients had significantly ($p < 0.01$) higher blood glucose levels than the control group. The mean plasma total protein (7.96 ± 0.04 g/dl) and thyroid hormones T_3 (1.44 ± 0.05 ng/ml) and T_4 (28.25 ± 0.86 ng/ml) were found to be within the normal range, and did not vary between sampling days or between groups. The peripartum supplementation of bypass fat did not influence the levels of plasma total cholesterol. However, the levels of β -hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) tended to be higher in control than treatment group at most of the peripartum intervals. The period of uterine involution (32.75 ± 0.57 vs 37.00 ± 0.56 days) and service period (107.10 ± 4.43 vs 133.65 ± 6.04 days) were significantly ($p < 0.05$) shorter with higher postpartum fertility (85 vs 50% CR) in nutrient supplemented than control group. It was concluded that peripartum bypass fat and mineral supplementation prevented negative energy balance and/or ketosis and improved postpartum fertility in high yielding Jafarabadi buffaloes.

Key Words: Blood metabolites, Jafarabadi buffalo, Nutrient supplementation, Postpartum fertility, Thyroid hormones profile, Transitional period.

Ind J of Vet Sci and Biotech (2020): 10.21887/ijvsbt.15.3.5

INTRODUCTION

Buffaloes are preferred over cattle in India because they are well adapted to hot and hot-humid climate, have better feed conversion efficiency, greater resistance to diseases and higher milk fat percentage. Parturient events and postpartum fertility are crucial in dairy animals. Fats and minerals in the diet can influence postpartum reproduction positively by altering both ovarian follicle and corpus luteum function via improved energy status and by increasing precursors and catalysts for the synthesis of reproductive hormones such as sex steroids and prostaglandins (Rahbar *et al.*, 2014; Mane *et al.*, 2016). Factors such as limited energy intake, lower body reserves, and postpartum diseases can delay the uterine involution and thereby return to cyclicity. The peripartum nutritional management, event of parturition and the time thereafter play a key role in resuming the postpartum reproductive cycle (Dhami *et al.*, 2017). A trouble-free calving predisposes to prompt resumption of postpartum ovarian activity. Ideally, this should be followed by a minimal period of negative energy balance (NEB). The nutritional, managemental and environmental factors have

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How to cite this article: Vala, K.B., Dhami, A.J., Kavani, F.S., Bhanderi, B.B. and S.C. Parmar (2020). Impact of Peripartum Nutritional Supplementation on Thyroid Hormones, Metabolites and Reproductive Peridata in Jafarabadi Buffaloes. *Ind J Vet Sci and Biotech*, 15(3): 16-20.

Source of support: Nil

Conflict of interest: None.

Submitted: 17/12/2019 **Accepted:** 31/12/2019 **Published:** 09/03/2020

impact on postpartum fertility. Such studies on Jafarabadi buffaloes are scarce. Hence, this investigation was aimed to evaluate the influence of nutrient management of transition period on plasma profile of thyroid hormones, metabolites and postpartum fertility in Jafarabadi buffaloes.

MATERIALS AND METHODS

Forty advanced pregnant Jafarabadi buffaloes of 2-4 parity were selected from University Farm, Junagadh. They were maintained in well ventilated hygienic sheds and were fed green fodder, hay and compounded concentrate, as per feeding schedule followed on the farm. The buffaloes were divided into two equal groups, viz., control and treatment (n = 20 each). The control animals were maintained on routine farm feeding schedule and the animals of treatment group were given additional oral supplements daily with 50 g of chelated mineral mixture and 150 g of bypass fat along with concentrates for 6 weeks prepartum and 2 weeks postpartum. Bypass fat was then given @ 15 g/lit of milk limiting to 200 g/day/head till 60 days postpartum. Uterine involution was monitored by per rectal palpation and trans-rectal ultrasonography at weekly intervals. The puerperal events and periods for uterine involution, conception rate and service period were recorded for each group.

Blood samples were collected from all animals in heparinized vacutainers on days -45, -30, -7, 0 (day of parturition), 7, 15, 30, 45 and 60 peripartum. The plasma was separated immediately by centrifugation of blood samples at 3000 rpm for 10 minutes and stored at -80°C with a drop of merthiolate until analyzed. Blood glucose was determined by direct strip method using Accu-Chec Integra Kit (NIPRO Diagnostics, Mumbai). The levels of plasma thyroxine (T₄) and tri-iodothyronine (T₃) were determined by employing standard RIA techniques using kits procured from Immunotech-SA, France. The sensitivity of the T₃ and T₄ assay was 0.24 and 0.5 ng/ml, respectively. The cross-reactivity of the T₃ antisera against tri-iodothyronine (T₃) and thyroxine (T₄) was 100 and 0.1 %, and that of T₄ antisera against thyroxine (T₄) and tri-iodo-thyronine (T₃) was 100 and 5 %, respectively. Plasma total protein and total cholesterol concentrations were estimated by Biuret and CHOD/PAP method, respectively, using assay kits procured from Crest Biosystems, Goa on Chemistry Analyzer (Nova 2021, Analytical

Technol. Pvt. Ltd., Vododara). Estimation of non-esterified fatty acids (NEFA) was done by colorimetric method using diagnostic kits (Randox Lab Ltd., Crumlin, UK), while that of beta-hydroxybutyrate (BHBA) was done using ELISA kits (Cayman Chemicals, USA).

The data on thyroid hormones and metabolites within group were analyzed using ANOVA and NMRT, and between groups by 't' test for each parameter employing SPSS software version 20.00. The puerperal events were compared between groups by 't' test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The mean levels of blood metabolites and thyroid hormones recorded during peripartum periods in Jafarabadi buffaloes of control and treatment groups are presented in Tables 1-3.

Blood Glucose

There was significant ($p < 0.05$) increase in blood glucose level from day 45 prepartum to day of calving with values of 67.50 ± 0.96 to 69.20 ± 1.27 mg/dl and 68.65 ± 0.63 to 76.60 ± 1.39 mg/dl in control and peripartum nutrient supplemented group respectively, which reduced back to prepartum level within next 7 days postpartum in both the groups, and thereafter it fluctuated non-significantly till day 45-60 postpartum. The overall peripartum mean blood glucose level of nutrient supplemented group increased significantly ($p < 0.01$) to 73.91 ± 0.41 mg/dl from 66.72 ± 0.35 mg/dl in control group (Table 1). It is noteworthy that the buffaloes supplemented with peripartum nutrients had significantly ($p < 0.01$) higher blood glucose levels than the control group from day 30 prepartum till day 60 postpartum. This can be attributed to the effect of bypass fat supplementation in the diet of treatment group. The mean blood glucose levels found in the animals under both the groups were however within the normal limit during peripartum period ((Table 1). The present findings were in agreement with Phawa (2012) and Kalasariya *et al.* (2017) in buffaloes and Theodore *et al.* (2017) in cattle, who found higher blood glucose levels in nutrient supplemented than in control group. Butler *et al.* (2000), Abdulkareem (2013) and

Table 1: Mean (\pm SEM) blood glucose and plasma total cholesterol concentrations in Jafarabadi buffaloes of control group and those given peripartum nutrients supplements

Peripartum Period	Blood glucose (mg/dl)		Plasma total cholesterol (mg/dl)	
	Control (n = 20)	Supplemented (n = 20)	Control (n = 20)	Supplemented (n = 20)
-45 day	67.50 \pm 0.96 ^a	68.65 \pm 0.63 ^a	106.47 \pm 2.08 ^b	105.43 \pm 1.98 ^b
-30 day	66.85 \pm 1.15 ^{ab}	*71.25 \pm 1.04 ^{ab}	101.19 \pm 1.75 ^{ab}	106.50 \pm 2.61 ^b
-7 day	65.95 \pm 0.79 ^{ab}	*72.00 \pm 0.92 ^{bc}	97.05 \pm 2.29 ^a	103.66 \pm 2.78 ^b
0 day	69.20 \pm 1.27 ^b	*76.60 \pm 1.39 ^{def}	92.85 \pm 1.88 ^a	95.24 \pm 2.46 ^a
7 day	65.50 \pm 0.89 ^a	*73.35 \pm 1.40 ^{bcd}	101.34 \pm 1.74 ^{ab}	102.22 \pm 1.89 ^{ab}
15 day	66.95 \pm 1.36 ^{ab}	**73.95 \pm 1.42 ^{bcd}	109.53 \pm 2.38 ^b	106.83 \pm 2.62 ^b
30 day	65.40 \pm 1.04 ^a	**76.85 \pm 0.97 ^{ef}	122.17 \pm 3.30 ^c	124.29 \pm 3.30 ^c
45 day	65.95 \pm 0.77 ^{ab}	**75.15 \pm 0.99 ^{cdef}	131.69 \pm 4.79 ^d	134.34 \pm 4.22 ^d
60 day	67.15 \pm 1.03 ^{ab}	**77.35 \pm 0.74 ^f	136.58 \pm 4.88 ^d	134.62 \pm 3.17 ^d
Overall	66.72 \pm 0.35	**73.91 \pm 0.41	110.99 \pm 1.48	112.57 \pm 1.39

n = Number of animals; * $P < 0.05$, ** $P < 0.01$ between control and supplemented groups.

Means bearing uncommon superscripts within the column differ significantly between periods ($p < 0.05$).

Table 2: Mean (\pm SEM) plasma NEFA and BHBA concentrations in Jafarabadi buffaloes of control group and those given peripartum nutrients supplements

Peripartum Period	Plasma NEFA concentrations ($\mu\text{mol/l}$)		Plasma BHBA concentrations ($\mu\text{mol/l}$)	
	Control (n = 20)	Supplemented (n = 20)	Control (n = 20)	Supplemented (n = 20)
-45 day	162.80 \pm 7.24 ^a	159.12 \pm 09.49 ^a	747.54 \pm 20.76 ^a	726.13 \pm 18.47 ^a
-30 day	178.57 \pm 12.59 ^{ab}	163.88 \pm 07.77 ^{ab}	780.77 \pm 20.91 ^{ab}	768.69 \pm 23.96 ^{ab}
-7 day	176.88 \pm 10.33 ^{ab}	179.42 \pm 07.04 ^{ab}	986.25 \pm 26.52 ^{de}	878.74 \pm 22.84 ^{cd}
0 day	197.60 \pm 12.85 ^{abc}	187.99 \pm 11.03 ^{ab}	1113.7 \pm 40.48 ^g	1013.5 \pm 46.12 ^f
7 day	230.32 \pm 11.29 ^c	216.16 \pm 14.54 ^c	1073.8 \pm 30.51 ^{gf}	1024.7 \pm 27.40 ^f
15 day	212.66 \pm 16.36 ^{bc}	199.23 \pm 12.07 ^{bc}	1020.3 \pm 17.73 ^{ef}	950.68 \pm 15.42 ^d
30 day	191.85 \pm 14.69 ^{ab}	198.52 \pm 14.55 ^{bc}	940.98 \pm 25.77 ^d	913.49 \pm 20.16 ^{de}
45 day	205.22 \pm 16.65 ^{bc}	194.24 \pm 12.82 ^{ab}	856.33 \pm 20.90 ^c	828.84 \pm 21.94 ^{bc}
60 day	197.34 \pm 11.77 ^{ab}	165.37 \pm 11.02 ^{ab}	837.57 \pm 14.87 ^{bc}	814.98 \pm 14.14 ^{bc}
Overall	194.80 \pm 4.26	*184.88 \pm 03.97	928.57 \pm 12.35	879.98 \pm 11.00

n = Number of animals; * $P < 0.05$ between control and supplemented groups.

Means bearing uncommon superscripts within the column differ significantly between periods ($p < 0.05$).

Table 3: Mean (\pm SEM) plasma total protein, thyroxine (T4) and tri-iodothyronine (T3) concentrations in Jafarabadi buffaloes of control group and those given peripartum nutrients supplements

Peripartum Period	Plasma total protein (g/dl)		Plasma T4 concentration (ng/ml)		Plasma T3 concentration (ng/ml)	
	Control (n = 20)	Supplemented (n = 20)	Control (n = 20)	Supplemented (n = 20)	Control (n = 20)	Supplemented (n = 20)
-45 Day	8.12 \pm 0.11	8.09 \pm 0.13	30.72 \pm 2.61	29.00 \pm 2.65	1.32 \pm 0.10	1.35 \pm 0.15
-30 Day	7.99 \pm 0.14	8.14 \pm 0.15	30.11 \pm 2.54	29.01 \pm 2.84	1.30 \pm 0.08	1.28 \pm 0.12
-7 Day	7.77 \pm 0.11	7.89 \pm 0.15	27.90 \pm 2.27	32.78 \pm 2.55	1.27 \pm 0.08	1.39 \pm 0.13
0 Day	7.81 \pm 0.08	7.89 \pm 0.11	31.17 \pm 2.97	32.30 \pm 2.86	1.59 \pm 0.21	1.47 \pm 0.12
7 Day	7.89 \pm 0.11	7.81 \pm 0.12	27.33 \pm 2.59	27.70 \pm 2.81	1.36 \pm 0.11	1.59 \pm 0.16
15 Day	7.93 \pm 0.10	7.78 \pm 0.13	26.54 \pm 2.74	28.16 \pm 3.11	1.46 \pm 0.09	1.66 \pm 0.14
30 Day	8.10 \pm 0.10	7.88 \pm 0.13	26.12 \pm 2.70	27.61 \pm 2.78	1.46 \pm 0.16	1.62 \pm 0.16
45 Day	7.98 \pm 0.13	7.97 \pm 0.12	27.84 \pm 2.87	30.30 \pm 2.94	1.59 \pm 0.14	1.79 \pm 0.11
60 Day	8.07 \pm 0.14	7.97 \pm 0.14	26.54 \pm 2.17	34.31 \pm 3.20	1.59 \pm 0.18	1.52 \pm 0.14
Overall	7.96 \pm 0.04	7.92 \pm 0.04	28.25 \pm 0.86	30.13 \pm 0.95	1.44 \pm 0.05	1.52 \pm 0.05

n = Number of animals; None of the values differed significantly between periods within column or between groups at any of the periods.

Garverick *et al.* (2013) however reported steady mean glucose concentrations around calving and postpartum period.

The temporary rise in blood glucose around parturition and then coming back to the prepartum or reduced level in both the groups is attributed to increased gluconeogenesis under the influence of elevated cortisol concentration and the stress of calving (Vala *et al.*, 2019). The relatively higher blood glucose maintained during postpartum period till day 60 as compared to 45 days prepartum level in treatment group could be due to nutrient supplemented particularly bypass fat in that group. Moreover, the nutrients supplemented buffaloes having higher blood glucose level had early initiation of postpartum ovarian activity and estrus compared to control ones. These findings corroborated with observations of Kalasariya *et al.* (2017) in buffaloes. According to Butler *et al.* (2000), negative energy balance delayed the time of first ovulation postpartum through inhibition of LH pulse frequency and low level of blood glucose. In the present study, bypass fat supplement prevented negative energy balance and perhaps maintained positive and stimulating effect on LH pulse frequency that initiated early ovarian activity in treated animals.

Plasma Total Cholesterol

The prepartum mean plasma total cholesterol concentrations in animals of both control and treatment groups (106.47 \pm 2.08 and 105.43 \pm 1.98 mg/dl) gradually decreased as parturition approached with the lowest values at calving (92.85 \pm 1.88 and 95.24 \pm 2.46 mg/dl, respectively). Thereafter, the values again increased to reach the highest ($p < 0.01$) at day 60 postpartum in both the groups concurrent with ovarian follicular activity. The trend in the reduction of plasma total cholesterol concentration as seen from 7 days prepartum through 7 days postpartum and trend of escalation from 7 days postpartum onwards to 60 days postpartum in our study (Table 1) corroborated with the findings of Ranjan *et al.* (2012) and Dhami *et al.* (2017). The observed trend in increasing plasma total cholesterol might be associated with the initiation of ovarian activity and establishment of postpartum cyclicity.

There was no significant variation in mean plasma total cholesterol concentrations of treatment and control groups at any of the peripartum intervals. Ranjan *et al.* (2012), Dhami *et al.* (2017) and Kalasariya *et al.* (2017), however, noted elevated plasma cholesterol concentrations in



nutrients supplemented groups. The low levels of plasma total cholesterol at parturition may be attributed to its increased coupling with estrogen and thyroxine, which normally inhibits cholesterologenesis (Prakash and Tandon, 1979). Lactation probably also affect the level of plasma total cholesterol, which act as a fatty acid carrier in the form of cholesterol ester for milk synthesis, as a result there is gradual increase in plasma cholesterol level with advancing lactation (Theodore *et al.*, 2017). These reports and the present findings clearly proved that plasma total cholesterol, being precursor of steroid hormone, is closely associated with physiological status of animal reproduction.

Plasma Non-Esterified Fatty Acids and Beta-Hydroxybutyrate

The mean plasma concentrations of NEFA in buffaloes presented in Table 2 revealed that there was a significant ($p < 0.01$) increase in the values from 162.80 ± 7.24 and 159.12 ± 09.49 $\mu\text{mol/l}$ at 45 days prepartum to the highest 230.32 ± 11.29 and 216.16 ± 14.54 $\mu\text{mol/l}$ on day 7 postpartum, respectively, in control and treatment group and thereafter the values again declined significantly around day 30-45 postpartum in both the groups. The plasma NEFA concentrations were non-significantly higher in buffaloes of control group than treatment group almost throughout the study period with a significant difference in overall pooled peripartum mean values of control and supplemented group at the end of experiment (Table 2). Peripartum supply of bypass fat probably reduced the process of lipogenesis by adipose tissues which caused increased lipolysis resulting into the higher NEFA values (Staples *et al.*, 1998).

The mean plasma BHBA values of 747.54 ± 20.76 and 726.13 ± 18.47 $\mu\text{mol/l}$ observed at day 45 prepartum increased highly significantly ($p < 0.01$) as parturition approached with the peak levels of 1113.6 ± 40.48 and 1013.0 ± 46.12 $\mu\text{mol/l}$ on the day of calving for control and treatment groups, respectively. Thereafter the values declined gradually and significantly in subsequent days postpartum reaching values of day 60 postpartum at par with 30 days prepartum values in both the groups. The BHBA levels tended to be higher in control than treatment group at most of the peripartum intervals including overall peripartum mean but the differences were statistically non-significant (Table 2).

The trend of the mean total NEFA and BHBA in the buffaloes during pre- and post-partum periods of the present study were found to be comparable with the observations of Cerri *et al.* (2009), Garverick *et al.* (2013) and Benzaquen *et al.* (2015). It can be seen that supplementation of bypass fat and chelated mineral mixture had beneficial effect in lowering the plasma NEFA and BHBA levels. These findings corroborated with Theodore *et al.* (2017), but contradicted Staples *et al.* (1998), who observed higher plasma NEFA and BHBA values in treatment group.

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-Añon *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 parturient animals balance energy intake and energy demands in early lactation, but excessive elevation of NEFA or BHBA can indicate poor adaptation to NEB (Herdt, 2000). In the present study, bypass fat supplementation peripartum ameliorated the negative energy balance postpartum as indicated by higher blood glucose, and lower NEFA and BHBA profile, and thus concurred with the observations of Theodore *et al.* (2017) in crossbred cows.

Plasma Total Protein and Thyroid Hormones

Bypass fat and mineral supplementation did not evince any effect on the total protein values in buffaloes (Table 3) at day of calving (7.81 ± 0.08 vs 7.89 ± 0.11 g/dl) and overall peripartum means (7.96 ± 0.04 vs 7.92 ± 0.04 g/dl) at the end of experiment for 60 days postpartum in control vs. supplemented group. These observations concurred well with report of Theodore *et al.* (2016) in crossbred cows fed with ASMM and bypass fat. The trend of mean total protein in buffaloes of the present study was also found to be comparable with the observations of Lone *et al.* (2003) and Ashmawy (2015). Kalasariya *et al.* (2017), however, reported significant ($p < 0.05$) variation in the plasma protein levels between days within the group, with the lowest values on the day of calving in both the groups, and also higher ($p < 0.05$) values on day 30 and 60 postpartum in buffaloes fed peripartum high dietary protein as compared to control group. The non-significant difference observed between periods and groups in the present study could be due to iso-nitric diet in both the groups and adding only minerals and bypass fat in the experimental group of animals.

The mean plasma concentrations of tri-iodothyronine (T_3) and thyroxine (T_4), did not show any change in any of the groups during peripartum period studied (Table 3). These observations contradicted the reports of Garg *et al.* (1997) and Theodore *et al.* (2017), who showed that serum T_3 and T_4 levels were low from day 1 to 28 after calving as compared with advance pregnancy, and again increased on day 35 and 42 postpartum postulating 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 lactation (Garg *et al.*, 1997). However, no such trend was noticed in the present study, though the postpartum ovarian activity and fertility showed significant benefit from peripartum nutrients supplementation in buffaloes.

ACKNOWLEDGEMENTS

Authors thank the Officer in Charge of CBF, JAU, Junagadh and their staff for permitting to use their animals with kind cooperation, and PI, Dr. A.J. Dhami of "AICRP on nutritional & physiological interventions for enhancing reproductive performance in animals" of AAU, Anand for financial support during this study.

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