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Peripartum supplementation of nutrients in Gir cows improves the feed intake, blood metabolic profile and postpartum fertility

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ABSTRACT

The study evaluated the effect of supplementation of rumen protected choline (RPC) and fat (RPF) alone and in combination, and injection Vit-E + selenium on feed intake, blood metabolic profile and postpartum fertility in transition Gir cows. Forty advanced (8 months) pregnant cows were divided into five equal groups (T1 to T5, n=8) and were managed individually from 30 days prepartum till 60 days postpartum. Cows in T1 group were fed basal diet, those in T2 to T5 groups received additional oral supplements of RPC @ 45 g/d (T2), RPF @ 80 g/d (T3), RPC + RPF as above (T4) and injection Vit-E 500 mg + Se 15 mg fortnightly (T5). The mean fortnightly dry matter intake increased significantly by days 0, 15 and 45 of lactation with higher overall value in T2 than other groups. The blood glucose levels were significantly higher on day of calving and dropped suddenly around day 15 postpartum in all groups. Plasma total cholesterol was significantly lower in T5 and T2, and higher in T4, and it declined significantly from 30 days prepartum till day of calving with lowest values on day 15 postpartum, which increased gradually till day 60 postpartum. The TAG and VLDL concentrations dropped 15-20 fold on day 0 and 15 postpartum over prepartum values, and again increased 5-10 fold on days 30 and 45 postpartum in most groups with higher values in T3, T4 and T5 than in T1 and T2 groups. BHBA levels were significantly increased on day 15 postpartum over day of calving or prepartum values, declined by day 45 postpartum, and were significantly higher in T2 and lower in T5 as compared to other groups. The uterine involution, first estrus postpartum and conception rates were significantly enhanced with all nutrient supplements, and T5 in particular.

Keywords: Fat, Fertility, Gir cows, Metabolic profile, Peripartum supplementation, Rumen protected choline, Selenium, Vitamin-E

Transition period i.e. the period from 3 weeks before to 3 weeks after calving, is very crucial for dairy cows due to profound changes in their metabolic and endocrine status (Hayirli *et al.* 2002). The dietary energy intake during this period is not sufficient to meet the demands for reduced appetite (Shahsavari *et al.* 2016) leading to marked decrease in dry matter intake (DMI) (Grummer 2012). Due to decline in DMI during the last 3 weeks of pregnancy, the dairy animals enter into the state of negative energy balance (NEB) (Esposito *et al.* 2014). NEB is a physiological adaptive mechanism in high yielding dairy animals (Wankhade *et al.* 2017) by mobilizing adipose tissue reserve through lipolysis, as a result more non-esterified fatty acids (NEFAs) are drained towards liver leading to accumulation of triacylglycerides in liver causing fatty liver (FL) syndrome (Drackley *et al.* 2014). Liver fails to use the excessive amounts of NEFA as fuel and leads to a ketotic state with increased production of β-hydroxybutyrate and acetoacetate, and this remains a major challenge for production, health and welfare of dairy cows (Goff 2006).

A multi-functional B-complex vitamin Choline is involved in the metabolism of fatty acids in the liver and serves as a methyl donor (Shahsavari *et al.* 2016). Choline can indirectly affect DMI in transition cows by restricting the detrimental effects of ketosis on general health (Esposito *et al.* 2014). However, the dietary choline gets degraded rapidly in the rumen, hence it must be supplemented in the protected form (Elek *et al.* 2008). Energy density of ration and energy intake during early lactation in high yielding cows can be enhanced without compromising rumen cellulolytic bacterial activity through fat supplementation in protected form, thus mitigating the deleterious effect of NEB (Ganjkhanlou *et al.* 2009). Vit-E and selenium are established antioxidants with known positive effects in body metabolism and fertility (Modi *et al.* 2016, Hosnedlova *et al.* 2017). Hence the present

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study was aimed to evaluate the effect of peripartum supplementation of rumen protected choline (RPC), rumen protected fat (RPF) alone and in combination, and injection Vit-E + selenium on feed intake, blood metabolic profile and postpartum fertility in Gir cows.

MATERIALS AND METHODS

This study was carried out from August, 2020 to May, 2021 at Cattle Breeding Farm, Junagadh Agricultural University (JAU), Junagadh (India), following ethical approval by the Institutional Animal Ethics Committee, vide No. JAU/JVC/IAEC/LA /64/2020. Forty advanced (8 months) pregnant Gir cows in their first to third lactation were divided into five equal groups (T1 to T5, n=8) on the basis of their parity, body weight and previous lactation yield. In T1 (control) group, cows were fed with basal diet of green sorghum 10 kg, mature pasture grass/hay *ad lib.*, and compound cattle feed added with 250 g maize bhardo and cotton seed cake to meet their nutrient requirement as per ICAR (2013) feeding standards followed on the farm. The cows in other groups received additional oral supplementation of RPC @ 45 g/d (Kemin Industries South Asia Pvt. Ltd) in T2, RPF @ 80 g/d (Kemin Industries South Asia Pvt. Ltd) from -30 to +15 days peripartum and then @ 10 g/kg milk yield in T3, RPC plus RPF as above in T4, and injection Vit-E 500 mg + Se 15 mg (Inj. E-Care Se$^{(R)}$, 10 ml fortnightly, Cadila-Zydus Pharma) in T5 group, starting from 30 days before expected date of calving to 60 days postpartum. All the cows were maintained in well ventilated hygienic sheds with free access to wholesome drinking water. Daily and fortnightly dry matter intake was recorded for each cow.

Blood samples were collected from jugular vein in heparinised vacutainers on days -30, 15, 0, 15, 30, 45 and 60 of calving. The plasma was separated immediately by centrifugation of samples and stored at -80°C until analyzed. The blood glucose level was determined in fresh samples with glucometer and Gluco-One BG-03 strips (Morpen Laboratories Limited, Delhi). Plasma concentrations of blood urea nitrogen (BUN), total cholesterol and triacylglycerides (TAG) were analyzed by using standard kits (Greiner Diagnostic GmbH, Germany) with fully automatic biochemistry analyzer (Dia-Chem 240 plus, Diatek). Very low density lipoprotein (VLDL)

Table 1. Effect of RPC, RPF and Se + Vit-E supplementation on fortnightly dry matter intake, blood glucose and BUN profile in periparturient Gir cows (Mean±SE)

Days peripartum	Dietary treatment group					
	T1	T2	T3	T4	T5	Overall***
DMI (kg/d)						
-30	6.68^{A}±0.24	7.16^{A}±0.14	7.07^{A}±0.30	6.89^{A}±0.37	6.63^{A}±0.16	6.89^{A}±0.08
-15	6.88^{A}±0.14	7.15^{A}±0.10	7.59^{A}±0.48	7.26^{A}±0.53	7.02^{AB}±0.43	7.18^{A}±0.10
0	6.83^{A}±0.28	6.82^{A}±0.43	7.09^{A}±0.38	7.50^{A}±0.85	6.93^{AB}±0.31	7.03^{A}±0.10
15	8.65^{B}±0.73	9.74^{B}±0.30	9.16^{B}±0.44	8.98^{B}±0.64	8.23^{BC}±0.52	8.95^{B}±0.20
30	8.94^{aB}±0.52	10.79^{bB}±0.47	9.50^{abB}±0.78	9.61^{abB}±0.74	8.87^{aDC}±0.63	9.54^{B}±0.27
45	9.03^{aB}±0.46	10.95^{bB}±0.35	9.64^{abB}±0.71	9.89^{abB}±0.64	9.75^{abD}±0.63	9.85^{C}±0.24
60	9.18^{aB}±0.36	11.00^{bB}±0.39	9.69^{abB}±0.57	9.77^{abB}±0.57	9.89^{abD}±0.48	9.91^{C}±0.24
Overall***	8.03^{a}±0.41	9.09^{b}±0.69	8.53^{a}±0.43	8.56^{a}±0.46	8.19^{a}±0.48	
Blood glucose (mg/dL)						
-30	56.13^{bB}±2.26	53.50^{abA}±3.98	48.25^{abAB}±1.91	46.88^{aA}±1.74	56.75^{bA}±1.36	52.30^{A}±1.60
-15	57.50^{B}±4.70	53.63^{A}±2.77	56.63^{BC}±2.70	52.25^{AB}±2.43	60.38^{AB}±2.74	56.08^{B}±1.14
0	68.13^{C}±2.49	67.75^{B}±3.19	63.13^{C}±2.93	60.38^{B}±2.45	68.38^{B}±2.16	65.55^{B}±1.28
15	46.88^{aA}±4.15	51.50^{aA}±3.49	47.38^{aA}±1.99	51.50^{aA}±3.38	63.00^{bAB}±2.40	52.05^{A}±2.30
30	47.00^{abA}±4.42	47.75^{abA}±3.58	45.88^{aA}±1.93	46.38^{aA}±2.32	55.75^{bA}±2.53	48.55^{A}±1.44
45	43.13^{aA}±3.63	48.50^{aA}±4.54	44.38^{aA}±3.55	49.38^{aA}±4.69	58.25^{bA}±3.24	48.73^{A}±2.10
60	45.88^{aA}±3.01	45.50^{aA}±4.47	50.88^{abAB}±2.12	47.75^{aA}±2.75	58.13^{bA}±2.43	49.63^{A}±1.84
Overall***	52.09^{a}±3.14	52.59^{a}±2.59	50.93^{a}±2.37	50.64^{a}±1.71	60.09^{b}±1.54	
BUN (mg/dL)						
-30	22.04±2.38	22.25±2.24	21.72^{AB}±1.61	20.10^{AB}±1.28	21.48±0.99	21.52^{B}±0.30
-15	20.58±2.42	20.63±1.19	16.98^{A}±1.69	17.87^{A}±1.61	18.40±1.60	18.89^{A}±0.58
0	22.92±1.54	22.71±2.35	22.88^{B}±2.63	24.98^{B}±1.83	21.78±2.19	23.05^{B}±0.41
15	22.54±1.24	24.14±1.59	25.60^{B}±2.81	20.76^{AB}±1.47	21.29±0.68	22.87^{B}±0.71
30*	20.58^{a}±2.35	26.66^{b}±2.57	23.80^{abB}±2.74	21.12^{abB}±2.35	23.32^{ab}±1.74	23.10^{B}±0.86
45	23.33±1.89	24.30±2.13	24.32^{B}±1.59	24.63^{B}±2.46	23.79±1.19	24.07^{B}±0.18
60	23.63±1.59	23.44±1.60	25.93^{B}±1.99	22.42^{AB}±1.98	24.36±1.30	23.96^{B}±0.46
Overall	22.23±0.44	23.45±0.67	23.03±1.08	21.70±0.89	22.06±0.71	

T1, Control; T2, RPC; T3, RPF; T4, RPC + RPF; T5, Se + Vit-E;*P<0.05, ***P<0.001. Means bearing different small superscripts (a, b, c) within the row and capital superscripts (A, B, C, D) within the column differ significantly (P<0.05) for a parameter.

was calculated from the value of TAG using standard formula, and plasma beta-hydroxybutyric acid (BHBA) concentration was measured by using colorimetric assay kit and procedure described by Cayman Chemical Company, USA.

The puerperal event including uterine involution was monitored using transrectal ultrasonography as well as per rectal palpation. The occurrence of first estrus and fertile estrus postpartum, conception rates and service period in each group were recorded for cows conceived within 10 months postpartum. Cows showing estrus 60 days postpartum were only inseminated and pregnancy was confirmed by palpation per rectum 45 days after last AI.

The data on DMI and different blood parameters were analyzed by two way analysis of variance (ANOVA) for treatment and period effects and on reproductive traits by one way ANOVA for treatment effect using SPSS software version 20.0 (Snedecor and Cochran 1994). Pair-wise mean differences between groups were compared by Duncan's Multiple Range test for significance at $p<0.05$.

RESULTS AND DISCUSSION

Effect on feed intake: The overall mean DMI (kg/d) was significantly ($p<0.01$) higher in RPC supplemented cows, while it was numerically higher in T3, T4 and T5 groups as compared to control group. The significant effect of the treatment on DMI was observed from 30th day onwards. The DMI was observed significantly ($p<0.05$) lower on the day of parturition in all groups, which was significantly ($p<0.05$) increased during 1st fortnight in all groups, except in T5 group and the increased was found highest in T2 group (Table 1, Fig. 1). These observations concurred well with earlier reports (Hayirli *et al.* 2002, Zom *et al*. 2011, Soltan *et al.* 2012, Chavda *et al.* 2021) in transition HF and Gir cows supplemented with RPC and/or RPF. A meta-analysis of thirteen studies by Grummer (2012) also showed that feeding RPC does not affect feed intake before calving, but increases DMI by 0.8 kg/d in early lactation. However, others reported non-significant effect of supplementation of RPC and/or RPF (Elek *et al.* 2008, Acharya *et al.* 2019[a], Anonymous 2020) on DMI in periparturient dairy cows.

Effect on blood glucose and BUN profile: The overall mean fortnightly blood glucose level (mg/dL) was found

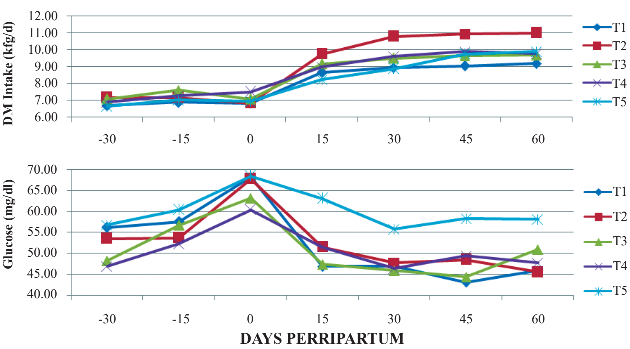

Fig. 1. Impact of peripartum supplementation of RPC (T2), RPF (T3), RPC+RPF (T4) and Se+Vit-E (T5) over control (T1) on DM Intake and blood glucose profile in Gir cows.

significantly higher in T5 group as compared to other treatment groups. The mean fortnightly blood glucose concentrations recorded in cows of different nutrients supplemented groups varied non-significantly at most periods, except on day 15, 45 and 60 postpartum, where the levels in group T5 supplemented with Se + Vit-E were significantly ($p<0.05$) higher (Table 1, Fig. 1). The mean blood glucose levels were significantly higher on the day of parturition as compared to peripartum periods in all the groups. Overall, the values were found to be within the normal range of 45-75 mg/dl in all groups and periods. Similar non-significant influence on blood glucose concentration was reported earlier in transition cows supplemented with varying levels of RPC (Zom *et al.* 2011, Amrutkar *et al.* 2015, Leiva *et al.* 2015, Acharya *et al.* 2019[b]) or RPF (Tyagi *et al.* 2010, Chavda *et al.* 2022). On the other hands, in certain other studies plasma glucose concentration had increased significantly with supplemented RPC (Gupta *et al.* 2018, Anonymous 2020) and RPF (Waghmare *et al.* 2016, Nirwan *et al.* 2019).

The mean plasma BUN levels across the groups and periods revealed non-significant effect of treatments as the levels were more or less similar, except on day 30th in the treatment group receiving RPC. Very similar results have also been reported by earlier workers on BUN levels in transition dairy cows supplemented with RPC, RPF or its combination (Tyagi *et al.* 2010, Acharya *et al.* 2019[b], Anonymous 2020, Chavda *et al.* 2022).

Effect on plasma cholesterol, triglycerides and VLDL profile: The overall mean plasma cholesterol levels were found to be significantly ($p<0.001$) higher in groups T3 and T4 as compared to T1 (control). The values in cows under all treatment groups tended to be lower on the day of parturition, and thereafter increased again in the subsequent days postpartum to reach highest ($p<0.05$) level at day 60 postpartum (Fig. 2). Cholesterol is a component of the serum lipoproteins and its concentration in serum gives an indication of overall lipoprotein concentrations. Higher plasma cholesterol levels, being precursor of steroid hormones, are associated with better reproductive performance in high yielding cows (Tyagi *et al.* 2010). In earlier studies, significantly (Soltan *et al.* 2012, Nirwan *et al.* 2019) and non-significantly (Tyagi *et al.* 2010, Gupta *et al.* 2018, Acharya *et al.* 2019[b]) higher serum cholesterol levels in transition cows receiving bypass fat or RPC alone or a combination of both RPC and RPF (Chavda *et al.* 2022) has been reported.

The mean plasma TAG and VLDL values in cows under all five groups were significantly higher in prepartum period as compared to all postpartum periods, being significantly lowest on the day 15th postpartum and again tended to increase in the subsequent days to reach significantly ($p<0.05$) high at day 45 and 60 postpartum. None of the treatments influenced significantly the overall mean plasma TAG and VLDL concentrations in transition Gir cows. However, both were significantly higher in treatment groups T3, T4 and T5 on 30th day postpartum as compared

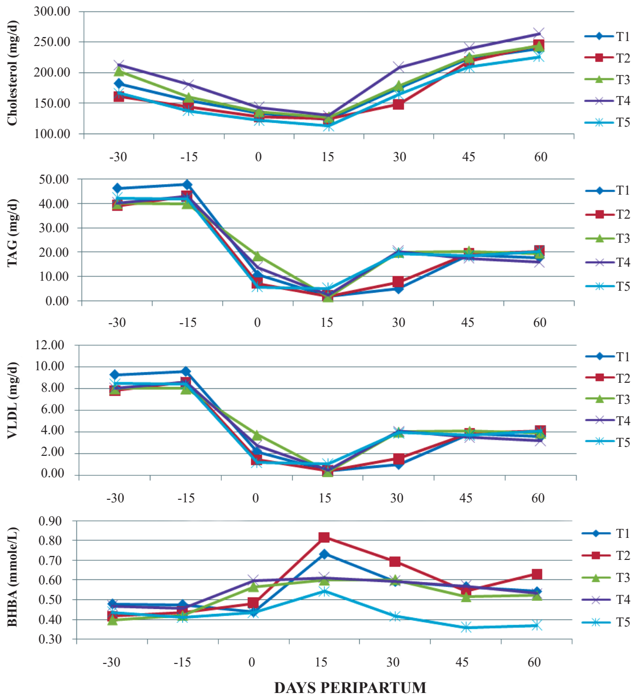

Fig. 2. Impact of peripartum supplementation of RPC (T2), RPF (T3), RPC+RPF (T4) and Se+Vit-E (T5) over control (T1) on certain blood biochemical profiles in Gir cows.

to T1 (control) and T2 groups (Fig. 2). Accumulation of TAG increases in the liver around parturition due to re-esterification of plasma NEFA, and TAG requires VLDL as a carrier to export NEFA from liver. Cooke *et al.* (2007) reported that transition dairy cows are choline deficient, which negatively affects liver status. In present study, supplementation with different nutrients during peripartum period might have helped in synthesis of phosphatidylcholine, a precursor of VLDL synthesis and improved the liver status. Present findings are in agreement with similar non-significant effects of supplementing RPC and/or RPF alone (Chavda *et al.* 2022) or in combination (Tyagi *et al.* 2010, Chavda *et al.* 2022) on blood TAG and VLDL concentrations reported by earlier researchers. However, significantly higher TAG and VLDL levels were reported with RPC (Acharya *et al.* 2019[b]) and RPF (Singh *et al.* 2014) supplementation in some studies, while others reported significant decrease in values in the dairy cows supplemented with RPC during peripartum (Amrutkar *et al.* 2015, Gupta *et al.* 2018) and RPF during postpartum period (Waghmare *et al.* 2016).

Effect on plasma BHBA status: Supplementation of both RPC and RPF alone or in combination did not affect plasma BHBA concentration, however it was found to be significantly ($p<0.001$) lower, overall as well as from 15th day until 60th day postpartum in cows receiving Se+Vit-E supplementation as compared to control, suggesting that supplementation of RPC and RPF alone or in combination did not alter BHBA production in the liver, while that of Se+Vit-E improved the oxidative status of liver (Fig. 2). Similar non-significant effect of supplementation of RPC on plasma BHBA concentration has been reported previously (Leiva *et al.* 2015, Pirestani and Aghakhani 2018, Anonymous 2020). However, Acharya *et al.* (2019[b]) found significantly higher plasma BHBA in cows supplemented with RPC during peripartum period. Non-significant effect of RPF supplementation on plasma BHBA was in line with Singh *et al.* (2014) using prill fat and Garnsworthy *et al.* (2008) in cows supplemented with bypass fat at various dose rates during postpartum period.

Effect on postpartum fertility: The cows of all the nutrients supplemented groups showed uterine involution on an average 2 days earlier with significantly earlier ($p<0.001$) commencement of cyclicity as compared to control group, and the periods (days) for T2, T3 and T4 groups were however found to be statistically similar (49.63±3.09, 51.75±5.43 and 50.88±5.72) with either T1 (63.50 ±9.52) or T5 (33.63±3.44) groups. Service period (days) was also significantly shortened with reduced number of services per conception in T5 group supplemented with Se + Vit-E (96.29±14.78) than that of control T1 (214.25±60.17), and the values for RPC, RPF and its combined supplementation were statistically similar (137.17±11.26, 193.40±40.69 and 186.83±29.36) with either T1 or T5 group, suggesting beneficial effect of all four supplementation in transition cows. Significantly higher conception rate in Vit-E + Se supplemented group (87.50%) was also recorded followed by RPC and RPC + RPF groups (75.00% each), RPF alone (62.50%) as compared to control T1 group (50.00%). A significant positive effect of supplementation of bypass fat and choline (Tyagi *et al.* 2010, Khalil *et al.* 2012, Dhami *et al.* 2017, Acharya *et al.* 2019[a], Vala *et al.* 2019) and Vit-E and Se (Modi *et al.* 2016, Hosnedlova *et al.* 2017) on uterine involution, occurrence of first estrus postpartum with reduced service period and improved conception rate has also been reported by earlier workers. However, Amrutkar *et al.* (2015) and Anonymous (2020) did not find significant influence on postpartum cyclicity with RPC, and Nirwan *et al.* (2019) with RPF supplementation in cows, though the service period was reduced. Fats and choline in the diet improve energy status increasing precursors (insulin and IGF-I) for the synthesis of reproductive hormones such as steroids and prostaglandins and has positive influence on reproduction by altering both ovarian follicle and CL function (Rahbar *et al.* 2014, Anonymous 2020).

Based on the results of peripartum nutrients supplementation in Gir cows, it can be concluded that DMI (kg/d) increased significantly ($p<0.001$) with RPC alone, while supplementation of RPF alone and its combination with RPC or Vit-E + Se supplementation did not influence it significantly. Vit-E + Se supplementation significantly ($p<0.001$) improved the concentration of blood glucose, plasma BHBA and total cholesterol followed by supplementation of RPC along with RPF as compared to control. Postpartum fertility in terms of reduced periods of uterine involution, postpartum first estrus ($p<0.05$) and service period with higher pregnancy rate ($p<0.01$) were noted in all the groups of nutrient supplemented cows than the non-supplemented cows.

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