RESEARCH ARTICLE

Influence of Peripartum Supplementation of Rumen Protected Choline and Rumen Protected Fat on Blood Biochemical Profile and Postpartum Reproductive Performance in Gir Cows

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Abstract

This study was conducted on 24 advanced pregnant Gir cows in their first to third lactation to evaluate the effects of supplementing rumen protected choline (RPC) and rumen protected fat (RPF) alone or in combination on various blood biochemical parameters and postpartum reproductive performance. The cows were divided into four equal treatment groups (n=6 each), *viz.,* T1 (control), T2 (RPC), T3 (RPF) and T4 (RPC and RPF), based on their parity, body weight and previous lactation yield. In T1 group, cows were fed with basal diet to meet their nutrient requirement as per ICAR (2013) feeding standards. In T2 group, cows were supplemented with RPC @ 45 g/day, in T3 with RPF @ 80 g/d and in T4 with RPC @ 45 g/day + RPF @ 80 g/d along with basal diet of T1 starting from 30 days before expected date of calving to 60 days postpartum and blood samples were collected at monthly intervals. Supplementation of RPC and RPF alone or in combination had no significant effect on plasma BUN, cholesterol, TAG, VLDL, NEFA and BHBA levels. Days to first observed heat were reduced significantly (p<0.001), while service period and number of services per conception were reduced non-significantly in supplemented groups compared to control. There was no advantage of combined supplementation of RPC and RPF in periparturient Gir cows. It was concluded that RPC or RPF alone can be supplemented in the ration of periparturient Gir cows to optimize the blood biochemical profile and improve postpartum reproductive performance.

Keywords: Gir cows, Peripartum metabolic profile, Postpartum fertility, Rumen protected choline, Rumen protected fat.

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Introduction

The negative energy balance (NEB) is an important characteristic of transition cows and is a normal adaptive mechanism in high yielding dairy animals (Wankhade *et al.,* 2017). Dairy cows try to adapt with NEB by mobilizing adipose tissue reserve through lipolysis, because of which more non-esterified fatty acids (NEFAs) are drained towards liver (Drackley *et al.,* 2014) and are accumulated as triacylglycerol (TAG) (LeBlanc, 2010). Esterification to form TAG is acceptable if they are exported as very low-density lipoprotein (VLDL). Though the hepatic capacity to esterify imported NEFA to TAG increases at the time of parturition (Litherland *et al.,* 2011), the influx of NEFA into the liver overwhelms hepatic oxidation and the capacity of the liver to secrete esterified fatty acids (FA) or TAG as VLDL (Grummer, 2008). The failure of the liver to use the excessive amounts of NEFA as fuel leads to increased production of ketone bodies (Goff, 2006). About 50-60% of transition dairy cows experience moderate to severe fatty liver and ketosis, and this remains a major challenge for production, health and welfare of dairy cows (Duffield, 2000). Resumption of ovarian activity is reported to be initiated during NEB, but NEB in early lactation has subsequent adverse effects on fertility that leads to delayed postpartum ovarian activity and poor conception rates (Wathes *et al.,* 2007; Chavda *et al.,* 2021).

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Choline being involved in the metabolism of fatty acids in the liver plays an important role in VLDL synthesis and thereby contributes to fat export from the liver (Acharya *et al.,* 2019[b]). The bioavailability of choline from the feedstuffs is very low in ruminants (<30%) because of extensive degradation by rumen micro-organisms, hence it must be supplemented in the protected form in the diet (Elek *et al.,* 2008). Supplementing RPF to high producing lactating cows can enhance energy density of ration and energy intake in early lactation without compromising rumen cellulolytic bacterial activity (Thakur and Shelke, 2010) and reduces the deleterious effect of NEB during early lactation (Ganjkhanlou *et al.,* 2009) and improves lactation performance (Duske *et al.,* 2009) and reproduction (Chavda *et al.,* 2021). Therefore, the present study was planned to evaluate the effects of peripartum supplementation of rumen protected choline (RPC) and rumen protected fat (RPF) alone or in combination on blood biochemical profile and postpartum reproductive performance in Gir cows.

Materials and Methods

Selection and Management of Animals

This study was carried out from August 2020 to May 2021 at the Department of Animal Nutrition, College of Veterinary Sciences, Kamdhenu University, Junagadh in collaboration with Cattle Breeding Farm, Junagadh Agricultural University, Junagadh on 24 advanced pregnant Gir cows in their first to third lactation. The cows were divided equally into four treatment groups, *viz.,* T1 (control), T2 (RPC), T3 (RPF) and T4 (RPC and RPF), based on their parity, body weight and previous lactation yield. In T1 group, cows were fed with basal diet of 250 g maize bhardo, 10 kg green sorghum, mature pasture grass hay *ad lib*., and compound cattle feed and cotton seed cake to meet their nutrient requirement as per ICAR (2013) feeding standards. In T2 group, the cows were supplemented with RPC @ 45 g/day, in T3 with RPF @ 80 g/d and in T4 with RPC @ 45 g/day + RPF @ 80 g/d along with basal diet of T1 starting from 30 days before expected date of calving to 60 days postpartum. All the cows were maintained in well ventilated hygienic sheds and clean drinking water was made available *ad lib.* RPC and RPF were purchased from Kemin Industries South Asia Pvt. Ltd. Ethical permission was granted by the Institutional Animal Ethics Committee of the College Junagadh vide experimental protocol No. JAU/JVC/IAEC/LA/64/2020.

Blood Sampling and Postpartum Events

From all the cows, blood samples were collected from jugular vein in heparinised vacutainers on days -30, 0, 30, and 60 of calving. The plasma was separated immediately by centrifugation of samples and stored at -20°C with a drop of merthiolate until analyzed. The reproductive parameters postpartum were recorded. Cows showing estrus 60 days after calving were only inseminated and pregnancy was confirmed 45 days after last AI.

Biochemical Assays

The biochemical parameters, *viz.,* blood urea nitrogen (BUN), plasma cholesterol and TAG were analyzed by using standard kits (Greiner Diagnostic GmbH, Germany) with fully automatic biochemistry analyzer (Dia-Chem 240 plus, Diatek). Plasma VLDL was calculated from the value of TAG using standard formula and plasma NEFA was estimated by colorimetric method using standard procedure and diagnostic kits (Randox Lab Ltd., Crumlin, UK). Plasma beta-hydroxybutyric acid (BHBA) concentration was measured by using colorimetric assay kit and procedure described by Cayman Chemical Company, USA as per the method of Galan *et al.* (2001).

Statistical Analyses

The data on different blood biochemical parameters were analyzed by using two way analysis of variance (ANOVA) for treatment and period effects, and on reproductive traits by one way ANOVA for treatment effect (Snedecor and Cochran, 1994). Pair-wise mean differences between groups were compared by Tukey's post-hoc test for significance at $p<0.05$.

Results and Discussion

Plasma Cholesterol Level

The mean plasma total cholesterol values in cows under all four groups tended to be lowered on the day of parturition, and thereafter the values again increased in the subsequent days postpartum to reach the highest ($p<0.05$) at day 60 postpartum (Table 1; Fig. 1). Supplementation of both RPC and RPF alone or in combination however did not significantly affect the plasma cholesterol concentration on any day of experiment including overall values, although it was higher in cows supplemented with RPF alone or in combination with RPC. Cholesterol is a component of the serum lipoproteins and its concentration in serum gives an indication of overall lipoprotein concentrations. Higher plasma cholesterol levels are associated with better reproductive performance in high yielding dairy cows (Tyagi *et al.*, 2010), as cholesterol acts as a precursor of steroid hormones (Staples *et al.,* 1998). Significant drop in plasma cholesterol on the day of calving may be associated with its utilization for greater synthesis of estrogens and corticosteroids required for the initiation of parturition and to combat related stress.

Gupta *et al.* (2018) and Acharya *et al.* (2019[b]) found no significant difference in plasma cholesterol concentration on supplementation of RPC at various dose rates to transition dairy cattle. However, Soltan *et al.* (2012) reported significant increase in blood cholesterol level in lactating dairy cows supplemented RPC postpartum. Tyagi *et al.* (2010) observed non-significantly higher cholesterol in crossbred cows receiving 2.5% bypass fat (on DMI basis) 40 days prepartum to 90 days postpartum than control. Yadav *et al.* (2015) found significant decrease in blood cholesterol levels in crossbred

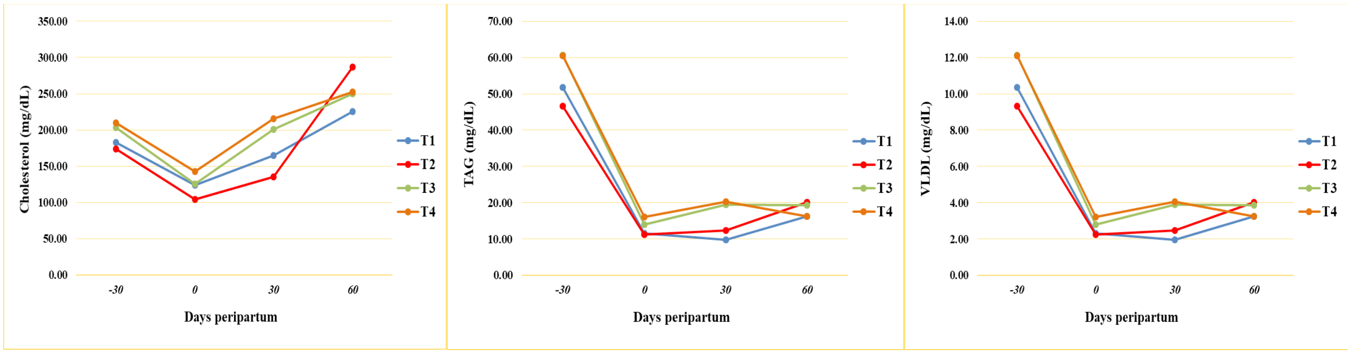

Fig. 1: Effect of RPC and RPF supplementation peripartum on monthly plasma cholesterol, triacyl glycerides and very low-density lipoprotein levels in Gir cows

Table 1: Effect of rumen protected choline (RPC) and rumen protected fat (RPF) supplementation peripartum on monthly plasma cholesterol, VLDL and TAG levels in Gir cows (Mean ± SE)

		Dietary treatment groups			
Parameter studied	*Peripartum Days*	*T1*	*T2*	*T3*	*T4*
Plasma cholesterol (mg/dL)	-30	183.00^{AB} ± 16.16	173.67^{A} ± 35.68	203.67^{AB} ± 10.72	210.17^{AB} ± 9.68
	0	123.83^{A} ± 16.62	103.83^{A} ± 22.70	125.67^{A} ± 10.94	142.33^{A} ± 5.12
	30	164.67^{AB} ± 21.40	135.17^{A} ± 32.99	201.00^{AB} ± 24.71	215.67^{AB} ± 16.82
	60	225.17^{B} ± 24.91	287.00^{B} ± 37.51	250.17^{B} ± 29.88	252.83^{B} ± 12.35
	Overall	174.17 ± 15.57	174.92 ± 27.46	195.13 ± 16.40	205.25 ± 7.60
Very low-density lipoprotein (mg/dL)	-30	10.37^{B} ± 1.25	9.33^{B} ± 1.19	12.13^{B} ± 2.04	12.10^{B} ± 2.02
	0	2.30^{A} ± 0.25	2.23^{A} ± 0.16	2.80^{A} ± 0.31	3.20^{A} ± 0.23
	30	1.97^{A} ± 0.14	2.47^{A} ± 0.41	3.90^{A} ± 0.44	4.07^{A} ± 0.42
	60	3.23^{A} ± 0.20	4.03^{A} ± 0.19	3.87^{A} ± 0.41	3.23^{A} ± 0.34
	Overall	4.47 ± 0.34	4.52 ± 0.29	5.68 ± 0.61	5.65 ± 0.52
Plasma triacyl-glycerides (mg/dL)	-30	51.83^{B} ± 6.25	46.67^{B} ± 5.93	60.67^{B} ± 10.21	60.50^{B} ± 10.12
	0	11.50^{A} ± 1.23	11.17^{A} ± 0.79	14.00^{A} ± 1.57	16.00^{A} ± 1.13
	30	9.83^{A} ± 0.70	12.33^{A} ± 2.04	19.50^{A} ± 2.22	20.33^{A} ± 2.12
	60	16.17^{A} ± 1.01	20.17^{A} ± 0.95	19.33^{A} ± 2.06	16.17^{A} ± 1.70
	Overall	22.33 ± 1.70	22.58 ± 1.45	28.38 ± 3.05	28.25 ± 2.61

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF,
Means bearing different upper case superscripts (A, B) within the column differ significantly ($p<0.05$) for periods or days for a parameter. No differences were observed between treatments for any of the parameters/periods.

dairy cows supplemented with RPF during peripartum period, while Nirwan *et al.* (2019) reported significantly increased serum cholesterol level in peripartum bypass fat supplemented Holstein Friesian cows.

Plasma TAG and VLDL Levels

The mean plasma TAG and VLDL values in cows under all groups were significantly higher in prepartum period as compared to postpartum periods, and it reflected mobilization of body reserve with initiation of lactation. Supplementation of both RPC and RPF alone or in combination did not significantly influence the plasma TAG and VLDL concentrations in periparturient Gir cows. However, they were non-significantly higher in cows supplemented with RPF alone or in combination with RPC, particularly on 30 days prepartum and 30 days postpartum as compared to control and RPC supplemented groups (Table 1; Fig. 1). Accumulation of TAG increases in the liver around parturition due to re esterification of plasma NEFA and this TAG requires VLDL as a carrier to be exported from liver. It was reported that periparturient dairy cows are choline deficient, which negatively affects liver status (Cooke *et al.*, 2007). In present study, supplementation of RPC and RPF during peripartum period might have helped in synthesis of phosphatidylcholine, a precursor of VLDL synthesis and improved the liver status.

Gupta *et al.* (2018) reported significantly decreased TAG and VLDL concentrations, while Acharya *et al.* (2019[b]) found significantly higher plasma TAG and VLDL levels in dairy cows supplemented with RPC during peripartum period. Similarly, Waghmare *et al.* (2016) observed significant decrease in blood TAG level, while Singh *et al.* (2014) recorded

Table 2: Effect of rumen protected choline (RPC) and rumen protected fat (RPF) supplementation peripartum on monthly BUN, plasma BHBA and NEFA levels in Gir cows (Mean ± SE)

		Dietary treatment groups			
Parameter studied	*Peripartum Days*	*T1*	*T2*	*T3*	*T4*
Blood urea nitrogen (mg/dL)	-30	24.04 ± 2.23	24.06 ± 2.48	21.15 ± 1.94	21.18 ± 1.35
	0	22.75 ± 2.04	21.55 ± 2.62	23.43 ± 3.53	27.18 ± 1.30
	30	21.42 ± 2.99	26.09 ± 3.20	22.88 ± 2.57	22.48 ± 2.47
	60	24.31 ± 1.86	24.24 ± 1.84	28.18 ± 1.73	22.73 ± 1.81
	Overall	23.13 ± 1.59	23.98 ± 1.87	23.91 ± 1.92	23.39 ± 0.97
Plasma BHBA (mmol/L)	-30	0.48 ± 0.01	0.42^{A} ± 0.02	0.40^{A} ± 0.05	0.47 ± 0.04
	0	0.44 ± 0.02	0.48^{AC} ± 0.02	0.57^{AB} ± 0.03	0.60 ± 0.05
	30	0.59 ± 0.04	0.69^{B} ± 0.05	0.60^{B} ± 0.10	0.59 ± 0.04
	60	0.54 ± 0.04	0.63^{BC} ± 0.02	0.52^{AB} ± 0.05	0.53 ± 0.05
	Overall	0.51 ± 0.03	0.56 ± 0.02	0.52 ± 0.05	0.55 ± 0.03
Plasma NEFA (mmol/L)	-30	0.73 ± 0.07	0.92 ± 0.19	0.75 ± 0.14	0.78 ± 0.10
	0	0.56 ± 0.03	0.86 ± 0.09	1.05 ± 0.14	0.75 ± 0.07
	30	1.00 ± 0.16	0.94 ± 0.16	0.87 ± 0.15	1.07 ± 0.13
	60	0.81 ± 0.14	1.11 ± 0.17	0.90 ± 0.18	0.86 ± 0.06
	Overall	0.78 ± 0.06	0.93 ± 0.11	0.91 ± 0.12	0.86 ± 0.06

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF; Means bearing different upper case superscripts (A, B) within the column for periods/days differ significantly ($p<0.05$) for BHBA, otherwise no significant differences were observed between treatments or periods within treatment for BUN and NEFA.

significantly higher levels of triglycerides and VLDL in the cows supplemented with RPF during postpartum period. However, serum triglycerides were reported to be increased non-significantly on feeding bypass fat with and without RPC by Tyagi *et al.* (2010), while Yadav *et al.* (2015) found no significant effect of peripartum bypass fat supplementation on blood triglycerides and VLDL concentrations in crossbred cows, which concurred with the present findings.

Blood Urea Nitrogen (BUN) Level

Supplementation of both RPC and RPF alone or in combination did not affect the BUN concentration in periparturient Gir cows, as the levels across the treatments and periods within treatment were similar (Table 2). Similar were the findings of Acharya *et al.* (2019[b]) with RPC supplementation peripartum. Anonymous (2020) also did not observe significant effect of supplementing RPC during transition period on BUN level in dairy cows. Non-significant effects of RPF supplementation on BUN level in periparturient crossbred dairy cows was also reported by Tyagi *et al.* (2010).

Plasma BHBA and NEFA Levels

Plasma BHBA concentration was not affected significantly by supplementation of both RPC and RPF alone or in combination (Table 2), suggesting that supplementation did not alter BHBA production in the liver. Although it was apparently higher on day 30 postpartum in most of the groups, and in RPC supplemented group over others at most intervals. Our results are comparable with findings of Pirestani and Aghakhani (2018) and Anonymous (2020), who supplemented RPC during transition period at various dose rates to dairy cows and found no significant difference in plasma BHBA concentration. However, significantly higher plasma BHBA in cows supplemented with RPC during peripartum period was reported by Acharya *et al.* (2019[b]). Non-significant effect on plasma BHBA level in RPF supplemented cows is in line with Singh *et al.* (2014), who also reported non-significant effect of prill fat supplementation @ 75 g/day on plasma BHBA level in lactating crossbred cows.

Supplementation of both RPC and RPF alone or in combination also did not show any significant effect on plasma NEFA concentration in periparturient Gir cows, even though it was higher in all nutrient supplemented groups, and on day 30 postpartum in most groups (Table 2) suggesting that supplementation of both RPC and RPF alone or in combination did not alter adipose tissue mobilization. We also observed non-significant effect of these supplements on blood glucose and plasma insulin levels, but with reduced IGF-1 levels compared to control group (Chavda *et al.*, 2021), which supported the present finding on NEFA and BHBA as well.

Zom *et al.* (2011) reported non-significant difference in plasma NEFA concentration in periparturient cows receiving different levels of RPC. However, Acharya *et al.* (2019[b]) reported significantly decreased concentrations of plasma NEFA in response to RPC supplementation peripartum in dairy cows. Like present finding, Tyagi *et al.* (2009) also did not detect any treatment effect on blood NEFA concentration in crossbred cows supplemented with bypass fat (2.5%

of total dry matter intake) 40 days pre-partum till 90 days postpartum, while others (Yadav *et al*., 2015; Nirwan *et al*., 2019) reported significantly decreased concentrations of plasma NEFA in response to RPF supplementation to dairy cows during peripartum period.

Postpartum Reproductive Performance

In the present study, the days to first observed heat postpartum in T1, T2, T3 and T4 groups of Gir cows were 81.67 ± 3.87, 51.17 ± 4.00, 46.17 ± 3.89 and 53.50 ± 4.92, respectively. It was almost similar and significantly shorter (p<0.001) in all three nutrients supplemented groups as compared to control. The service period or days open in the respective groups T1 to T4 were 155.00 ± 17.17, 125.33 ± 12.21, 126.50 ± 15.95 and 138.50 ± 6.05, which however was non-significantly lower in all three nutrient treatment groups than the control, suggesting beneficial effect of nutrients supplementation in transitional Gir cows. Pirestani and Aghakhani *et al.* (2018) observed very similar findings on these reproductive traits of cows supplemented with RPC.

The commencement of postpartum cyclicity, is related with the process of involution of uterus and postpartum genital health. Acharya *et al.* (2019[a]) and Anonymous (2020) observed non-significantly earlier commencement of cylicity in cows fed RPC during peripartum period than that of control. Similarly, Pandurang (2012) and Chavda *et al.* (2021) observed non-significant improvement in service period in the cows receiving RPC in peripartum periods. However, the service period was reported to be improved significantly due to supplementation of RPC in other studies (Acharya *et al.*, 2019[a]; Anonymous, 2020). The significant effect of peripartum RPF supplementation on commencement of cylicity in present study agreed with findings of Dhami *et al.* (2017) and Chavda *et al.* (2021), while Nirwan *et al*. (2019) observed no significant effect on onset of cyclicity, but there was significantly shorter service period in peripartum RPF supplemented dairy cows.

Conclusions

Based on the results, it can be concluded that supplementation of both RPC and RPF alone or in combination does not have significant effect on concentration of plasma cholesterol, TAG, VLDL, BUN, NEFA and BHBA in periparturient Gir cows. Supplementation of both RPC and RPF alone or its combination improves postpartum fertility by reducing days to first observed heat and service period.

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