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Influence of peripartum dietary supplementation of choline and fat in protected form on production performance of Gir cows

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ABSTRACT

In this experiment, the effects of supplementing choline and fat in rumen protected form during peripartum period on feed intake, milk production and composition of Gir cows were studied. Twenty four Gir cows of 1st to 3rd parity were used from 30 days pre-partum through 60 days post-partum and randomly assigned to four equal treatment groups (n=6) on the basis of their parity, body weight and previous lactation yield. Control diet was fed to cows in group T_1 (control). Additionally, rumen protected choline (RPC) @45 g/d in group T_2; rumen protected fat (RPF) 80 g/d in group T_3 and RPC @45 g/d + RPF @80 g/d in group T_4 were supplemented along with control diet. The treatments significantly affected dry mater intake (DMI) and milk production of cows. DMI was increased in the cows fed with RPC as compared to control. Milk yield, 4% fat-corrected milk, solid-corrected milk and energy-corrected milk were higher in the cows fed with RPC and RPF alone or in combination, as compared to control. No synergistic effect was observed with these supplements on DMI or milk production. None of these supplements influenced the milk composition significantly, however yield of milk fat, protein and lactose were higher in all the nutrient supplemented cows compared to control. Net return over feed cost was higher in supplemented cows compared to control. Results indicated that supplementation of RPC or RPF can improve feed intake and productive performance of Gir cows for overall economic benefits.

Keywords: Dairy cows, Milk yield, Negative energy balance, Rumen protected choline, Rumen protected fat, Transition period

Dairy cows face a metabolic challenge in the transition period, and in early lactation during which they prepare themselves first for parturition and then for subsequent lactation along with profound changes in their metabolic and endocrine status. At the same time, dairy cows show a marked decrease in dry matter intake (DMI) along with sudden increase in energy demand, by both foetus and dam which let the animals enter to a state of negative energy balance (NEB) (Esposito *et al.* 2014). In high producer, it is a normal adaptive mechanism (Wankhade *et al.* 2017) through which animals try to deal with high energy demands from body reserve by lipolysis. Level of non-esterified fatty acids (NEFAs) in blood increased by 5 to 10 fold during NEB which are accumulated as triacylglycerol (TAG) in the liver after re-esterification (LeBlanc 2010) and animal is likely to develop fatty liver syndrome (Bobe *et al.* 2004) as liver fails to secrete esterified fatty acids as very low density lipoprotein (VLDL) due to increased inflow of NEFAs beyond the hepatic oxidative capacity (Grummer 2008), even though the hepatic oxidative capacity increases by 20% few weeks before parturition (Drackley *et al.* 2001). It has been suggested that strategies to reduce fat mobilization during transition period could also reduce the impact of NEB on overall performance of dairy animals (Sordillo and Raphael 2013).

Strategic supplementation of choline and fat in the diet of transient dairy cows may obviate the adverse effects of NEB during early lactation. Choline contributes to fat export from the liver through synthesis of VLDL (Acharya *et al.* 2019), thereby improving the fat metabolism and energy intake during transition period can be enhanced by supplementing protected fat to high producing dairy cows without affecting rumen cellulolytic microbial activity. As most of the free choline present in the feedstuffs of dairy animals gets degraded rapidly in the rumen, it must be supplemented in the protected form. Looking at the facts, the present study was envisaged to rule out the deleterious effects of NEB on postpartum productive performance of Gir cows and thereby to augment the milk production and to improve dairy farmers' economy as well as their livelihood.

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MATERIALS AND METHODS

The experiment was carried out at Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh (Gujarat), India (21.5°N 70.4°E; 107 meter above sea level) under the approval of Institutional Animal Ethics Committee of the college vide protocol No. JAU/JVC/IAEC/LA/64/2020 in collaboration with Cattle Breeding Farm, Junagadh Agricultural University, Junagadh, India during the year 2020-21.

Experimental animals, treatments and management: Twenty four Gir cows of 1st-3rd parity in advance pregnant stage were selected from Cattle Breeding Farm, Junagadh, India. Based on lactation number, body weight and previous lactation yield cows were divided into four equal treatment groups (n=6) from T_1 to T_4. Each group had two animals each of 1st - 3rd parity. The average body weight (kg) and previous lactation yield (kg) of experimental cows in T_1, T_2, T_3 and T_4 groups were 411 and 1851; 440 and 1812; 455 and 1746 and 418 and 1781, respectively. Cows in control (T_1) group were fed with basal diet comprising of 250 g maize *bhardo* (ground maize), 10 kg green sorghum (*Sorghum bicolor*), mature mixed local pasture grass hay *ad lib*. and compound cattle feed and cotton seed cake to meet their nutrient requirements (ICAR 2013). Along with basal diet, the cows in other three groups were additionally supplemented with 45 g/d rumen protected choline (RPC) in group T_2, 80 g/d rumen protected fat (RPF) in group T_3, and 45 g/d RPC + 80 g/d RPF in group T_4 for a period of 90 days (30 days pre-partum to 60 days post-partum). The feeds and fodder used in the experiment were analysed for proximate composition (Table 1) as per the methods of AOAC (2012). Free access to wholesome drinking water and well-ventilated hygienic sheds were made available to all experimental cows.

Feed intake: Dairy DMI was calculated by recording the daily feed offered and feed left over during the entire experimental period. The dry matter of different feed ingredients was recorded fortnightly.

Milk yield and composition: Animals were hand milked twice daily (5:00 h and 17:00 h) and average daily milk yield of individual animal was recorded at each milking. The average milk production of individual animal was calculated on fortnightly basis. Representative milk samples were analyzed fortnightly for fat, solids-not-fat (SNF), total solids, protein and lactose by using pre-calibrated milk analyzer (Lacto scan); total ash was analysed as per the method described by AOAC (2012). Milk yield data was converted to 4% fat-corrected milk (FCM), solid-corrected milk (SCM) and energy-corrected milk (ECM) using standard equations described by Gaines (1928), Tyrrell and Reid (1965) and Sjaunja *et al.* (1990), respectively.

Economics: Economics of milk production was calculated based on farm price of various feeds (compound cattle feed ₹25.58/kg; cotton seed cake ₹25.31/kg; maize *bhardo* ₹21.80/kg), fodders (green fodder ₹2.00/kg; dry fodder ₹3.25/kg), and milk (₹34.00/kg), while for supplements actual market price (RPC ₹390/kg; RPF ₹100/kg) was taken into consideration.

Statistical analysis: Treatment and period effects on dry matter intake, yield of milk and milk components as well as milk composition were statistically analysed by two-way analysis of variance according to methods described by Snedecor and Cochran (1994). Tukey's post-hoc test was used to compare means between the groups at the 5% level of significance.

RESULTS AND DISCUSSION

Feed intake: Treatments had significant effect on DMI as it was significantly ($P<0.01$) higher in cows supplemented with RPC, while supplementation of RPF alone or in combination with RPC showed non-significant ($P>0.05$) numerically higher values for DMI as compared to control group (Table 2). The significant effect of the treatment on DMI was observed from 45th day onwards. Period had significant ($P<0.05$) effect on DMI in all experimental groups as DMI was found significantly ($P<0.05$) lower on the day of parturition, which then significantly ($P<0.05$) increased during 1st fortnight in T_2, 2nd fortnight in T_3 and 4th fortnight in T_1 and T_4 groups (Fig. 1). Treatment × period had no any significant ($P>0.05$) effect on DMI.

In the present study, DMI was significantly increased by 1.15 kg/d over the control in the cows supplemented with RPC, which corroborated with previous studies. Significant increase in DMI of 1.6 kg/d (from 14.4 to 16.0) in Holstein Friesian cows supplemented with RPC @60 g/d during peri-parturient period (Zom *et al.* 2011) and 8.4%

Table 1. Proximate composition of feeds and fodder

Particular	% DM basis						
	DM	OM	CP	EE	CF	NFE	TA
Compound cattle feed	90.37	87.6	20.47	3.65	10.62	52.85	12.4
Cotton seed cake	92.57	94.21	20.77	8.43	33.24	31.77	5.79
Ground maize	91.03	97.43	10.83	3.41	2.97	80.23	2.57
Sorghum green	24.35	92.13	5.58	2.9	32.05	51.6	7.87
Mature pasture grass/hay	92.13	91.39	3.1	1.16	39.83	47.3	8.61

DM, Dry matter; OM, Organic matter; CP, Crude protein; EE, Ether extract; CF, Crude fibre; NFE, Nitrogen free extract; TA, Total Ash.

Table 2. Effect of RPC and RPF supplementation on dry matter intake, milk yield, yield of milk components and milk composition

Item	Treatment				SEM	P-value		
	T_1	T_2	T_3	T_4		Treatment	Period	Treatment × Period
DMI (kg/d)	8.13[a]	9.28[b]	8.88[ab]	8.77[ab]	0.24	0.009	<0.001	0.933
Yield (kg/d)								
Milk	6.68[a]	9.30[b]	8.85[b]	8.68[b]	0.42	<0.001	0.717	0.998
4% FCM	7.62[a]	10.51[b]	10.49[b]	10.15[b]	0.57	0.001	0.750	0.996
SCM	7.36[a]	10.19[b]	10.22[b]	9.80[b]	0.54	<0.001	0.801	0.996
ECM	7.37[a]	10.20[b]	10.21[b]	9.81[b]	0.54	<0.001	0.798	0.996
Fat	0.33[a]	0.45[b]	0.46[b]	0.45[b]	0.03	0.005	0.743	0.986
Protein	0.21[a]	0.29[b]	0.29[b]	0.28[b]	0.01	<0.001	0.907	0.984
Lactose	0.32[a]	0.45[b]	0.43[b]	0.42[b]	0.02	<0.001	0.907	0.986
Milk composition (%)								
Fat	4.88	4.86	5.18	5.17	0.20	0.521	0.518	0.638
Protein	3.15	3.16	3.23	3.18	0.12	0.560	0.151	0.947
Lactose	4.76	4.78	4.89	4.81	0.23	0.264	0.172	0.888
Solids-not-fat	8.67	8.71	8.91	8.76	0.05	0.580	0.181	0.939
Total solids	13.55	13.57	14.09	13.93	0.07	0.559	0.151	0.944
Total ash	0.70	0.71	0.72	0.71	0.01	0.510	0.128	0.968

T_1, Control; T_2, RPC; T_3, RPF; T_4, RPC + RPF. Means bearing different superscripts (a, b) within the row differed significantly (P<0.05). SEM, standard error of mean.

increase due to RPC supplementation @30 g/d in the first 12 weeks of lactation in Holstein cows (Soltan *et al.* 2012) has been reported earlier. However, a non-significant effect of RPC supplementation on DMI in peri-parturient dairy cows (Elek *et al.* 2008) and in early lactating dairy cows (Rahmani *et al.* 2014, Pineda and Cardoso 2015) has also been reported in some studies. In a meta-analysis of thirteen studies, Grummer (2012) founded 0.8 kg increase in DMI per day in early lactation due to RPC supplementation, however feed intake was not affected before calving. Supplementation of RPF alone or along with RPC did not influence DMI significantly in this study. Similar non-significant effect of supplementing dairy cows with bypass fat during peri-partum period on DMI was observed by Shankhpal *et al.* (2016). In other studies, the lactating crossbred cows receiving RPF @300 g/d (Sirohi *et al.* 2010) or 100 g of RPF alone and along with 10 g of RPC (Garg *et al.* 2012) or 75 g/d RPF (Singh *et al.* 2014) had no significant influence on their DMI.

Milk yield: The fortnightly mean daily milk yield, 4% FCM, SCM and ECM for the whole experimental period are given in Table 2. There was significant (P<0.001) effect of supplementing RPC and RPF alone or in combination on these traits across lactation weeks. The actual milk yield (P<0.001) and 4% FCM (P=0.001) were greater for RPC followed by RPF and RPC+RPF fed cows, while SCM (P<0.001) and ECM (P<0.001) were greater for RPF followed by RPC and RPC+RPF fed cows than control,

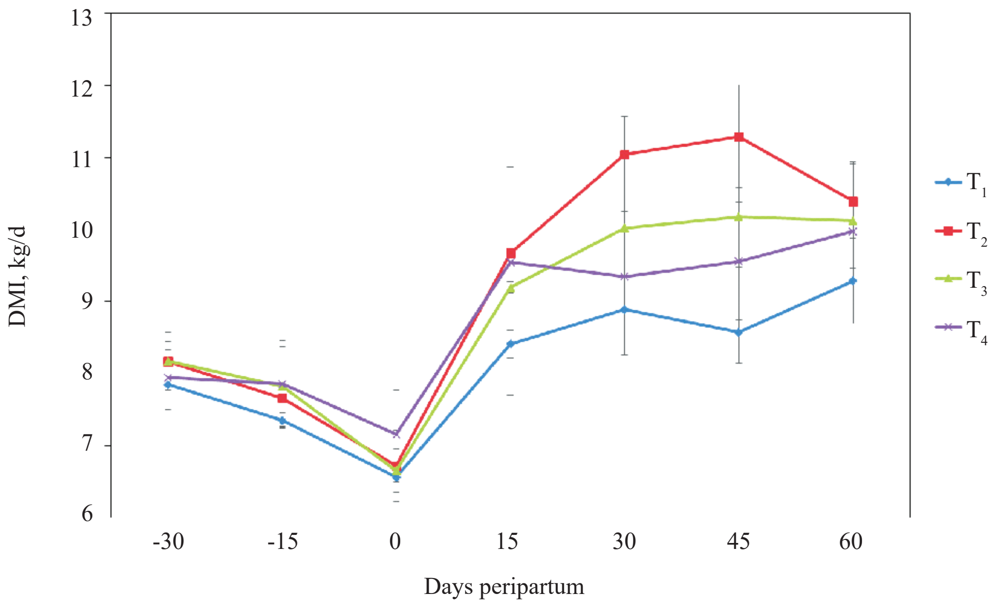

Fig. 1. Effect of RPC and RPF supplementation on dry matter intake (T_1, Control; T_2, RPC; T_3, RPF; T_4, RPC + RPF).

Table 3. Economics of peripartum RPC and RPF supplementation in Gir cows

Particular	Treatment			
	T_1	T_2	T_3	T_4
Average cost of feeding (₹/head/d)	118.91	158.98	145.64	151.76
Receipt from sale of milk (₹/head/d)	227.12	316.20	300.90	295.12
Net return over feed cost (₹/head/d)	108.21	157.22	155.26	143.36
Net return over control (₹/head/d)	---	49.01	47.05	35.15

T_1, Control; T_2, RPC; T_3, RPF; T_4, RPC + RPF.

however the differences for these traits among the nutrient supplemented cows were non-significant.

Significant improvement in yield of actual milk, 4% FCM, SCM and ECM in experimental cows fed with RPC and RPF alone or in combination was observed. Similar significant ($P<0.001$) improvement in milk yield and 4% FCM by 4.4 and 2.5 kg/d in the cows receiving supplementary choline @100 g/d from 21 days pre-partum and @200 g/d 60 days post-partum has been documented (Elek *et al.* 2008). Many other researchers also have observed a tendency for higher milk and 4% FCM yield with supplementation of RPC in cows during transition period (Zahra *et al.* 2006, Ardalan *et al.* 2010, Grummer 2012, Soltan *et al.* 2012). Significant increase in yield of milk and 4% FCM due to RPF supplementation in present study corroborated well with studies of Sirohi *et al.* (2010) and Gowda *et al.* (2013) in crossbred cows. However, others could not find positive effect of RPC (Zom *et al.* 2011, Leiva *et al.* 2015) or RPF (Singh *et al.* 2014, Shankhpal *et al.* 2016) supplementation on milk and 4% FCM yield in dairy cows during the transition period. Furthermore, significant increase in ECM as observed in current study has been also reported in dairy cows supplemented with RPC (Ardalan *et al.* 2010, Acharya *et al.* 2020) or prill fat (Singh *et al.* 2014); others did not observe significant effect of RPC supplementation on ECM (Rahmani *et al.* 2014, Pineda and Cardoso 2015). Significant increase in SCM yield noted in supplemented Gir cows under study however contradicted the findings of Leiva *et al.* (2015) in cows supplemented with 50 and 100 g of RPC 21 days before and 45 days after calving.

Milk production in lactating dairy cows was reported to be improved with increased supply of intestinal choline (Baldi and Pinotti 2006). Dietary choline can directly or indirectly promote milk production in dairy cows. In transition cows, indirect effect may be due to improvement in general health condition leading to positive production response (Shahsavari *et al.* 2016), whereas direct effect is based on enhanced gluconeogenesis in liver due to lower hepatic fat and higher availability of spared methionine for milk synthesis (Goselink *et al.* 2013). Methionine has been identified as the most limiting amino acid for the synthesis of milk and milk protein by the dairy cows fed diets on corn (Overton *et al.* 1998). Choline can reduce methionine utilization for methylation reaction as it acts as methyl group donor (Pinotti *et al.* 2008) and decrease demand of methyl group from methionine pool that contributes to production benefits to dairy cows (Lobley *et al.* 1996). Increased milk yield in RPC fed cows was also reported due to increased (Zahra *et al.* 2006) or similar dry matter intake (Elek *et al.* 2008). The significant increase in milk yield in present study may be due to increased dry matter intake, which may lead to increased nutrient flow towards milk production or due to increased energy density of ration that leads to more TDN intake and associative effects of rumen protected fat or due to the fact that supplementation of RPC may improve methyl group status in cows during transition period.

Milk composition: Any significant differences in fat, SNF, total solids, protein, lactose or total ash contents in the milk of cows supplemented with either RPC and RPF alone or in combination were not observed ($P>0.20$, Table 2), however milk of the cows fed RPF alone or along with RPC had non-significantly higher values for these traits in general. Yields of milk fat ($P=0.005$), protein ($P<0.001$) and lactose ($P<0.001$) from nutrient supplemented cows were greater than that of control, which was due to significantly higher milk production in all nutrient supplemented cows, however the differences among the nutrient supplemented cows were non-significant. In general, the treatment × period had no any significant ($P>0.05$) effect on milk yield and composition.

In previous researches, concentration of milk fat was reported to not be affected by the supplementation of RPC (Elek *et al.* 2008, Ardalan *et al.* 2010) or RPF (Gowda *et al.* 2013) to dairy cows. Similarly, others (Xu *et al.* 2006, Pawar *et al.* 2015) also did not observe significant effect of supplementing RPC on concentration of various milk components in dairy cows. Findings of present study are in agreement with these studies. However, significant increase in milk fat, protein and total solids with RPC (Leiva *et al.* 2015, Acharya *et al.* 2020) and RPF (Garg *et al.* 2012, Singh *et al.* 2014, Shankhpal *et al.* 2016) supplementation to dairy cows has been reported in various other studies.

Economics: The economics of feeding RPC and RPF alone or in combination to Gir cows throughout the trial has been shown in Table 3. The average cost of feeding (₹/head/d) was higher in T_2, T_3 and T_4 groups as compared to T_1 due to additional cost of supplements. While the net return over feed cost of milk yield (₹/head/d) was higher in T_2 (₹49.01) followed by T_3 (₹47.05) and T_4 (₹35.15) groups over that of T_1 (control) group. Thus, feeding RPC and RPF alone or in combination to Gir cows during peripartum period was cost effective. However, highest net return over control was found in RPC supplemented cows. Net return over control/animal/day was increased by 45.29, 43.48 and 32.48% in cows supplemented with RPC, RPF alone and in combination, respectively, over the non-supplemented cows, which was attributed to higher milk yield in supplemented groups, and relatively high cost of two supplements in T_4 group.

The study concludes that the supplementation of rumen protected choline in the diet of periparturient Gir cows significantly increases dry matter intake. Milk production was improved significantly in cows supplemented with rumen protected choline and rumen protected fat alone or in combination. However, none of these supplements influenced the milk composition significantly. No synergistic effect was observed for these two supplements on dry matter intake or milk production. Results of this study indicated that supplementation of rumen protected form of choline or fat can be beneficial and is recommended in the diet of periparturient Gir cows to improve their feed intake and productive performance for overall economic benefits.

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