Indian J Dairy Sci 76(1): 73-77
https://doi.org/10.33785/IJDS.2023.v76i01.010

RESEARCH ARTICLE

Effect of rumen-protected choline supplementation on production performance and haemato-biochemical profile of Kankrej cows

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Received: 27 April 2022 / Accepted: 09 September 2022 / Published online: 20 February 2023

Abstract: A study was conducted to examine the effect of supplementation of rumen-protected choline chloride (RPC) on production performance and haemato-biochemical parameters of Kankrej cows. Twenty Kankrej cows were randomly allocated to two treatment groups: control (CON: fed basal diet without supplement) and treatment (RPC: basal diet supplemented with 50 g/d/cow of RPC) from 4-weeks pre-partum through 8-weeks post-partum. Results showed that average body weights and dry matter intake were not affected (P<0.05) by the supplementation of RPC. There was significant (P<0.05) improvement in yields of milk (11.10 *vs.* 12.45 kg/d), FCM (10.93 *vs.* 12.49 kg/d) and ECM (12.07 *vs.* 13.76 kg/d) with RPC supplementation when compared with cows fed on the control diet. The milk fat, SNF, total solids, protein and lactose contents did not differ (P>0.05) between the treatment groups.Supplementation of RPC improved feed efficiency in terms of kg of 4% FCM/kg of DMI and kg of ECM/kg of DMI in lactating cows.The higher (P<0.05) serum glucose concentration (65.72 *vs.*62.05 mg/dL) was observed in RPC than the CON. Supplementation of RPC reduced (P<0.05) concentration of serum triglycerides (27.27 *vs.*31.38 mg/dL) as compared to the CON group. Other estimated blood metabolites were not influenced (P>0.05) by the supplementation of RPC. In conclusion, supplementation of 50 g/d rumen-protected choline during peripartum period improved milk production, feed efficiency and blood glucose in Kankrej cows.

Keywords: Blood metabolites, Choline, Kankrej cow, Milk yield, Transition phase

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Introduction

Nutritional care during the transition period influences productivity, health and fertility of dairy cows. Due to the reduced dry matter intake during periparturient period, the energy intake is usually insufficient in dairy cows to meet the high energy needs for milk production, resulting in a negative energy balance (Ospina et al. 2013). This leads to production of non-esteriûed fatty acids (NEFA)as a source of energy through the mobilization of fat from adipose tissue. The excessive amounts of NEFA overwhelm hepatic oxidation, leading to ketosis because of increased production and secretion of ketone bodies and “fatty liver” syndrome due to accumulation of triacylglycerol in the liver (Morrison et al. 2018). The elevated levels of ketone bodies and NEFA further contribute to oxidative stress, inûammatory responses, and a compromised immune system, therefore enhancing the susceptibility to infectious diseases, impairing fertility and reduced productive performance (Shahsavari et al. 2016).

Choline is a nutrient required for the synthesis of phosphatidylcholine, aphospholipid found in the membranes of VLDL (NRC, 2001). Choline improves lipid metabolism, increasing the VLDL synthesis, availability of fatty acids for the mammary gland, and its incorporation into phospholipid membranes around fat globules (Lopreiato et al. 2020). The dietary choline is rapidly degraded by rumen microbes, which lead to less than 20% bioavailability ofcholine in the ruminants (Bollatti et al. 2020a), the only effective method of increasing choline availability to dairy cows is to feed it in a form that is protected from ruminal degradation (Arshad et al. 2020). Recent studies have shown that supplementation of rumen-protected choline (RPC) during the periparturient period has led to increase in milk yield (Bollatti et al. 2020b; Potts et al. 2020; Holdorf and White, 2021). However, the effects of RPC supplementation in Indigenous dairy cows

have not been studied widely yet. Kankrej cows maintained at Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Gujarat had average standard (305 days) lactation yield of 2479 litres during the year 2020 (Anonymous, 2021). To test the hypothesis that RPC supplementation during periparturient period in Kankrej cows improves yields of milk and milk components without affecting feed intake, a study was conducted to evaluate the effect of feeding RPC from 4-weeks pre-partum through 8-weeks post-partumon milk production, composition and haemato-biochemical profile of Kankrej cows.

Materials and Methods

The use of the animals and the experimental protocol of this study were approved by the Institutional Animal Ethics Committee of College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India (VETCOLL/IAEC/2019/13/PROTOCOL-08).

Twenty Kankrej cows (BW,457.1±6.22 kg;parity,3.2±0.28;average previous lactation milk yield,8.30±0.18 kg/d)from Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India were randomly divided into two groups: 1) control (CON), fed with basal diet (consisting of concentrate mixture, green oat fodder and jowar hay) and 2) treatment (RPC), fed with basal diet supplemented with 50 g/cow daily of rumen-protected cholinechloride (CholiPEARL™, Kemin Industries South Asia Pvt. Ltd., Chennai, India). RPC was mixed thoroughly into concentrate and provided once daily from 4-weeks pre-partum through 8-weeks post-partum. The basal diet was formulated to meet ICAR (2013) nutrient requirements. The chemical composition of feeds and fodders fed to the experimental animals (Table 1) was within the normal range for Indian feed stuffs (ICAR, 2013).

The offered feeds and subsequent left-over were recorded at fortnightly interval for each animal in order to calculate feed intake. The samples of feeds and fodders were collected, composited and dried at 60°C in a forced air oven for 48 h, ground to pass through a 1-mm screen using a Wiley mill (Star Scientific Instruments, Delhi, India). The feeds samples were analyzed for dry matter (DM, method 934.01), ash (method 942.05), crude protein (method 976.05) and ether extract (method 973.18) according to AOAC (2007). Neutral detergent fibre and acid detergent fibre were determined as per Van Soest et al. (1991). Non-fiber carbohydrate was calculated according to NRC (2001). Cows were milked twice a day and individual milk yield for each cow was recorded daily by using electronic weighing balance. The 4% fat corrected milk (FCM) was calculated using formula: milk yield (kg) x 0.4 + fat yield (kg) x 15. Energy corrected milk (ECM) was determined using formula: 0.327 × milk yield (kg/d) + 12.86 × fat yield (kg/d) + 7.65 × protein yield (kg/d). Milk samples were collected at fortnightly interval for analysis of milk composition (fat, solids-not fat (SNF), protein and lactose) using EKOMILK Ultra Pro Milk Analyzer (Everest Instruments Pvt. Ltd.).

On 56th day of experimental feeding, blood samples from jugular vein were collected from each animal in the vials (BD Vacutainer® Spray-coated K2EDTA Tubes, BD Franklin Lakes, NJ, USA) with and without EDTA (BD Vacutainer® Plus Plastic Serum Tubes, BD Franklin Lakes, NJ, USA). The fresh blood samples were analyzed for haemoglobin, haematocrit, erythrocytes and leucocytes using Exigo EOS Vet Haematology Analyser(Boule Medical AB, Sweden).The serum samples were analyzedfor concentrations of glucose, total proteins, albumin, urea, triglycerides and cholesterol using Randox Monaco Analyser (Randox Laboratories Ltd., UK).

All the experimental data obtained were statistically analyzed by using statistical software SPSS v.16.0 (SPSS Inc., Chicago IL). One-way ANOVA was used to test the level of significance. Significant differences between means of treatments were assessed by the Duncan's test, and the differences among treatments were declared significant at $P<0.05$.

Results and Discussion

The effect of rumen-protected choline chloride supplementation on production performance of Kankrej cows is given in Table 2. Supplementation of RPC increased yields of milk (1.35 kg/d; P=0.046), 4% FCM (1.69 kg/d; P=0.022) and ECM (1.81 kg/d; P=0.024) as compared to the CON group. The percentages of

Table 1 Chemical composition (% DM basis) of feeds and fodders fed to Kankrej cows

Composition	Concentrate mixture	Oat forage	Jowar hay
Dry matter	94.91	17.44	11.83
Crude protein	19.57	8.92	6.39
Crude fibre	6.58	27.67	32.25
Ether extract	4.88	3.12	1.34
Ash	8.02	7.34	9.07
NFC	42.38	23.81	21.79
NDF	25.15	56.81	61.41
ADF	15.27	32.06	40.98

NFC: Non-fiber carbohydrate, calculated by equation: NFC (% of DM) =100 - (CP + NDF + EE + Ash); NDF: Neutral detergent fiber; ADF: Acid detergent fiber

milk fat, SNF, total solids, protein and lactose were not influenced (P>0.05) by the supplementation of RPC. There were increased yields of milk fat (18.2%), SNF (11.4%), total solids (13.6%), protein (12.8%) and lactose (13.7%) due to feeding of RPC when compared to the CON group. The improved production performance in dairy cows due to supplementation of RPC may be because of role choline in intermediary metabolism, in particular as a component of phospholipids and lipoproteins, which are critical for lipid absorption and transport, and enhancing uptake of fatty acids in early lactation, thereby making transition cows responsive to supplementation (Bollatti et al. 2020b). Choline also influences nutrient partition in the mammary gland toward milk synthesis mediated by increases in growth hormone (Kawamura et al. 2012). Choline kinase, an enzyme involved in the conversion of choline to phosphocholine, regulates mammary cell proliferation (Ramirez de Molina et al. 2004). Thus, an increased supply of choline in early lactation might have stimulated the enzyme choline kinase to enhance mitosis in mammary cells in RPC supplemented cows. These results are in agreement with those obtained by Potts et al. (2020) who found that feeding of 60 g/d of RPC in primiparous Holstein cows increased milk yield by 3.1 kg/d when compared to the control. Bollatti et al. (2020b) reported that yields of milk (36.5 *vs.* 34.8 kg/d), 3.5% FCM (43.1 *vs.* 39.6 kg/d) and ECM (42.0 *vs.* 38.9 kg/d) were improved due to supplementation of 12.9 g/d of choline ion (60 g/d of RPC) in Holstein cows. Arshad et al. (2020) in their meta-analysis reported a 1.6 kg/d response in milk production in multiparous cows fed 12.9 g/d of choline ion (60 g/d of RPC). Recently, Holdorf and White (2021) reported that milk yield tended (P<0.10) to increase when 60 g/d of RPC was supplemented in multiparous Holstein cows.

Feeding of RPC did not affect (P>0.05) average body weight and DM intake (Table 3). Feed efficiency in terms of 4% FCM/DMI (P=0.044) and ECM/DMI (P=0.049) was significantly improved in RPC supplemented group than the CON. Similar to the present finding, recent studies also reported that there were no difference

Table 2 Effect of rumen-protected choline chloride supplementation on production performance ofKankrej cows (n=20)

Parameters	CON	RPC	P value
Yield (kg/d)			
Milk	11.42^{a}±0.51	12.77^{b}±0.41	0.046
4% FCM	11.21^{a}±0.53	12.90^{b}±0.42	0.022
ECM	12.39^{a}±0.58	14.20^{b}±0.46	0.024
Fat	0.44^{a}±0.02	0.52^{b}±0.02	0.013
Solids not fat	0.88±0.04	0.98±0.03	0.056
Total solids	1.32^{a}±0.06	1.50^{b}±0.05	0.028
Protein	0.39^{a}±0.02	0.44^{b}±0.01	0.042
Lactose	0.51^{a}±0.02	0.58^{b}±0.02	0.046
Milk composition (%)			
Fat	3.93±0.10	3.97±0.05	0.702
Solids not fat	7.62±0.12	7.65±0.09	0.846
Total solids	11.55±0.16	11.63±0.11	0.698
Protein	3.37±0.02	3.40±0.02	0.354
Lactose	4.46±0.09	4.50±0.01	0.635

FCM: fat corrected milk; ECM: energy corrected milk.
CON: Basal diet without additive; RPC: Basal diet + 50 g/animal/day of rumen-protected choline
^{ab}Means in a row with different superscripts differ significantly (P≤0.05)

Table 3 Effect of rumen-protected choline supplementation on body weight, dry matter intake and feed efficiency in Kankrej cows (n=20)

Parameters	CON	RPC	P value
BW (kg)	456.10±8.80	458.00±10.15	0.889
DMI (kg/d)	11.18±0.22	11.22±0.25	0.888
Feed efficiency			
Milk (kg)/DMI (kg)	1.03±0.06	1.14±0.03	0.114
4% FCM (kg)/DMI (kg)	1.01^{a}±0.06	1.15^{b}±0.03	0.044
ECM (kg)/DMI (kg)	1.12^{a}±0.06	1.27^{b}±0.03	0.049

DMI: dry matter intake; FCM: fat corrected milk; ECM: energy corrected milk
CON: Basal diet without additive; RPC: Basal diet + 50 g/animal/day of rumen-protected choline
^{ab}Means in a row with different superscripts differ significantly (P≤0.05)

Table 4 Effect of rumen-protected cholinesupplementation on haemato-biochemical profile of Kankrej cows (n=20)

Parameters	CON	RPC	P value
Haematological parameters			
Haemoglobin (g/dL)	10.41±0.28	10.48±0.18	0.835
Hematocrit (%)	33.53±1.26	33.93±1.54	0.843
Erythrocytes (10^6/µL)	6.82±0.16	6.89±0.20	0.780
Leukocytes(10^3/µL)	8.00±0.46	8.12±0.31	0.830
Blood biochemical parameters			
Glucose (mg/dL)	62.05^a±1.40	65.72^b±0.58	0.027
Total protein (g/dL)	6.48±0.08	6.40±0.10	0.547
Albumin (g/dL)	2.96±0.07	2.89±0.06	0.461
Globulin (g/dL)	3.52±0.14	3.51±0.13	0.940
Urea (mg/dL)	42.08±1.71	38.68±1.82	0.190
Triglycerides (mg/dL)*	31.38^b±0.85	27.27^a±0.95	0.005
Cholesterol (mg /dL)	149.03±3.29	154.14±4.17	0.349

CON: Basal diet without additive; RPC: Basal diet + 50 g/animal/day of rumen-protected choline
^{ab}Means in a row with different superscripts differ significantly (*P<0.05)

(P>0.05) in DM intake and body weight in Holstein cows fed 60 g/d of RPC (Bollatti et al. 2020b; Potts et al. 2020; Salman and Alan, 2020).Improved conversion efficiency of DMI into FCM and ECM in the present experiment might be related to improved nutrient absorption due to better gastrointestinal function with RPC supplementation. Moreover, less disrupted gastrointestinal barrier due to choline supplementation might have reduced the nutritional costs necessary to support an activated immune system (Kvidera et al. 2017), which would have spared more nutrients for milk synthesis.

The effect of supplementation of rumen-protected choline on haemato-biochemical parameters of Kankrej cows is given in Table 4. There was no significant (P>0.05) difference in the concentrations of haemoglobin, haematocrit, erythrocytes and leucocytes between the CON and RPC groups. The supplementation of rumen-protected choline in Kankrej cows significantly (P<0.05) increased glucose concentration (65.72 *vs*. 62.05 mg/dL) as compared to the CON group. Higher blood glucose levels in RPC group may be resulted because of feeding RPC increases mRNA for GLUT2, a hepatic protein that facilitates glucose release from the liver into blood (Zhao and Keating, 2007). Similar to the present findings, Arshad et al. (2020) in their meta-analysis reported that feeding of 12.9 g/d of choline ion (60 g/d of RPC) increased (P<0.05) blood glucose concentrations in dairy cows. In contrast to the present findings, previous studies observed that plasma glucose concentration was unaffected by RPC supplementation in cows (Zhou et al. 2016; Zenobi et al. 2018; Bollatti et al. 2020c; Potts et al. 2020). The concentration of triglycerides was significantly (P<0.05) reduced in RPC (27.27 mg/dL) group when compared to the CON (31.38 mg/dL) group. This may be attributed to role of choline which acts in intermediary metabolism, in particular as a component of phospholipids and lipoproteins, which are critical for lipid absorption, transportation and has been shown to attenuate triacylglycerol infiltration into the liver (Zenobi et al. 2018). In agreement with our results, Mohsen et al. (2011) reported that RPC supplementation led to a significant decrease (P<0.05) in the concentrations of plasma triglycerides in dairy cows. Also, Holdorf and White (2021) observed that 60 g/d of RPC supplementation tended (P<0.10) to reduce blood triglycerides in Holstein cows. The concentrations of serum total proteins, albumin, globulin, urea and cholesterol did not differ (P>0.05) between the CON and RPC groups. Previous studies also observed no change in other blood biochemical parameters in dairy cows supplemented with RPC (Zenobi et al. 2018; Bollatti et al. 2020c; Potts et al. 2020).

Conclusions

Based on the results of the present study, it may be concluded that dietary inclusion of 50 g/day of rumen-protected choline in Kankrej cows from four weeks pre-partum up to eight weeks post-partum for improved milk yield with higher concentration of serum glucose and lower concentration of triglycerides.

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