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Effect of Rumen Protected Methionine and Choline on Blood Biochemical Metabolites, Milk Yield and its Composition during Transition Period in Surti Buffaloes

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ABSTRACT

Background: Methionine and choline supplementation can aid in nutritionally managing transition Surti buffaloes. Present study has evaluated blood biochemical metabolites, milk yield and its changes in composition on supplementation of rumen protected methionine (RPM) and choline (RPC) in transition Surti buffaloes.

Methods: Twenty-seven pregnant multiparous Surti buffaloes in three groups (n=9) from -15 d to 30 d postpartum received supplementation as: T_1 (Control: basal diet), T_2 (basal diet + RPM@10 gm/animal/day) and T_3 (basal diet + RPM@10 gm/animal/day + RPC@ 50 gm/animal/day). Sample was collected at beginning, 1st, 3rd and 6th week for blood and at 1st, 3rd, 6th, 9th and 12th week postpartum for milk. Milk yield was recorded upto 100 days postpartum.

Result: Postpartum TC, HDL and VLDL differed significantly (P<0.05) being highest in T_3 and lowest in control (T_1) whereas it was reverse for NEFA and BHBA. Supplemented groups had significantly lower TG levels at 1st and 3rd week postpartum. Milk fat upto 9th and SNF, protein, lactose, TAS, Ca, P and Mg upto 6th week were significantly (P<0.05) highest in T_3, followed by T_2 and T_1. It was concluded that RPC along with RPM supplementation is more beneficial than only RPM supplementation in terms of enhancing liver health, reducing negative energy balance and improving milk quality.

Key words: Choline, Methionine, Rumen protected, Transition surti buffaloes.

INTRODUCTION

Transition period is most critical phase of dairy animal spanning 3 week before and after parturition (Batisel *et al.,* 2017). High metabolic demands for rapid fetal development prepartum and postpartum onset of lactation pushes animal in negative energy balance (NEB) (NRC, 2001). Low feed intake and fodder with insufficient nutrient and energy level does not fulfil energy demands of dairy animals (Zhou *et al.,* 2016). NEB triggers fat mobilization to meet energy demands of lactating animals causing higher circulatory non-esterified fatty acids (NEFA) production from adipose tissue and its transportation to liver for further metabolism and energy production. Incomplete oxidation and esterification of NEFA produces β-hydroxy butyric acid (BHBA) and triglyceride (TG), respectively. These TG either accumulates in liver or is exported out of liver in the form of very low density lipoprotein (VLDL). Hepatic accumulation of TG and increased circulatory BHBA causes fatty liver condition during transition further reducing lactation performance, impairs reproduction and causes various metabolic disorders (Sun *et al.,* 2016 and Michelotti *et al.,* 2021).

Methionine and choline supplementation play metabolic roles especially during transition. They are lipotropic, act as methyl group donor, enhance fat metabolism and minimizes risk of fatty liver and ketosis. Their limited dietary availability due to extensive microbial degradation in rumen (Zhou *et al.,* 2016 and Potts *et al.,* 2020) can be overcome by resistant coating. Therefore supplementing RPM and RPC may meet daily demand of methyl group, improve milk quality and health of dairy animals during transition. Considering the benefits of dietary supplementation of rumen protected forms of methionine and choline (RPM and RPC) and the dearth of studies on these amino acids in buffaloes present study was planned to evaluate blood biochemical, milk yield and its composition on supplementation of rumen protected methionine (RPM) and choline (RPC) in transition Surti buffaloes.

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MATERIALS AND METHODS

Present study was done at Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NAU, Navsari during 2018-20 and was approved by Institutional Animal Ethics Committee (066-VCN-VPY-2018). 27 pregnant multiparous Surti buffaloes were selected and divided based on their parity, total milk yield of previous lactation and body condition score into three groups of 9 animals each. Animals were maintained under standard housing and management at Livestock Research Station, NAU, Navsari. Feeding and watering was done as per ICAR feeding standards, 1998. Following dietary treatment was given from -15d prepartum to 30d postpartum to each groups: T_1 (Control: basal diet), T_2 (basal diet+ RPM@10 gm/animal/day) and T_3 (basal diet+RPM @10 gm/ animal/day+RPC@50 gm/animal/day). Proximate analysis of feed was done (AOAC, 2007).

10 ml blood was collected in K_3EDTA and vacutainers for serum at 15 days prepartum and 1st, 3rd and 6th week postpartum. Vacutainers were centrifuged (3000 rpm for 15 minutes) to separate plasma and serum and stored at -20°C until further analysis. Plasma was used to estimate non-esterified fatty acid (NEFA) (Shipe *et al*., 1980) and serum was used to estimate total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), triglyceride (TG) and β-hydroxy butyric acid (BHBA) by Randox kit.

Daily milk yield was recorded upto 100 days postpartum. 100 ml milk was collected at 1st, 3rd, 6th, 9th and 12th week postpartum for analysing milk composition, total antioxidant status (TAS), macro and micro elements. Milk fat, solid not fat (SNF), lactose, protein, total solids (TS), urea, free fatty acids (FFA) and lactic acid (LA) were estimated using fully automated FOSS made milkoscan FT1 milk analyser (FOSS, Slangerupgade 69, Denmark) at Surat Milk Union Limited (SUMUL), Surat.

Milk serum (whey) was separated (Naser *et al.,* 2014) for estimation of milk macro elements such as calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K). Milk Ca, P, Mg were estimated using Randox kits and Milk Na and K were estimated by flame photometer 128 (SYSTRONICS). Milk micro elements were determined by ashing milk samples in muffle furnace, solubilizing with diluted HCL (1:4) and making final volume 100 ml with triple glass distilled water. Milk iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) were estimated by atomic absorption spectrophotometer (Model AAS141, Electronics Corporation of India Ltd.). Milk TAS was estimated by method of Alyaqoubi *et al.* (2014). Extraction of antioxidant compounds in fresh milk was carried out according to method of Li *et al.* (2007).

Data was analysed statistically by ANOVA using DMRT. Means were compared at P<0.05 (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Chemical composition of feed samples (Table 1) were within normal range for Indian feedstuff (ICAR, 2013).

Blood biochemical parameters

Among blood biochemical parameters (Table 2), postpartum TC, HDL and VLDL differed significantly (P<0.05) being highest in T_3 and lowest in control (T_1) whereas it was reverse for NEFA and BHBA. Supplemented groups had significantly lower TG levels at 1st and 3rd week postpartum. LDL levels differed non-significantly between groups.

TC, HDL, LDL, VLDL and TG are lipid profile parameters. Cholesterol an important inflammatory biomarker (Osorio *et al.,* 2014; Batisel *et al.,* 2017) acts as precursor of steroid hormone that aids revival of postpartum ovarian activity. Increase in HDL might occur due to synthesis of paroxonase in liver that protects HDL from oxidative damage (Turk *et al.,* 2004). Lower TG and higher VLDL in treatment groups might be due to RPM and RPC acting as lipotropic agent thus preventing TG accumulation in liver and stimulating hepatic VLDL formation (Sun *et al.,* 2016).

Plasma NEFA and BHBA are indicators of energy status in dairy animals especially during transition. Adipose tissue releases NEFA into circulation that is transported to liver for metabolism and energy production. TG can either be accumulated in hepatocyte or can be exported out of liver in the form of VLDL (Sun *et al.,* 2016). Hepatic accumulation of TG and increased BHBA in plasma causes fatty liver condition during transition period, which further reduces lactation performance, impairs reproduction and causes various metabolic disorders (Tsiplakou *et al.,* 2017). RPM+RPC causes decrease in NEFA that may be due to better metabolism and its use in energy production. Further, it may also prevent increase in triglyceride in liver and protects liver health during transition.

Milk yield and composition

Among milk parameters (Table 3, 4 and 5), RPM and RPC supplementation had non-significant effect on milk yield. However, Milk fat upto 9th and SNF, protein, lactose, TAS, Ca, P and Mg upto 6th week were significantly (P<0.05)

Table 1: Chemical composition (%, DM basis) of feed.

Name		Dry mater	Crude protein	Ether extract	Crude fiber	Total ash	NFE
Concentrate		91.07	20.2	2.4	12	6.93	58.47
Green fodder	Hybrid napier	27.57	8.3	1.3	29	13.15	48.23
	Jowar (Green)	25.43	7.3	1.28	37	8.36	45.51
	Lucerne	25.26	18.1	1.79	27	12.15	40.95
	Green grass (Para grass)	25.52	6.7	1.66	33	12.09	46.51
Dry fodder	Paddy straw	89.76	2.3	1.52	40	17.28	38.94
	Jowar hay	89.6	7.4	1.52	39	8.34	43.69

Table 2: Changes in biochemical parameters (Mean±SE) in different supplemental groups at -15, 1st, 3rd and 6th week of parturition in Surti buffaloes.

	T_1					T_2					T_3				
	Pre partum	Post partum				Pre partum		Post partum			Pre partum		Post partum		
	-15 day (n=9)	1 week (n=9)	3 week (n=9)	6 week (n=9)	Overall (N=27)	-15 day (n=9)	1 week (n=9)	3 week (n=9)	6 week (n=9)	Overall (N=27)	-15 day (n=9)	1 week (n=9)	3 week (n=9)	6 week (n=9)	Overall (N=27)
TC	118.67	92.11^{A}	92.69^{A}	96.07^{A}	93.63^{A}	121.20	100.11^{B}	117.11^{B}	113.19^{B}	110.14^{B}	122.36	108.55^{C}	119.37^{C}	118.08^{C}	115.33^{C}
(mg/dl)	±4.07	±1.67	±3.51	±2.45	±2.04	±4.17	±2.38	±2.11	±0.96	±0.73	±4.12	±3.73	±0.76	±0.44	±1.14
HDL	117.24	88.96^{A}	77.19^{A}	73.69^{A}	79.95^{A}	116.33	90.99^{B}	98.35^{B}	89.37^{B}	93.07^{B}	117.59	101.73^{C}	111.69^{C}	112.99^{C}	108.80^{C}
(mg/dl)	±5.46	±1.27	±1.46	±1.31	±1.29	±8.83	±2.64	±2.07	±1.98	±1.92	±3.47	±1.32	±2.05	±2.63	±1.53
LDL	56.89	57.19	56.24	60.91	58.11	54.73	55.88	55.42	58.02	56.44	54.78	54.95	53.98	55.89	49.23
(mg/dl)	±3.68	±3.53	±3.24	±2.28	±2.99	±3.91	±2.31	±2.22	±2.70	±1.70	±3.29	±2.87	±3.40	±2.96	±1.07
VLDL	3.68	4.89^{A}	4.53^{A}	3.80^{A}	4.40^{A}	3.70	5.28^{B}	5.02^{B}	4.70^{B}	5.00^{B}	3.61	6.01^{C}	5.83^{C}	5.30^{C}	5.71^{C}
(mg/dl)	±0.10	±0.20	±0.36	±0.19	±0.14	±0.31	±0.17	±0.28	±0.16	±0.12	±0.23	±0.19	±0.24	±0.16	±0.06
TG	19.38	30.05^{B}	29.15^{B}	22.72	27.31^{B}	22.48	19.58^{A}	20.40^{A}	21.23	0.40^{A}	21.53	18.83^{A}	19.98^{A}	20.49	19.77^{A}
(mg/dl)	±0.48	±0.99	±1.81	±1.78	±1.07	±1.53	±1.38	±0.87	±0.62	2±0.78	±1.13	±1.22	±0.96	±1.17	±0.35
NEFA	0.36	0.88^{C}	0.82^{C}	0.60^{B}	0.77^{C}	0.37	0.47^{B}	0.48^{B}	0.59^{B}	0.51^{B}	0.37	0.33^{A}	0.35^{A}	0.37^{A}	0.35^{A}
(µmol/l)	±0.06	±0.05	±0.05	±0.06	±0.04	±0.09	±0.03	±0.02	±0.08	±0.03	±0.11	±0.02	±0.05	±0.03	±0.01
BHBA	0.31	0.67^{C}	0.62^{C}	0.58^{C}	0.62^{C}	0.29	0.45^{B}	0.45^{B}	0.42^{B}	0.44^{B}	0.29	0.38^{A}	0.36^{A}	0.36^{A}	0.37^{A}
(mmol/l)	±0.03	±0.02	±0.02	±0.01	±0.01	±0.02	±0.01	±0.01	±0.01	±0.01	±0.02	±0.01	±0.01	±0.01	±0.01

^{A,B,C}Different upper case superscripts within row shows significant difference between the groups at $p<0.05$.

Table 3: Weekly milk yield (Mean±SE) from calving to 100 days of lactation in different supplemental groups of Surti buffaloes.

Group	W 1 (N=9)	W 2 (N=9)	W 3 (N=9)	W 4 (N=9)	W 5 (N=9)	W 6 (N=9)	W7 (N=9)	W 8 (N=9)	W 9 (N=9)	W 10 (N=9)	W 11 (N=9)	W 12 (N=9)	W 13 (N=9)	W 14 (N=9)	W 15 (N=9)	Overall (N=27)
T_1	16.40	20.60	23.23	24.81	25.48	25.73	26.41	26.68	26.68	27.22	27.49	27.72	28.63	28.91	29.17	25.68
	±1.09	±1.51	±1.43	±1.32	±1.53	±1.60	±1.24	±1.87	±2.09	±1.94	±1.64	±1.75	±1.80	±1.81	±2.08	±1.43
T_2	17.32	21.58	22.20	22.52	24.26	25.73	26.32	26.44	27.23	28.14	28.69	28.72	28.86	29.16	29.37	26.33
	±1.36	±1.93	±2.79	±2.56	±2.54	±1.60	±2.74	±2.95	±2.61	±4.07	±4.08	±4.16	±4.46	±3.75	±4.08	±2.31
T_3	15.16	17.67	21.11	22.04	22.87	23.11	23.90	24.90	25.94	25.44	25.22	25.61	26.01	26.04	26.86	26.39
	±2.26	±3.17	±3.32	±3.49	±3.29	±3.40	±3.52	±3.87	±3.91	±3.76	±3.66	±3.96	±3.88	±3.93	±3.77	±2.09

Table 4: Changes in milk composition (Mean±SE) in different supplemental groups at 1^{st}, 3^{nd}, 6^{th}, 9^{th} and 12^{th} week of parturition in Surti buffaloes.

Group	Week	Fat (%)	SNF (%)	Protein (%)	Lactose (%)	Lactic acid (mEq/L)	FFA (mmol/l)	Urea (mg/l)	Total solids (%)	Density (g/cm^3)
T_1	1^{st} (n=9)	$7.93^{A}\pm0.18$	$9.93^{A}\pm0.15$	$6.29^{A}\pm0.05$	$3.29^{A}\pm0.05$	0.15±0.04	0.71±0.12	258.08±33.52	17.00±0.27	1025.02±1.74
	3^{rd} (n=9)	$7.45^{A}\pm0.17$	$9.45^{A}\pm0.17$	$6.09^{A}\pm0.06$	$3.69^{A}\pm0.04$	0.13±0.05	0.81±0.22	264.68±23.19	16.06±0.21	1022.33±4.98
	6^{th} (n=9)	$6.95^{A}\pm0.16$	$8.95^{A}\pm0.16$	$5.59^{A}\pm0.04$	$4.29^{A}\pm0.06$	0.14±0.04	0.95±0.20	248.65±27.20	16.36±0.21	1018.04±3.07
	9^{th} (n=9)	$6.61^{A}\pm0.15$	$8.57^{A}\pm0.15$	5.39±0.05	4.89±0.04	0.14±0.03	0.97±0.12	230.59±28.25	15.80±0.44	1019.32±2.05
	12^{th} (n=9)	6.19±0.16	8.19±0.16	5.19±0.06	5.10±0.05	0.14±0.04	0.98±0.12	229.94±31.61	16.00±0.27	1016.79±2.12
	Overall (N=27)	$7.03^{A}\pm0.05$	$9.02^{A}\pm0.04$	$5.71^{A}\pm0.05$	$4.25^{A}\pm0.05$	0.14±0.00	0.88±0.08	246.39±9.04	16.24±0.19	1020.30±1.41
T_2	1^{st} (n=9)	$8.77^{B}\pm0.17$	$10.77^{B}\pm0.13$	$6.61^{B}\pm0.03$	$3.61^{B}\pm0.04$	0.14±0.05	0.73±0.12	257.35±39.08	16.90±0.28	1022.92±2.98
	3^{rd} (n=9)	$8.26^{B}\pm0.15$	$10.26^{B}\pm0.14$	$6.41^{B}\pm0.04$	$4.01^{B}\pm0.05$	0.13±0.04	0.88±0.18	260.36±29.95	17.11±0.26	1020.98±2.07
	6^{th} (n=9)	$7.76^{B}\pm0.12$	$9.76^{B}\pm0.15$	$5.91^{B}\pm0.06$	$4.61^{B}\pm0.04$	0.14±0.03	0.98±0.12	248.26±24.29	16.44±0.24	1018.69±1.77
	9^{th} (n=9)	$7.04^{B}\pm0.16$	$8.84^{A}\pm0.12$	5.42±0.04	4.92±0.05	0.14±0.05	0.97±0.14	227.98±24.46	16.05±0.31	1016.39±1.86
	12^{th} (n=9)	6.22±0.19	8.24±0.16	5.22±0.03	5.12±0.05	0.14±0.04	0.98±0.15	226.05±22.82	15.91±0.32	1017.51±2.02
	Overall (N=27)	$7.67^{B}\pm0.10$	$9.64^{B}\pm0.11$	$5.96^{B}\pm0.05$	$4.41^{B}\pm0.05$	0.14±0.00	0.90±0.06	244.01±16.52	16.56±0.22	1019.68±1.16
T_3	1^{st} (n=9)	$9.20^{C}\pm0.18$	$11.20^{C}\pm0.13$	$6.83^{C}\pm0.03$	$3.83^{C}\pm0.05$	0.15±0.05	0.79±12	258.64±16.94	17.08±0.26	1024.39±2.43
	3^{rd} (n=9)	$8.71^{C}\pm0.17$	$10.71^{C}\pm0.14$	$6.63^{C}\pm0.04$	$4.23^{C}\pm0.04$	0.13±0.04	0.95±0.13	258.27±20.62	16.64±0.32	1021.34±3.26
	6^{th} (n=9)	$8.21^{C}\pm0.18$	$10.21^{C}\pm0.14$	$6.13^{C}\pm0.05$	$4.83^{C}\pm0.05$	0.14±0.03	1.02±0.14	244.38±17.74	15.64±0.49	1017.54±1.52
	9^{th} (n=9)	$7.26^{B}\pm0.15$	$9.22^{B}\pm0.15$	5.43±0.04	4.93±0.06	0.14±0.05	0.99±0.12	230.70±28.16	16.21±0.26	1018.08±2.33
	12^{th} (n=9)	6.24±0.16	8.22±0.13	5.23±0.03	5.13±0.05	0.14±0.04	0.99±0.16	233.75±29.14	16.08±0.26	1019.00±2.03
	Overall (N=27)	$7.92^{B}\pm0.13$	$9.91^{B}\pm0.13$	$6.05^{B}\pm0.03$	$4.59^{C}\pm0.03$	0.14±0.00	0.95±0.04	245.15±11.80	16.33±0.25	1020.07±1.58

^{A,B,C}Different upper case superscripts shows significant difference between the groups at $P<0.05$.

Table 5: Changes in milk TAS, macro and micro minerals (Mean±SE) in different supplemental groups at 1st, 3rd, 6th, 9th and 12th week of parturition in Surti buffaloes.

Group	Week	TAS (µM)	Ca (mg/dl)	P (mg/dl)	Mg (mg/dl)	Na (mEq/l)	K (mEq/l)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
T_1	1st (n=9)	150.41^A±9.61	11.69^A±0.18	5.49^A±0.18	2.29^A±0.18	140.50±3.50	3.85±0.21	2.20±0.16	0.46±0.02	0.84±0.01	1.39±0.02
	3rd (n=9)	162.44^A±9.62	11.08^A±0.19	4.88^A±0.17	1.68^A±0.19	139.82±3.28	3.94±0.21	2.01±0.17	0.39±0.03	0.79±0.01	1.38±0.02
	6th (n=9)	188.47^A±9.62	10.67^A±0.20	4.47^A±0.19	1.27^A±0.18	138.43±3.64	3.84±0.19	1.94±0.18	0.36±0.01	0.71±0.02	1.31±0.01
	9th (n=9)	324.54±9.63	10.65±0.19	4.45±0.18	1.25±0.19	142.92±3.27	4.09±0.18	1.66±0.19	0.33±0.01	0.71±0.01	1.28±0.02
	12th (n=9)	340.50±9.65	10.63±0.17	4.43±0.17	1.23±0.17	141.14±3.01	4.20±0.17	1.62±0.10	0.26±0.02	0.69±0.02	1.19±0.01
	Overall (N=27)	233.27^A±9.62	10.95^A±0.15	4.75^A±0.15	1.55^A±0.15	140.56±2.10	3.98±0.09	1.89±0.05	0.36±0.01	0.75±0.01	1.31±0.01
T_2	1st (n=9)	254.87^B±7.33	12.83^B±0.17	6.63^B±0.17	3.43^B±0.17	138.21±3.27	3.82±0.20	2.17±0.11	0.49±0.01	0.85±0.01	1.40±0.02
	3rd (n=9)	266.92^B±7.34	12.23^B±0.17	6.03^B±0.18	2.83^B±0.18	137.56±3.65	3.91±0.18	2.08±0.19	0.40±0.02	0.82±0.01	1.38±0.03
	6th (n=9)	292.91^B±7.24	11.83^B±0.18	5.63^B±0.19	2.43^B±0.18	139.42±3.64	3.84±0.14	1.93±0.19	0.35±0.02	0.76±0.02	1.31±0.02
	9th (n=9)	342.96±8.31	10.66±0.18	4.46±0.21	1.26±0.21	140.69±3.27	4.10±0.14	1.63±0.18	0.33±0.03	0.73±0.03	1.27±0.02
	12th (n=9)	348.03±8.29	10.63±0.21	4.43±0.14	1.23±0.14	141.99±3.21	4.09±0.20	1.72±0.10	0.28±0.03	0.68±0.01	1.20±0.02
	Overall (N=27)	299.65^B±5.25	11.71^B±0.15	5.51^B±0.15	2.31^B±0.15	139.33±1.25	3.95±0.11	1.92±0.03	0.38±0.02	0.77±0.02	1.32±0.01
T_3	1st (n=9)	312.77^C±6.83	13.76^C±0.21	7.56^C±0.16	4.36^C±0.19	140.18±2.29	3.78±0.16	2.17±0.11	0.50±0.01	0.83±0.01	1.41±0.02
	3rd (n=9)	324.77^C±6.90	13.16^C±0.16	6.96^C±0.18	3.76^C±0.18	137.36±2.35	3.86±0.20	2.03±0.12	0.41±0.02	0.80±0.02	1.39±0.03
	6th (n=9)	350.82^C±6.78	12.76^C±0.18	6.56^C±0.17	3.36^C±0.17	137.62±2.81	3.80±0.18	1.97±0.15	0.37±0.02	0.73±0.02	1.32±0.01
	9th (n=9)	340.80±6.89	10.70±0.19	4.50±0.18	1.40±0.15	140.28±1.93	4.11±0.19	1.66±0.13	0.34±0.01	0.72±0.02	1.28±0.03
	12th (n=9)	342.78±6.90	10.66±0.15	4.46±0.15	1.26±0.15	141.35±3.36	4.29±0.19	1.67±0.20	0.28±0.01	0.70±0.02	1.21±0.02
	Overall (N=27)	334.39^C±16.88	12.21^B±0.25	6.01^B±0.25	2.83^B±0.23	139.36±1.39	3.97±0.10	1.90±0.08	0.38±0.01	0.75±0.01	1.31±0.01

[A,B,C]Different upper case superscripts shows significant difference between the groups at P<0.05.

highest in T_3, followed by T_2 and T_1. Remaining parameters did not differ significantly between groups.

MF percentage is reported to increase as acetate to propionate ratio (A:P) ratio increases up to 2.2 (Davis, 1967). Methionine and choline supplementation increases the A:P ratio and enhances MF (Ray *et al.,* 1983). Observed higher MF percentage in T_3 and T_2 groups might be due to increased ruminal A:P ratio and/or it might have helped mammary fat synthesis. Similar findings for MF were observed by Amrutkar *et al.* (2015), Potts *et al.* (2020) and Mavrommatis *et al.* (2021).

Methionine is limiting amino acid (AA) for milk protein (MP) synthesis (NRC, 2001). Choline supplementation spares methionine for MP synthesis. RPM and RPC supplementation enhances MP content in transition dairy cows and improves efficiency of MP synthesis (Sun *et al.,* 2016). Higher MP percentage in treatment groups might be due to supplemental effect of RPM and RPC in transition Surti buffaloes. Similar results were observed by Osorio *et al.* (2014), Amrutkar *et al.* (2015), Sun *et al.* (2016), Zhou *et al.* (2016) and Mavrommatis *et al.* (2021). RPM has been shown to have direct stimulatory effect on lactose biosynthesis in mammary gland (Amrutkar *et al.,* 2015). Higher milk lactose percentage in present study may be attributed to higher blood glucose level in T_3 and T_2 group that helps in mammary lactose biosynthesis. Milk SNF primarily consists of lactose, caseins, whey protein and minerals. Increased milk SNF at week 2 was consistent with the increased milk yield of protein and lactose (Socha *et al.,* 2005). Observed higher ($P<0.05$) milk SNF percentage in treatment groups might be due to higher milk protein and lactose percentage. Milk Ca, P and Mg level in T_3 and T_2 groups might be due to their optimal availability in blood. Methionine and choline plays an important role in maintaining antioxidant level during transition period in dairy animals (Tsiplakou *et al.,* 2017). Hence in present study higher milk TAS levels in treatment groups indicate that methionine and choline elevates TAS of milk that may help in improving keeping quality of milk.

CONCLUSION

The results indicated that RPM and RPC supplementation increase blood cholesterol, HDL and VLDL and decreases TG, NEFA and BHBA level during transition period in Surti buffaloes. RPM and RPC supplementation also enhances the milk quality by increasing milk FAT, SNF, lactose, protein, Ca, P, Mg and milk TAS level in Surti buffaloes. RPC along with RPM supplementation is more beneficial than only RPM supplementation.

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