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Physiological, Hematological, Biochemical and Thermographic Changes on Supplementation of Rumen Protected Methionine and Choline in Transition Surti Buffaloes

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ABSTRACT

Background: Methionine and choline may play role in maintaining health by metabolic optimization in transition Surti buffaloes. Present study has evaluated physiological, hematological, biochemical and thermographic changes on supplementation of rumen protected methionine and choline in transition Surti buffaloes.

Methods: 27 Surti buffaloes were divided in 3 groups (n=9) as G-I, G-II and G-III. Apart from basal diet offered to G-I (control), supplementation of RPM in G-II and RPM+RPC in G-III was done from -15 d to 30 d postpartum. Dose per/animal/day of RPM and RPC was 10 g and 50 g respectively. Blood collection, measurement of meteorology, BCS, physiological parameters and infrared thermography were done -15 d, 1st, 3rd and 6th week of calving.

Result: RPM and RPC supplementation increased BCS, blood glucose, TP, ALB, T_3, T_4, Ca, P and Mg levels and decreased BUN, Cr, AST, ALT, GGT, GLDH and cortisol levels during transition period in Surti buffaloes. RPM and RPC supplementation also decreased RT, TT and ET at 1st week postpartum period in Surti buffaloes. Supplementation of RPM +RPC as compared to RPM alone, gave better results in terms of biochemical analytes in transition Surti buffaloes.

Key words: Rumen protected methionine and choline, Surti buffaloes, Transition period.

INTRODUCTION

Transition period is associated with highest incidence of metabolic and infectious diseases in dairy animals (Batisel *et al.,* 2017b). Dairy animals undergo a sequence of stressful events during this period (Sun *et al.,* 2016). Increased nutritional requirement in last trimester owing to fetal growth and during early lactation is further aggravated by low feed intake thus inducing stress (Zhou *et al.,* 2016) and creating negative energy balance (NEB). NEB decreases BCS by mobilizing fat. During transition reproductive organs and liver are highly susceptible to injury predisposing to infection and inflammatory responses. Inflammatory process switches the acute phase response to synthesis of positive acute phase protein to alter the liver function to re-establish homeostasis (Batisel *et al.,* 2017b). Increased body temperature and anorexia may ensue as inflammatory response.

Methionine and choline have metabolic roles for maintaining health during transition period. They enhance hepatic fat metabolism and minimize fatty liver and ketosis. They act as methyl donors and helps in synthesis of each other. Methionine minimizes inflammatory processes by triggering acute phase protein synthesis. Bioavailability of these from diet is limited due to microbial degradation in rumen (Zhou *et al.,* 2016) thus they are coated with resistant material. Methionine levels in common feedstuffs are usually lower than that found in milk of dairy cows. Therefore, routine diet fails to supply metabolizable methionine in optimum amount to dairy animals. Present study was therefore planned to supplement rumen protected methionine and choline (RPM and RPC) in diet during transition period in Surti buffaloes.

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MATERIALS AND METHODS

Present study was approved by IAEC (066-VCN-VPY-2018) and conducted at Department of Veterinary Physiology and Biochemistry, Veterinary College, NAU, Navsari (Gujarat). Based on BCS, previous lactation yield and parity; twenty-seven multiparous Surti buffaloes in advanced gestation were divided in 3 groups as G-I, G-II and G-III with 9 buffaloes in each. G-I (control) was offered basal diet. Basal diet of G-II was supplemented with rumen protected methionine (RPM)@10 gm/buffalo/day and of G-III with both rumen

protected methionine@10 gm/buffalo/day and rumen protected choline (RPC) @50 gm/buffalo/day. Dietary supplementation was done for 45 days (-15 days to +30 days of parturition). Feed samples were collected for proximate analysis.

Collection of blood samples (with and without anticoagulant K_3EDTA), recordings of meteorological variables (temperature-humidity index by NRC, 1971), body condition scoring (BCS) (Edmonson *et al.,*1989), physiological parameters (rectal temperature by clinical thermometer-Rossmax Swiss GnbH), tympanic temperature (tympanic thermometer-BPLAccuDiGiT), respiration rate-observing left flank movements) and infrared thermography were done at -15 day, 1st, 3rd and 6th week of calving. Hematological analysis was done using fully automated hematology cell counter machine (MEDONIC-CA-620/530VET). Plasma and serum were separated and stored for further analysis. Randox kits for blood biochemical metabolites, bovine specific ELISA assay kits (Labor-Diagnostica-Nord-GmbH and Co, KG, Nordhorn) for hormones, flame photometer 128 (SYSTRONICS) for electrolytes and atomic absorption spectrophotometer (Model:AAS141, ECIL) for trace elements were used their analysis. Infrared thermography was done using camera FLIR-T420 (FLIR Systems Commercial co., USA).

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

RESULTS AND DISCUSSION

Meteorological conditions

Minimum and maximum ambient temperature (°C) was 22.8 and 34.8, relative humidity was (%) 36.00 and 85.00 and THI was 65.06 and 87.77 respectively.

Chemical composition of feed

Composition (Table 1) was in normal range (ICAR, 2013).

Physiological, BCS and hematological parameters

Results for physiological parameters and BCS are presented in Table 2 and for hematological parameters in Table 3.

RT and TT were significantly (P<0.05) lower at 1st week postpartum in GP-III and GP-II than GP-I whereas no effect was observed for RR. BCS was significantly (P<0.05) higher in both treatment groups at 1st and 3rd week of calving than control. Highest BCS was observed at 3rd week postpartum in GP-III.

Supplementation of RPM and RPC reduces calving stress and minimizes inflammatory processes during transition period (Batisel *et al.,* 2017b). This may explain body temperature lowering at 1st week postpartum in GP-III and GP-II. Calving and early lactation stress aggravates negative energy balance with excessive fat mobilization and loss of BCS as in GP-I of present study. Higher BCS in treatment groups may be due to higher DMI due to RPM and RPC supplementation.

Serum biochemical parameters

Results for biochemical metabolites, enzymes, hormones, macro and micro-elements are presented in Table 4, 5, 6 and 7 respectively.

Metabolites

Results show highest (P<0.05) postpartum glucose concentration in GP-III followed by II and I. Similarly, postpartum TP level was significantly (P<0.05) higher in GP-III and GP-II than control. GP-III had highest (P<0.05) TP level during 1st week postpartum than GP-II. ALB concentration was significantly (P<0.05) high in both supplemented group during 1st and 3rd week postpartum. Higher (P<0.05) ALB level at 6th week postpartum was observed in GP-III than GP-I. BUN level was significantly (P<0.05) lower in GP-III and GP-II than control. It was lowest (P<0.05) in GP-III at 1st and 3rd week postpartum. Similarly, lower (P<0.05) Cr level was observed in GP-III and II than control and lowest concentration (P<0.05) in GP-III animals. RPM and RPC supplementation provides energy *via* hepatic β-oxidation of NEFA sparing glucose in circulation and also stimulates hepatic gluconeogenesis during transition period in dairy cows (Zhou *et al.,* 2016). Higher TP level in both treatment groups can be attributed to protein anabolic effects of RPM and RPC during transition period. Being inflammatory and hepatic biomarker, lower levels of ALB in control suggests hepatic damage during transition (Osorio *et al.,* 2014) thereby implying anti-inflammatory responses of RPM and RPC. This was corroborated by Batisel *et al.* (2017b)

Table 1: Proximate analysis of feed sample (%, DM basis).

Feed	Proximate analysis of feed (%, DM basis)					
	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 physiological parameters and body condition score (Mean±SE) in different supplemental groups at -15, 1st, 3nd and 6th week of parturition in Surti buffaloes.

Para meters	GP-I					GP-II					GP-III				
	Pre-partum	Postpartum				Pre-partum	Postpartum				Pre-partum	Postpartum			
	-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)
RT (°F)	99.68± 0.11	101.14^{B}± 0.19	100.64± 0.18	99.63± 0.20	100.46± 0.14	99.67± 0.10	100.71^{A}± 0.20	100.62± 0.19	99.64± 0.17	100.31± 0.10	99.68± 0.11	100.66^{A}± 0.20	100.61± 0.22	99.62± 0.18	100.28± 0.13
RR (breath /min)	30.44± 0.65	30.78± 0.36	31.22± 0.46	31.11± 0.31	31.04± 0.20	30.00± 0.69	31.00± 0.50	30.00± 0.44	30.67± 0.44	30.56± 0.40	30.00± 0.73	30.67± 0.37	31.22± 0.32	30.89± 0.31	30.93± 0.23
TT (°C)	35.60± 0.11	36.78^{B}± 0.23	36.33± 0.23	35.32± 0.24	36.13± 0.16	35.62± 0.13	36.47^{A}± 0.21	36.31± 0.21	35.33± 0.24	36.02± 0.14	35.67± 0.13	36.43^{A}± 0.16	36.30± 0.17	35.31± 0.21	35.99± 0.13
BCS	4.03± 0.08	3.53^{A}± 0.08	3.69^{A}± 0.04	4.03± 0.08	3.75^{A}± 0.06	4.03± 0.07	3.81^{B}± 0.06	3.86^{B}± 0.04	4.00± 0.06	3.89^{A}± 0.05	4.08± 0.06	3.92^{B}± 0.06	4.08^{C}± 0.06	4.08± 0.06	4.03^{B}± 0.06

Different uppercase superscripts within row shows significant difference between the groups at P<0.05.

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

Parameters	GP-I					GP-II					GP-III				
	Pre-partum	Postpartum				Pre-partum	Postpartum				Pre-partum	Postpartum			
	-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)
TEC (10^6×µl)	7.19± 0.07	5.69± 0.07	6.38± 0.08	6.90± 0.07	6.32± 0.07	7.23± 0.08	5.71± 0.10	6.41± 0.09	6.92± 0.09	6.35± 0.09	7.19± 0.08	5.68± 0.08	6.41± 0.08	6.90± 0.08	6.33± 0.08
Hb (g/dl)	11.44± 0.50	9.57± 0.40	10.49 ±0.39	11.54± 0.41	10.52± 0.39	11.31± 0.41	9.32± 0.36	10.24± 0.36	10.94± 0.35	10.16± 0.35	11.36± 0.49	9.48± 0.40	10.37± 0.40	11.18± 0.40	10.33± 0.40
PCV (%)	33.67± 0.50	31.88± 0.40	32.59± 0.39	33.76± 0.40	32.73± 0.39	33.52± 0.41	31.63± 0.36	32.26± 0.42	33.06± 0.35	32.31± 0.37	33.57± 0.49	31.79± 0.40	32.47± 0.40	33.29± 0.40	32.51± 0.40
Platelets (10^3×µl)	263.33± 22.42	280.11± 22.59	258.00± 18.41	290.56± 19.12	276.22± 15.75	263.11± 23.61	280.56± 22.87	252.78± 18.31	297.00± 19.28	276.78± 13.69	265.44± 23.27	286.11± 22.36	253.11± 18.66	286.89± 19.08	275.37± 15.51
MCV (fl)	49.39± 0.96	49.87± 1.08	50.72± 1.20	51.11± 1.09	50.57± 0.73	49.25± 0.81	49.80± 1.02	50.64± 1.12	51.50± 1.06	50.65± 0.57	49.17± 0.75	49.74± 1.07	50.74± 1.01	51.49± 1.03	50.66± 0.81
MCH (pg)	16.44± 0.57	16.84± 0.38	17.27± 0.43	17.19± 0.44	17.10± 0.19	16.27± 0.54	16.80± 0.34	17.21± 0.51	17.16± 0.47	17.06± 0.23	16.38± 0.55	16.97± 0.30	17.23± 0.49	17.34± 0.42	17.18± 0.16
MCHC (%)	33.15± 0.88	32.82± 0.78	32.97± 0.81	33.19± 0.81	32.99± 0.48	33.16± 0.80	32.86± 0.77	32.96± 0.81	33.17± 0.84	32.99± 0.61	33.17± 0.72	32.99± 0.75	32.80± 0.87	33.17± 0.77	32.99± 0.72

Different uppercase superscripts within row shows significant difference between the groups at P<0.05.

and Zhou *et al.* (2016). BUN concentration indicates excretion and utilization efficiency of nitrogen in dairy animals (Sun *et al.,* 2016). Choline spares methionine for protein synthesis by donating -CH_3. This might explain decreased BUN due to RPM and RPC supplementation. Creatinine indicates body muscle mass and its catabolism during transition. High BCS with lower muscle mass mobilization during transition is associated with lower Cr (Osorio *et al.,* 2014). In present study lowered Cr and higher BCS in GP-III and II than GP-I indicated lowered muscle mobilization due to RPM and RPC.

Liver enzymes

Lowest (P<0.05) postpartum AST level was in GP-III. GP-II had lower (P<0.05) AST level at 1st and 3rd week postpartum than GP-I. Similarly, lowest (P<0.05) ALT concentration was at 1st and 3rd week post-partum in GP-III followed by GP-II and highest (P<0.05) was in GP-I. GGT and GLDH levels were significantly (P<0.05) lowered in GP-III and II during 1st and 3rd week postpartum than GP-I.

Around calving AST, ALT, GGT and GLDH are key liver health biomarkers (Osorio *et al.,* 2014) and their high concentration indicates hepatic damage (Batisel *et al.,* 2017a). Results of present study imply improvement of liver health by RPM and RPC supplementation.

Hormones

Amongst hormones GP-III had significantly (P<0.05) higher T_3 level at 1st and 6th week postpartum than control. Highest (P<0.05) thyroxine (T_4) level was observed in GP-III followed by II and I during 6th week postpartum period. Cortisol level was significantly (P<0.05) lower in GP-III and I at 1st and 6th week postpartum than control.

Thyroid hormones play metabolic roles and their postpartum reduction reflects decreased secretion rate due to energy deficiency and high demand for these hormones by mammary tissue (Fiore *et al.,* 2018). Similar to findings of Fiore *et al.* (2018) and Steinhoff *et al.* (2019) we observed significantly (P<0.05) lower T_3 level in control during 1st and 6th week postpartum while T_4 level was lower only at 6th week postpartum. This might be due to adaptive change for NEB at calving in 1st week postpartum as well as during increased metabolic demand of approaching peak lactation at 6th week postpartum. Higher (P<0.05) level of T_3 in GP-III and of T_4 in GP-III and II might be attributed to alleviation of NEB by RPM and RPC supplementation. Conversion of T_4 to T_3 may explain non-significant T_4 differences at 1st week postpartum.

In the present study, significantly (P<0.05) higher cortisol concentration in control at 1st and 6th week postpartum indicates stress without RPM and RPC supplementation.

Macro and micro elements

Results show highest (P<0.05) level of Ca, P and Mg at 1st and 3rd week postpartum in GP-III followed by II and I. Higher pre-partum calcium levels decreased at 1st and 3rd week of parturition probably due to its partitioning for milk. This was

Table 4: Changes in biochemical metabolites (Mean±SE) in different supplemental groups at -15, 1st, 3rd and 6th week of parturition in Surti buffaloes.

Parameters	GP-I					GP-II					GP-III				
	Pre-partum	Postpartum				Pre-partum	Postpartum				Pre-partum	Postpartum			
	-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)
Glucose	62.83±	49.69^{A}±	52.02^{A}±	54.89^{A}±	52.20^{A}±	64.88±	55.77^{B}±	58.30^{B}±	60.16^{B}±	58.08^{B}±	65.48±	61.47^{C}±	63.04^{C}±	64.60^{C}±	63.03^{C}±
(mg/dl)	0.97	1.65	1.29	1.21	0.76	2.13	1.26	1.71	1.09	0.83	1.72	1.29	1.50	1.56	1.08
TP (g/dl)	9.92±	6.89^{A}±	7.02^{A}±	7.56^{A}±	7.16^{A}±	9.95±	7.88^{B}±	8.86^{B}±	8.50^{B}±	8.41^{B}±	9.93±	9.25^{C}±	9.59^{B}±	9.04^{B}±	9.29^{C}±
	0.23	0.19	0.27	0.31	0.17	0.35	0.26	0.21	0.26	0.17	0.21	0.34	0.30	0.25	0.17
Albumin	4.19±	2.80^{A}±	2.92^{A}±	3.45^{A}±	3.06^{A}±	4.14±	3.67^{B}±	3.81^{B}±	3.96^{AB}±	3.81^{B}±	4.23±	3.91^{B}±	4.07^{B}±	4.11^{B}±	4.03^{B}±
(g/dl)	0.27	0.20	0.26	0.25	0.17	0.21	0.24	0.21	0.15	0.11	0.24	0.27	0.13	0.14	0.08
BUN	18.67±	25.54^{C}±	21.05^{C}±	19.05^{B}±	21.88^{C}±	19.35±	18.36^{B}±	17.48^{B}±	17.25^{A}±	17.70^{B}±	17.12±	13.89^{A}±	13.13^{A}±	15.82^{A}±	14.28^{A}±
(mg/dl)	2.23	1.28	0.90	0.67	0.72	1.69	1.31	0.58	0.58	0.65	1.28	1.07	0.63	0.59	0.59
Creatinine	1.36±	2.55^{C}±	2.35^{C}±	2.25^{C}±	2.38^{C}±	1.26±	1.72^{B}±	1.68^{B}±	1.65^{B}±	1.68^{B}±	1.24±	1.41^{A}±	1.17^{A}±	1.18^{A}±	1.25^{A}±
(mg/dl)	0.11	0.05	0.08	0.14	0.06	0.12	0.10	0.04	0.05	0.02	0.11	0.05	0.02	0.01	0.02

Different uppercase superscripts within row shows significant difference between the groups at P<0.05.

Table 5: Changes in liver enzymes (Mean±SE) in different supplemental groups at -15, 1st, 3rd and 6th week of parturition in Surti buffaloes.

Parameters	GP-I					GP-II					GP-III				
	Pre-partum	Postpartum				Pre-partum	Postpartum				Pre-partum	Postpartum			
	-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)
AST (U/l)	100.22± 7.42	128.11^{C} ± 1.25	116.11^{C} ± 1.25	101.44^{B} ± 1.46	115.22^{C} ± 1.31	100.67± 4.14	113.22^{B} ±2.70	101.56^{B} ± 2.63	99.78^{B}± 2.61	104.85^{B} ± 2.64	100.33± 5.77	103.11^{A} ± 2.87	92.89^{A}± 2.84	91.67^{A}± 3.13	95.89^{A}± 2.82
ALT (U/l)	26.00± 2.63	36.93^{C}± 0.97	34.73^{C}± 0.97	26.63± 0.97	32.76^{C} ± 0.97	25.91± 1.69	32.66^{B}± 0.75	30.46^{B}± 0.75	26.26± 0.75	29.80^{B} ± 0.75	28.27± 1.36	28.31^{A}± 1.02	26.11^{A}± 1.02	26.01± 1.02	26.81^{A} ± 1.02
GGT (U/l)	10.91± 0.43	17.16^{B}± 0.95	14.86^{B}± 0.95	10.86± 0.95	14.30^{B}± 0.95	10.94± 0.41	13.63^{A}± 0.48	11.33^{A}± 0.48	10.53± 0.48	11.83^{A}± 0.48	10.92± 0.41	11.98^{A}± 0.44	10.68^{A}± 0.44	10.48± 0.44	11.05^{A}± 0.44
GLDH (U/l)	12.37± 0.78	17.16^{B}± 0.95	15.10^{B}± 0.81	13.63± 0.75	15.3^{B} ± 0.57	12.51± 0.96	15.05^{A}± 0.55	12.84^{A}± 0.62	12.85± 0.74	13.58^{A}± 0.40	12.49± 0.55	14.13^{A}± 0.87	12.36^{A}± 0.60	12.33± 0.66	12.94^{A}± 0.50

Different uppercase superscripts within row shows significant difference between the groups at P<0.05.

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

Parameters	GP-I					GP-II					GP-III				
	Pre-partum	Postpartum				Pre-partum	Postpartum				Pre-partum	Postpartum			
	-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)
T_3 (ng/dl)	1.32± 0.026	1.30^{A}± 0.086	1.60± 0.051	1.61^{A}± 0.035	1.50^{A}± 0.03	1.32± 0.033	1.40^{AB}± 0.074	1.63± 0.026	1.61^{A}± 0.051	1.54^{A}± 0.02	1.33± 0.031	1.60^{B}± 0.041	1.70± 0.065	1.91^{B}± 0.048	1.73^{B}± 0.02
T_4 (µg/dl)	50.37± 2.94	47.60± 5.04	53.39± 2.69	51.34^{A}± 1.29	50.78^{A}± 1.66	47.16± 1.32	49.34± 4.16	35.76± 1.38	58.37^{B}± 2.07	54.49^{A}± 1.55	49.54± 1.32	55.35± 0.62	59.20± 1.70	63.88^{C}± 1.99	59.48^{B}± 0.87
Cortisol (µg/dl)	2.76± 0.09	3.76^{B}± 0.05	3.16± 0.05	2.98^{B}± 0.13	3.30^{B}± 0.05	2.60± 0.95	3.00^{A}± 0.13	2.81± 0.21	2.60^{A}± 0.15	2.81^{A}± 0.11	2.80± 0.08	2.93^{A}± 0.03	2.94± 0.145	2.45^{A}± 0.086	2.78^{A}± 0.07

Different uppercase superscripts within row shows significant difference between the groups at P<0.05.

Table 7: Changes in serum macro and micro minerals (Mean±SE) in different supplemental groups at -15, 1st, 3rd and 6th week of parturition in Surti buffaloes.

Parameters	GP-I					GP-II					GP-III				
	Pre-partum	Postpartum				Pre-partum	Postpartum				Pre-partum	Postpartum			
	-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)
Ca (mg/dl)	12.62± 0.34	9.43^{A}± 0.13	9.65^{A}± 0.10	12.55± 0.10	10.55 ^{A}± 0.08	12.65± 0.38	11.36^{B}± 0.22	11.60^{B}± 0.11	12.57± 0.11	11.84^{B}± 0.14	12.68± 0.28	12.29^{C}± 0.21	12.37^{C}± 0.14	12.57± 0.14	12.41^{C}± 0.15
P (mg/dl)	8.95± 0.70	5.77^{A}± 0.12	5.88^{A}± 0.11	8.38± 0.11	6.68^{A}± 0.11	9.02± 0.41	6.53^{B}± 0.19	7.26^{B}± 0.15	8.46± 0.15	7.42^{B}± 0.15	8.76± 0.49	7.93^{C}± 0.28	8.08^{C}± 0.15	8.40± 0.15	8.14^{C}± 0.15
Mg (mg/dl)	3.09± 0.19	1.84^{A}± 0.03	1.88^{A}± 0.03	3.08± 0.03	2.26^{A}± 0.03	3.08± 0.12	2.37^{B}± 0.04	2.57^{B}± 0.06	3.08± 0.06	2.67^{B}± 0.04	3.08± 0.10	2.97^{C}± 0.06	3.05^{C}± 0.13	3.08± 0.10	3.03^{C}± 0.06
Na (mEq/l)	142.11± 1.27	150.56± 1.55	147.62± 2.22	145.19± 1.63	147.79± 1.29	142.95± 1.57	150.05± 2.85	146.97± 2.26	145.11± 2.10	147.38± 1.24	143.39± 1.90	150.25± 1.57	148.22± 2.41	144.88± 2.63	147.78± 1.96
K (mEq/l)	5.64± 0.25	6.06± 0.27	5.51± 0.21	5.35± 0.24	5.64± 0.16	5.54± 0.33	5.97± 0.25	5.52± 0.19	5.28± 0.27	5.59± 0.13	5.72± 0.20	6.04± 0.26	5.54± 0.30	5.54± 0.22	5.70± 0.16
Cl (mmol/l)	95.39± 1.53	95.45± 1.65	94.74± 1.43	94.19± 2.34	94.80± 1.10	94.83± 2.17	94.79± 2.32	93.38± 2.33	91.68± 1.99	93.28± 1.14	95.35± 2.01	94.31± 1.96	94.95± 1.40	92.02± 1.89	93.76± 1.40
Fe (ppm)	1.79± 0.10	1.82± 0.15	1.94± 0.08	2.10± 0.11	1.95± 0.05	1.79± 0.07	1.80± 0.09	1.91± 0.08	2.10± 0.12	1.93± 0.06	1.78± 0.05	1.80± 0.08	1.93± 0.10	2.04± 0.11	1.92± 0.07
Mn (ppm)	0.42± 0.05	0.40± 0.01	0.43± 0.01	0.46± 0.03	0.43± 0.01	0.41± 0.05	0.40± 0.02	0.45± 0.03	0.48± 0.03	0.44± 0.01	0.42± 0.04	0.40± 0.01	0.44± 0.02	0.46± 0.03	0.43± 0.01
Zn (ppm)	0.47± 0.05	0.41± 0.03	0.43± 0.02	0.47± 0.03	0.44± 0.02	0.44± 0.04	0.41± 0.03	0.43± 0.02	0.47± 0.04	0.44± 0.02	0.47± 0.03	0.42± 0.03	0.43± 0.02	0.48± 0.03	0.44± 0.01
Cu (ppm)	0.85± 0.03	0.87± 0.03	0.88± 0.02	0.86± 0.04	0.87± 0.02	0.88± 0.03	0.89± 0.02	0.87± 0.03	0.89± 0.03	0.88± 0.01	0.85± 0.04	0.87± 0.03	0.85± 0.03	0.87± 0.05	0.86± 0.02
Co (ppm)	1.24± 0.05	1.11± 10.08	1.12± 0.06	1.20± 0.08	1.14± 0.05	1.25± 0.06	1.15± 0.09	1.16± 0.04	1.23± 0.07	1.18± 0.04	1.21± 0.07	1.16± 0.06	1.17± 0.05	1.24± 0.07	1.19± 0.03

Different uppercase superscripts within row shows significant difference between the groups at P<0.05.

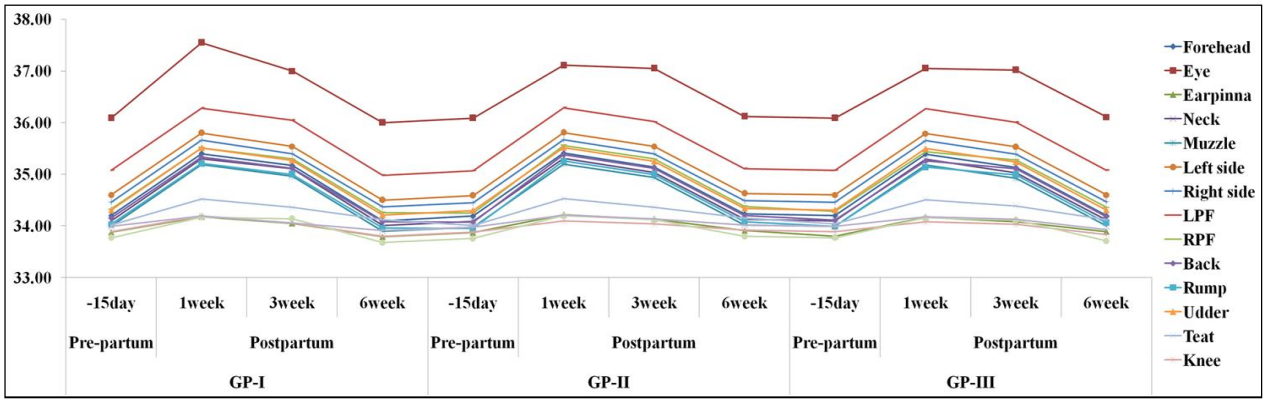

Fig 1: Skin surface temperature (°C) changes as measured by IRT in different supplemental groups at -15, 1st, 3rd and 6th week of parturition in Surti buffaloes.

also true for P and Mg. P under high energy demand during postpartum is used for ATP formation. Observed lower Ca, P and Mg level in GP-I buffaloes may be due to poor feed intake and NEB because of transitional stress. Thus levels of these elements in supplemented groups indicates improved feed intake.

Infrared thermography (IRT)

Among surface temperature of different body regions (Fig 1) eye temperature (°C) was significantly lower during 1st week postpartum in GP-III and II than I. More efficient cattle have lower surface temperature due to lower heat loss and methane production (Montanholi *et al.,* 2008).

Possibility of increase in body temperature due to supplementation of RPM and RPC was explored. Only eye temperature was significantly ($P<0.05$) low in GP-II and III during 1st week postpartum period. Montanholi *et al.* (2008) also found significant difference in eye temperature in beef steers with distinct feed efficiencies.

CONCLUSION

RPM and RPC supplementation increased BCS, blood glucose, TP, ALB, T_3, T_4, Ca, P and Mg levels and decreased BUN, Cr, AST, ALT, GGT, GLDH and cortisol levels during transition period in Surti buffaloes. RPM and RPC supplementation also decreased RT, TT and ET at 1st week postpartum period in Surti buffaloes. Supplementation of RPM +RPC as compared to RPM alone, gave better results in terms of biochemical analytes in transition Surti buffaloes.

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