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Effect of Dietary Rumen Protected Methionine on Postpartum Fertility Parameters in Multiparous Dairy Buffaloes (*Bubalus bubalis*)

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ABSTRACT

Present study was planned to assess dietary supplementation of rumen protected methionine (RPM) peripartum on blood profile and postpartum reproductive outcome in 18 multiparous Murrah buffaloes. Animals were randomly assigned to 3 treatment groups of 6 each, and fed diets supplemented with RPM 20 g day^{-1} (Group 1); 10 g day^{-1} (Group 2), and standard control diet (Group 3). RPM feeding was introduced from day 30 prepartum till day 60 postpartum. Progesterone, estradiol, IGF-I, and biochemical parameters were estimated starting day 21 prepartum to day 57 postpartum. Size of dominant follicle and corpus luteum were assessed across the treatment days postpartum. Progesterone concentration varied from 0.32±0.06 to 3.85±0.44 ngml^{-1}, 0.41±0.05 to 2.03±0.12 ngml^{-1}, and 0.35±0.08 to 1.82±0.11 ngml^{-1} in Groups 1, 2 and 3, respectively. Corresponding estradiol levels ranged from 16.00±1.09 to 243.16±4.67 pgml^{-1}, 13.16±0.60 to 228±5.06 pgml^{-1}, and 13.66±0.66 to 218.9±4.36 pgml^{-1} and IGF-I from 77.83±8.81 to 149.50±4.69 ngml^{-1}, 77.33±2.95 to 128.41±3.85 ngml^{-1}, and 62.66±2.20 to 124.50±5.57 ngml^{-1} in Groups 1, 2 and 3, respectively. Variations in blood glucose, BUN and creatinine were observed across the days in all the treatment groups. Estrogen to progesterone (E/P) ratio varied ($p<0.05$) among the treatment groups. Lower values of E/P in Group 1 were depictive of estrogen dominance, whereas higher E/P in Group 3 signified dominance of progesterone. Conception rates in Groups 1, 2 and 3 were 83.33%, 50.00% and 33.33%, respectively. Varying ($p<0.05$) progesterone and estradiol levels were indicative of positive role of dietary RPM. Dietary RPM improved follicular structures and increased production and reproduction outcomes.

Key words: Buffaloes, Corpus Luteum (CL), Dominant Follicle (DF), Estrogen, Murrah, Rumen Protected Methionine (RPM).

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INTRODUCTION

Regulatory systems involved in the estrous cycle are necessary to improve the reproductive efficiency of buffalo. During estrous cycle, majority of buffalo experience two waves of follicular activity (Roy and Prakash. 2009). Buffaloes have enlarged estrous cycle, length variability, higher incidences of excessively short and extended estrous cycles due to diverse environmental conditions, poor nutrition, and anomalies in ovarian steroid hormone release (Nanda *et al*., 2003). Nutritional inadequacies lower fertility, affect foetal and embryonic development at various stages of pregnancy, and sometimes result in abortion. Amino acid nutrition is one dietary component that may play a significant role in lactating dairy cattle's reproduction (Toledo *et al*., 2017).

Methionine is an important amino acid that limits reproduction in dairy cows during lactation (Brosnon *et al*., 2007). Expression of genes related to gluconeogenesis, fatty acid oxidation, the insulin signalling system, and inflammatory response was altered when pregnant bovines were supplemented with rumen-protected methionine (RPM) in the latter stages of pregnancy (Jacometo *et al*., 2016). Methionine is an essential element for milk production and reproduction (Ayyat *et al*., 2021). Methionine concentration in uterine and embryonic fluids depicts its role in appropriate embryonic growth and survival (Groebner *et al*., 2011). Methionine deficiency causes reduction in glutathione levels, and affects apoptosis, methylation status, and cleavage rate in *in vitro*-produced embryos (Bonilla *et al*., 2010).

Rumen-protected methionine supplementation impacts production and reproduction and is required for validating the optimal RPM feeding recommendations for buffaloes. Feeding rates for RPM are estimated *in vitro* in cattle, but there is limited literature available for buffalo. Feeding recommendations have been based upon the above notifications. The present study was formulated with the objective to evaluate the effect of peripartum RPM feeding on blood profile and reproductive parameters in buffaloes.

MATERIALS AND METHODS

Animals' Selection and Management

Buffaloes (n=18) above 4 years of age in the Dairy Farm, Directorate of Livestock Farms, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) were incorporated in the study. Experiment was conducted from January to June 2022. Buffaloes were maintained under optimum housing, feeding, management and health practices. The feed and water were available *ad lib* to these buffaloes. Procedures were approved by the Institutional Animal Ethics Committee (IAEC) vide letter no. GADVASU/2022/IAEC/65/18.

Feeding of Buffaloes

Nutrient requirements of the animals were mostly met with *ad-lib* green fodder and a measured amount of concentrate. Concentrate feeding was done at the rate of 2.5/kg/day per animal for body maintenance, plus 1 kg/day/animal up to seven months and 2 kg/day/animal in advanced pregnancy. Milking animals were given additional concentrate at the rate of 1 kg for every 1.5 kg milk production, above 5 kg milk yield. Buffaloes selected for study in the treatment part were fed with rumen protected methionine (RPM) from 21 days prepartum to 60 days postpartum. Pregnant buffaloes were fed RPM in Total Mixed Ration. Animals of Group 1 (n=6) were fed 20 g of RPM, Group 2 (n=6) 10 g of RPM, and Group 3 (n=6) were fed Standard control diet.

Sample Collection

Blood analysis and biochemical estimations were carried out in the Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Buffaloes were subjected to Jugular venous blood sampling in heparanized tubes as per the schedule; day 21 and 7 prepartum, followed by day 14, 25, 36, 45, 47, 54 and 57 postpartum. Blood samples were collected in heparanized tubes which were immediately placed in the ice box and transferred to the laboratory. Plasma was harvested by centrifugation (4000 rpm, 7 minutes) and stored at -20 degree Celsius until the hormone and biochemical assays. Frozen plasma samples were later thawed and used for estimation of Progesterone, Estrogen, Insulin like growth factor-1 (IGF-1), Blood urea nitrogen (BUN), Creatinine, and Glucose

Transrectal Ovarian Ultrasonography and Pregnancy Diagnosis

Transrectal ultrasonography examination was performed to evaluate the follicular dynamics postpartum. Evaluation of follicle number and size along with the status of CL using the brightness mode (B-mode) ultrasound device (M-turbo, Sonosite Inc. Bothell, USA) was done with linear transducer of frequency 5.0-7.5 MHz. Proper restraining, back-racking and application of coupling gel on the transducer was adopted to avoid any distortion in ultrasound

image. Each ovary was individually scanned through several planes by moving the transducer along its surface to identify various ovarian structures gently. Follicles appeared on the ultrasound images as anechoic structures surrounded by fine wall. CL appeared as greyish black structures with echogenic spots in the ultrasound image. Ultrasonography examination envisaged the observation of the diameter of dominant follicle and CL after freezing the best image with minimum distortion and maximum clarity. Ovulation was detected by the disappearance of the large follicle that was present at the previous examination and the subsequent formation of the luteal gland.

Transrectal ultrasonography was done on days 20, 27, 34, 41, 45, 47, 54, 56, and 57 postpartum until the day of fixed time artificial insemination (FTAI). Following observations were recorded; (a) Follicular dynamics (follicle number and follicle size), (b) Size of CL, and (c) Pregnancy diagnosis at 40-60 days post-artificial insemination.

Double-Synch Protocol

Buffaloes were subjected to FTAI after applying double synch protocol 45 days postpartum. Animals brought for insemination were confirmed for their proper stage of AI through rectal palpation for uterine tonicity and the presence of a dominant ovulatory follicle in the ultrasound image.

Statistical Analysis

Data generated by ultrasound examination, biochemical and hormone assays were subjected to statistical analysis using IBM SPSS Statistical Version 26. The conception rate was compared using "Chi-square test". The effect of treatment on various parameters over days within the group and between the groups was analyzed using "Repeated measures ANOVA". A probability level $P < 0.05$ was considered significant for mean differences.

RESULTS AND DISCUSSION

Hormonal Profile

Plasma progesterone values on different days of sampling in the control and the treatment groups of buffaloes fed peripartum RPM are given in Table 1. The maximum concentration of progesterone was 3.85 ± 0.44 ngml^{-1} on day 21 prepartum and the lowest 0.32±0.06 ngml^{-1} on day 57 postpartum in Group 1 fed 20 g RPM. In Group 2 fed 10 g RPM, it was highest *i.e.,* 2.03±0.12 ngml^{-1} on day 21 prepartum and lowest *i.e.,* 0.41±0.05 ngml^{-1} on the day of parturition (0 d). In the control Group 3 the highest progesterone concentration was 1.82±0.11 ngml^{-1} on day 21 prepartum and the lowest 0.35±0.08 ngml^{-1} on day 45 postpartum.

Table 1: Mean (±SEM) plasma progesterone levels (ng ml^{-1}) in buffaloes fed peripartum rumen protected methionine (RPM)

Days Peripartum (day)	Group 1 (RPM 20g d^{-1})	Group 2 (RPM 10g d^{-1})	Group 3 (Control)
-21	3.85±0.44^{a}	2.03±0.12^{b}	1.82±0.11
-7	2.15±0.15^{a}	1.53±0.09^{b}	1.33±0.08
0	0.47±0.03^{a}	0.41±0.05^{b}	0.50±0.03
14	0.56±0.10	0.45±0.11	0.46±0.10
25	0.49±0.11^{a}	0.55±0.13^{b}	0.44±0.09^{c}
36	1.11±0.24^{a}	1.10±0.25^{b}	0.98±0.19^{c}
45	0.45±0.11^{a}	0.44±0.10^{b}	0.35±0.08
47	0.45±0.13	0.51±0.14	0.43±0.12
54	1.12±0.25^{a}	1.01±0.22^{b}	1.13±0.25
57	0.32±0.06	0.46±0.11	0.38±0.06

^{abc} Mean values within the row having different superscripts differ significantly ($p<0.05$)

Mean plasma estradiol concentrations in Group 1 and 2 were highest 243.16 ± 4.67 and 228 ± 5.06 pgml^{-1} respectively, on the day of calving (0 d), and the lowest 16.00 ± 1.09 and 13.16 ± 0.60 pgml^{-1} on day 45 postpartum. In Group 1; fed 20 g RPM Estradiol was highest 228 ± 5.06 pgml^{-1} on the day of parturition (0d) and lowest 13.16 ± 0.60 pgml^{-1} on 45 day postpartum in Group 2 fed 10 g RPM. In the control group the highest estradiol concentration was 218.9 ± 4.36 pgml^{-1} on the day of calving (0d) and lowest 13.66 ± 0.66 pgml^{-1a} on day 25 postpartum (Table 2).

Table 2: Mean ± SEM Estradiol levels (pg ml^{-1}) in buffaloes fed Rumen Protected Methionine (RPM)

Peripartum (day)	GROUP 1 (RPM 20g d^{-1})	GROUP 2 (RPM 10g d^{-1})	GROUP 3 (CONTROL)
-21	36.57 ± 8.34^{a}	67.87 ± 12.3	85.91 ± 3.36
-7	76.43 ± 5.14	41.3 ± 3.19	49.40 ± 4.10
0	243.16 ± 4.67^{a}	228 ± 5.06^{b}	218.9 ± 4.36^{c}
14	19.50 ± 1.47^{a}	18.25 ± 1.35^{b}	15.16 ± 0.94
25	18.08 ± 1.22^{a}	13.83 ± 1.24^{b}	13.66 ± 0.66^{c}
36	20.16 ± 1.57^{a}	22.16 ± 1.88^{b}	21.25 ± 1.76
45	16.00 ± 1.09^{a}	13.16 ± 0.60^{b}	14.16 ± 0.79
47	26.66 ± 1.94	23.50 ± 1.25	23.66 ± 1.22
54	18.01 ± 0.49	15.66 ± 1.11	14.16 ± 1.14
57	30.16 ± 2.04	28.16 ± 1.01	27.50 ± 1.64

^{abc} Mean values within columns having different superscripts differ significantly ($p<0.05$)

IGF-1 was highest 149.50 ± 4.69 ngml^{-1} on 21-day prepartum in Group 1 and 131.33± 6.30 ngml^{-1} on day 54 postpartum in Group 2, with the lowest values 77.83 ± 8.81 and 77.33 ± 2.95 ngml^{-1} on the-day of calving (0d) in both the groups. In the control group IGF-1 was highest 124.50 ± 5.57 ngml^{-1} on day 7 prepartum and lowest 62.66 ± 2.20 ngml^{-1} on the day of parturition (Table 3).

Table 3: Mean ± SEM IGF-1 levels (ng ml^{-1}) in buffaloes fed Rumen Protected Methionine

Peripartum (day)	GROUP 1 (RPM 20g d^{-1})	GROUP 2 (RPM 10g d^{-1})	GROUP 3 (CONTROL)
-21	149.50 ± 4.69	128.41 ± 3.85	119.53 ± 4.07
-7	141.83 ± 3.60^{a}	124.66 ± 5.28^{b}	124.50 ± 5.57
0	77.83 ± 8.81	77.33 ± 2.95	62.66 ± 2.20
14	115 ± 9.50^{a}	113.33 ± 10.61^{b}	107.50 ± 10.03
25	100 ± 10.62	83.66 ± 8.73	76.33 ± 8.58
36	130 ± 22.47	130.33 ± 20.02	106.66 ± 16.73
45	125.16 ± 12.86	96 ± 9.05	88.00 ± 14.93
47	113.83 ± 11.06	98 ± 6.70	99.00 ± 11.84
54	135.66 ± 3.63	131.33 ± 6.30	94.33 ± 7.70
57	113 ± 15.29	126.33 ± 10.12	109.00 ± 16.4

^{abc} Mean values within columns having different superscripts differ significantly ($p<0.05$)

Serum IGF-1 concentrations rose linearly in multiparous late-gestation beef bovines fed RPM in increasing quantities by post-ruminal infusions (Waterman *et al.*, 2007). However, there was a decrease in IGF-1 levels in a study of Moriel *et al.* (2020). The drop in plasma IGF-1 concentrations suggests that micronutrients were being divided to support the immune system rather than for development. IGF-1 levels depict energy status and help in determining the fate of follicles and by feeding RPM the levels of IGF-1 improved and thus showed a positive impact.

Blood Biochemical Profile

Plasma glucose ranged from highest 95.00 ± 8.63 mgdl^{-1} on the day of calving (0d) and lowest 55.85 ± 9.45 mgdl^{-1} on 57-day post-partum in Group 1; fed 20 g Rumen Protected Methionine (RPM) and in Group 2 highest 105.83 ± 8.65 mgdl^{-1} on the day of calving (0d) and lowest 47.75 ± 11.05 mgdl^{-1} on 57-day post-partum fed 10 g Rumen Protected Methionine (RPM). In the Control group Plasma Blood Glucose was highest at 90.16±4.26 mgdl^{-1} on the day of calving (0d) and lowest 46.00±3.75 mgdl^{-1} on 14-day post-partum (Table 4).

Table 4: Mean ± SEM Plasma glucose levels (mg dl^{-1}) in buffaloes fed Rumen Protected Methionine (RPM)

Peripartum (day)	GROUP 1 (RPM 20g d^{-1})	GROUP 2 (RPM 10g d^{-1})	GROUP 3 (CONTROL)
-21	72.16 ± 2.18	74.33 ± 2.75	72.00 ± 3.62
-7	83.16 ± 2.30^{a}	54.00 ± 9.65^{b}	57.16 ± 3.58
0	95.00 ± 8.63^{a}	105.83 ± 8.65^{b}	90.16 ± 4.26^{c}
14	64.16 ± 2.68^{a}	51.16 ± 5.04^{b}	46.00 ± 3.75^{c}
25	76.00 ± 2.30	67.00 ± 3.84	75.00 ± 3.36
36	62.02 ± 4.96^{a}	68.6 ± 3.41^{b}	63.33 ± 3.98^{c}
45	70.50 ± 30	65.16 ± 8.28	72.00 ± 4.70
47	74.00 ± 2.91	67.66 ± 4.63	75.33 ± 5.14
54	74.33 ± 2.36	56.00 ± 10.63	78.5 ± 3.59
57	55.85 ± 9.45	47.75 ± 11.05	57.75 ± 13.03

^{abc} Mean values within columns having different superscripts differ significantly ($p<0.05$)

Blood urea nitrogen (BUN) values on different days of sampling are recorded in Table 5. BUN levels varied from highest 19.12 ± 4.47 mgdl^{-1} on -21-day prepartum to lowest 4.08 ± 1.51 mgdl^{-1} on 54-day postpartum in Group 1; fed 20 g Rumen Protected Methionine (RPM). In Group 2 BUN levels varied from highest 13.00 ± 3.28 mgdl^{-1} on 21-day prepartum to lowest 4.00 ± 1.46 mgdl^{-1} on 54-day postpartum fed 10 g Rumen Protected Methionine (RPM). Control Group BUN levels ranged from highest 11.50 ± 3.19 mgdl^{-1} on 21-day prepartum and lowest 4.54 ± 1.66 mgdl^{-1} on 25-day postpartum, respectively.

Table 5: Mean ± SEM Blood Urea Nitrogen (BUN) levels (mg dl^{-1}) in buffaloes fed Rumen Protected Methionine

Peripartum (day)	GROUP 1 (RPM 20g d^{-1})	GROUP 2 (RPM 10g d^{-1})	GROUP 3 (CONTROL)
-21	19.12 ± 4.47	13.00 ± 3.28	11.50 ± 3.19
-7	15.90 ± 4.79	9.58 ± 2.92	8.92 ± 2.82
0	8.57 ± 2.83	6.80 ± 2.27	6.25 ± 2.10
14	9.82 ± 3.54	8.16 ± 2.89	7.00 ± 2.47
25	4.80 ± 1.73^{a}	4.24 ± 1.54^{b}	4.54 ± 1.66^{c}
36	5.63 ± 1.98	4.96 ± 1.58	5.44 ± 1.99
45	4.44 ± 1.63	4.48 ± 1.64	4.88 ± 1.78
47	4.64 ± 1.70	4.76 ± 1.75	5.28 ± 1.96
54	4.08 ± 1.51^{a}	4.00 ± 1.46^{b}	5.28 ± 1.92
57	4.28 ± 1.56^{a}	4.72 ± 1.73^{b}	5.08 ± 1.86

^{abc} Mean values within columns having different superscripts differ significantly ($p<0.05$)

Plasma creatinine values on different days of sampling is recorded in Table 6. Plasma Creatinine levels varied from highest 1.73 ± 0.22 mgdl^{-1} on 54-day postpartum to lowest 0.40 ± 0.14 mgdl^{-1} on 14-day postpartum in Group 1; fed 20g Rumen Protected Methionine (RPM).In Group

2 fed 10 g Rumen Protected Methionine (RPM) the Plasma Creatinine levels were from highest 2.08 ± 0.10 mgdl-1 on 25-day postpartum and lowest 0.40 ± 14 $mgdl^{-1}$ on -7-day prepartum. In Control group Plasma Creatinine levels ranged from highest 2.35 ± 0.25 $mgdl^{-1}$ on 25-day postpartum to lowest 0.40 ± 15 $mgdl^{-1}$ on 7-day prepartum.

Table 6: Mean ± SEM Plasma Creatinine levels (mg dl^{-1}) in buffaloes fed Rumen Protected Methionine (RPM)

Peripartum (day)	GROUP 1 (RPM 20g d^{-1})	GROUP 2 (RPM 10g d^{-1})	GROUP 3 (CONTROL)
-21	0.42 ± 0.15	0.45 ± 0.16	0.50 ± 0.184
-7	0.50 ± 0.18	0.40 ± 0.14	0.40 ± 0.15
0	0.44 ± 0.17	0.65 ± 0.24	0.64 ± 0.24
14	0.40 ± 0.14	0.47 ± 0.17	0.43 ± 0.15
25	1.49 ± 0.17	2.08 ± 0.10	2.35 ± 0.25
36	1.73 ± 0.18	1.48 ± 0.10	1.51 ± 0.09
45	1.68 ± 0.04	1.73 ± 0.05	1.90 ± 0.12
47	1.55 ± 0.14	1.80 ± 0.14	2.06 ± 0.25
54	1.73 ± 0.22	1.58 ± 0.11	2.08 ± 0.12
57	1.43 ± 0.12	1.95 ± 0.15	2.11 ± 0.16

[abc] Mean values within columns having different superscripts differ significantly ($p<0.05$)

Ultrasonography of Ovarian Structures

Dominant follicle size (Mean ± SEM) ranged from 7.5 ± 0.38 mm to 13.22 ± 0.57 mm over the period of ultrasonography examination from day 20 to day 57 postpartum in different groups (Table 7). It ranged from maximum 13.22 ± 0.57 mm on day 57 (on the day of AI) to lowest 8.5 ± 0.58 mm on day 20 postpartum in group 1, with corresponding values of 12.50 ± 0.34 mm on day 57 and 8.1 ± 0.58 mm on day 41 postpartum in Group 2, and 12.3 ± 1.02 mm on day 45 and lowest 7.5 ± 0.38 mm on day 54 postpartum in control group 3.-

Table 7: Mean ± SEM Dominant follicle size (mm) in buffaloes fed Rumen Protected Methionine

Postpartum (day)	GROUP 1 (RPM 20g d^{-1})	GROUP 2 (RPM 10g d^{-1})	GROUP 3 (CONTROL)
20	8.5 ± 0.58^{b}	8.2 ± 0.61^{a}	7.6 ± 0.81^{a}
27	13 ± 0.74^{b}	11.2 ± 0.56^{a}	10.3 ± 0.57^{b}
34	10.5 ± 0.49^{a}	9.2 ± 0.36^{a}	9.0 ± 0.86^{b}
41	8.7 ± 0.76^{b}	8.1 ± 0.58^{b}	8.3 ± 0.78^{b}
45	13.3 ± 1.3^{b}	11.8 ± 1.05^{a}	12.3 ± 1.02^{a}
47	8.68 ± 0.42^{a}	7.42 ± 0.48^{b}	7.82 ± 0.52^{a}
54	9.2 ± 0.14^{a}	8.6 ± 0.34^{b}	7.5 ± 0.38^{b}
56	12.1 ± 0.22^{b}	10.16 ± 0.74^{a}	9.58 ± 0.54^{a}
57	13.22 ± 0.57^{a}	12.50 ± 0.34^{a}	11.6 ± 0.60^{a}

[abc] Mean values within columns having different superscripts differ significantly ($p<0.05$)

Corpus luteum size (mean ± SEM, Table 8) ranged from 3.40 ± 0.72 mm to 14.10 ± 0.54 mm. CL size over the period of ultrasonography examination from day 20 to day 57 postpartum varied from 3.8 ± 0.36 mm on day 57 postpartum to 12.6 ± 0.39 mm on day 37 postpartum for group 1. In group 2, CL size ranged from 3.6 ± 0.35 mm on day 57 of calving to 13.53 ± 0.34 mm on day 37 of calving. In control group 3, CL size ranged from 3.40 ± 0.72 mm on day 27 of calving to 14.10 ± 0.54 mm on day 37 of calving.

Table 8: Mean ± SEM Corpus Luteum size (mm) in buffaloes fed Rumen Protected Methionine

Postpartum (day)	GROUP 1 (RPM 20g d^{-1})	GROUP 2 (RPM 10g d^{-1})	GROUP 3 (CONTROL)
20	6.8 ± 0.76^{a}	7.4 ± 0.78^{a}	7.6 ± 1.06^{a}
27	3.50 ± 0.35^{a}	3.72 ± 0.51^{b}	3.40 ± 0.72^{b}
37	12.6 ± 0.39^{b}	13.53 ± 0.34^{a}	14.10 ± 0.54^{a}
41	7.1 ± 0.44^{a}	7.3 ± 0.28^{a}	6.9 ± 0.24^{a}
45	4.8 ± 0.35^{a}	4.2 ± 0.35^{a}	3.9 ± 0.63^{a}
47	5.2 ± 0.25^{a}	4.8 ± 0.28^{a}	4.5 ± 0.21^{b}
54	10.6 ± 0.24^{b}	10.5 ± 0.24^{b}	10.8 ± 0.28^{a}
56	4.3 ± 0.24^{a}	4.9 ± 0.28^{b}	4.8 ± 0.30^{b}
57	3.8 ± 0.36^{a}	3.6 ± 0.35^{a}	4.2 ± 0.39^{b}

[abc] Mean values within columns having different superscripts differ significantly ($p<0.05$)

Conception Rate

Conception rates in Groups 1, 2 and 3 fed 20 g RPM, 10 g RPM and Control were 83.33 %, 50.00 % and 33.33 %, respectively, the differences were found to be significant. The significantly higher conception rate observed in group 1 followed by group 2 may be due to higher levels of progesterone in them (Table 1). RPM feeding has an effect on the fertility of lactating dairy cows. Overall, fertility was excellent in this study for synchronised buffaloes using the Double synch protocol.

The results of the plasma biochemical analysis in the buffaloes fed RPM were within the normal ranges. The data of plasma glucose recorded were higher and significantly vary on day 7 prepartum and on day 0, 14 and 36 postpartum compared to the results reported by Ahmed *et al.* (2016). The blood glucose level was non-significantly ($P>0.05$) different among treatment and control groups. Plasma glucose levels were not affected by RPM supplementation in previous studies also (Giallongo *et al.,* 2015; Berthiaume *et al.*, 2006), although increase in levels of plasma glucose levels has been reported in another study (Berthiaume *et al.,* 2001).

Blood urea nitrogen levels were not significantly (P>.05) different between groups. These findings are consistent with those of Movaliya *et al.* (2013). Methionine supplementation in buffaloes showed no influence on BUN levels when compared to the control group. But Vudmaska *et al.* (2021) reported that after supplementation of RPM in TMR, the BUN levels were decreased to 4.64 ± 0.22 $mgdl^{-1}$ indicative of a positive role of RPM in feed.

Plasma creatinine was not affected by treatment and level of RPM supplementation, as was noted earlier (Giallongo *et al.,* 2015; Berthiaume *et al.,* 2006; Preynat *et al.,* 2009), although increases in plasma variables have been reported in some studies (Kröber *et al.*, 2000; Berthiaume *et al.*, 2001).

According to Krehbiel *et al.* (2001), there were no differences in overall pregnancy rates between treatments. However, in our study overall pregnancy rates were numerically higher than in the previous reports. The current findings suggest that the opportunity for reproductive improvement exists, as pregnancy rates were higher than in previous research, most likely due to nutritional deficiency in buffaloes.

CONCLUSIONS

RPM diet had a positive effect on the overall mean plasma concentration of progesterone, estrogen, IGF-1, glucose, BUN on different days of peripartum buffaloes. Overall mean size of dominant follicle and CL altered with positive follicular growth in the buffaloes fed RPM in diet. Conception rates varied significantly in different groups and increased by feeding higher (20g vs 10g) RPM in the diet. IGF-1 levels fluctuated and improved fate of follicles thus improving the fertility. RPM 20g Feeding enhances the follicular growth thus improving the estrus pattern as well as ovarian rebound thus improving the involution rates. Hormonal pattern improved significantly on different days of peripartum thus indicating a positive role of RPM in the diet. Nutrition-reproduction interaction if dealt and managed in an efficient way, it will lead to increase in the production outcomes vis-à-vis fertility outcomes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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