Reproductive Performance of Dairy Buffaloes Supplemented with Varying Levels of Vitamin E

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ABSTRACT : The effect of vitamin E supplementation on plasma α-tocopherol level, total antioxidant level and reproductive performance in Murrah buffaloes was studied during periparturient period. Twenty-four advance pregnant buffaloes were randomly divided into four equal groups as T_1, T_2, T_3 and T_4 and were supplemented with 0, 1,000, 1,500 and 2,000 IU of α-tocopheryl acetate (Merck) from 60 days prepartum to 30 days postpartum and 0, 500, 750 and 1,000 IU from 30 to 60 days postpartum, respectively. Blood samples were collected at -60, -45, -30, -15, -7, 0, 7, 15, 30 and 60 days of parturition and were analyzed for plasma α-tocopherol and total antioxidant activity (TAA). The intake of DM, CP and TDN did not vary among different groups. Plasma α-tocopherol and TAA around parturition (-7 to 15 day) in T_3 and T_4 were significantly higher than the control group. There was 17% reduction in retention of fetal membranes (RFM) and metritis in T_4 than control. The post partum estrus interval averaged 58.00, 55.33, 51.83 and 43.00 days in T_1, T_2, T_3 and T_4 respectively. There was significant reduction in days open in both T_3 and T_4 in comparison to T_1 group (127,130 Vs.146). All the vitamin E supplemented groups showed reduction in days open than their previous lactation performance. Supplementation of vitamin E at 1,500 IU d^{-1} from 60 day prepartum to 30 day post partum to buffaloes exhibited beneficial effect on plasma α-tocopherol level and TAA around parturition and continuation of its supplementation at 1,000 IU d^{-1} from 30 to 60 days of lactation improved post partum reproductive performance of buffaloes. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 1 : 19-25)***

Key Words : Vitamin E, α-Tocopherol, Total Antioxidant Activity, Reproduction, Buffaloes

INTRODUCTION

Feeding during peripartum period is most important as it affects the reproductive performance of dairy animals. In order to resume normal fertility after parturition, adequate balance of protein, energy, trace minerals and antioxidant vitamins must be maintained even during dry period. Role of vitamin E supplementation as an antioxidant vitamin has been well understood (Brzezinska et al., 1994; Mc Dowell, 2002). The fat soluble vitamin E acts as a membrane antioxidant to maintain the integrity of phospholipids against oxidative damage and peroxidation. During the peripartum period, there is increased generation of free radicals that overwhelm antioxidant defense mechanism and compromise cellular function (Dragel, 1992). The production of free radicals leads to infertility because steroidogenic enzymes (Miller et al., 1993), ovarian steriodogenic tissue (Margolin et al., 1990), spermatozoa (Aitken, 1994) and pre implantation of embryos (Fujitani et al., 1997) are sensitive to free radicals damage. Dietary and /or injectable form of vitamin E supplementation to dairy cows decreased the incidence of retention of placenta, reduced days to first observed estrus and decreased services/conception (Jukola et al., 1996; Kim et al., 1997). Delayed first estrus, longer first breeding and repeated breeding are major problems in dairy buffaloes. Being seasonal breeders, dairy buffaloes have more post partum reproductive problems than cows, but very little work has been conducted in buffaloes to reduce the reproductive disorders by supplementing vitamin E. The post partum estrus interval was reduced from 63 to 35 days in Egyptian buffaloes by supplementing 4,200 mg of vitamin E in combination with 4.2 mg Se from the last month of pregnancy till first month post partum (Ezzo, 1995). Anoestrus buffalo heifers supplemented with vitamin E at 3,500 IU/week had increased vitamin E level in the plasma and 80% buffaloes came to estrus within 133 days of supplementation (Nayyar et al., 2002). The present study was planned to see the effect of vitamin E supplementation on blood vitamin E level and reproductive performance of dairy Murrah buffaloes.

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MATERIALS AND METHODS

Feeding and management of buffaloes

Twenty-four primiparous Murrah buffaloes were randomly divided into four groups (T_1, T_2, T_3, T_4) of six each and were fed as per Kearl (1982) standards for feeding to buffaloes. The buffaloes were supplemented with 0, 1,000, 1,500 and 2,000 IU α-tocopheryl acetate/d (Merck) from 60 days prepartum to 30 days postpartum and 0, 500, 750 and 1,000 IU from 30 to 60 days postpartum, in the respective four groups. The feed grade DL α-tocopheryl acetate (Merck) of 50% potency dry powder was weighed accurately and put in a tissue paper fold for carrying to

experimental sheds and fed daily by mixing with small amount of concentrate in a tub. The remainder of the concentrate was fed afterwards.

The buffaloes were fed a minimum of 3.5 kg concentrate mixture and depending upon the requirement as per milk yield (1 kg concentrate mixture for every 2.5 kg milk produced after 5 kg), the quantity of concentrate was increased. The fodder source was silage 20 kg, wheat straw 3 kg and green fodder like maize/jowar/berseem around 10 kg. The intake and residuals of feed and fodders were recorded daily. Animals were milked twice daily (05:00 and 18:30 h) and milk yield was recorded. All the buffaloes were kept in individual pens throughout the experiment and were managed under similar conditions.

Sampling

All the feed and fodder samples were analyzed for DM, CP, NDF, ADF, EE, ash and vitamin E at fortnightly intervals. Blood samples from jugular vein were collected at -60, -45, -30, -15, -7, 0, 7, 15, 30 and 60 days of parturition into heparinized test tubes. Plasma was separated and was stored frozen in deep freezer (-20°C) until analysis of vitamin E and total antioxidant activity. The data obtained during prepartum period was adjusted with respect to date of calving and results have been presented in the tabular form from 60 days prepartum to 60 day post partum, taking day of calving as '0' day.

Reproduction criteria

Around parturition, the buffaloes were kept under special care and watch. After parturition, they were watched for recording the time of expulsion of foetal membranes. The buffaloes that did not shed the foetal membrane within 12 h of parturition were considered as cases of retained placenta (RP). After parturition, animals were observed daily up to 15 days for lochial discharge, diagnosis of post partum metritis and any abnormal discharge. The diagnosis of metritis (MET) was based on uterine size (Morrow et al, 1966) and when uterine size was intermediate, diagnosis was based on vaginal examination with speculum by the experts from Artificial breeding complex, cattle yard. Most buffaloes were examined per rectally and purulent discharge found during vaginal examination was considered diagnostic for MET. After 3 weeks of parturition, animals were examined rectally for involution of uterus and animals suspected for incomplete involution were taken for diagnosis of endometritis. Recovery from metritis was assessed by rectal examination of uterus (Sidhu et al., 2002) and physical characteristic of cervico-vaginal mucus (Luktuke and Roy, 1967). The animals were observed twice daily for signs of estrus. Estrus was detected with the help of a teaser bull. The option interval for first breeding was 60 days and all buffaloes were bred artificially 12 h after first detected estrus. Pregnancy was determined by rectal palpation between 60 days after last service. The animals failed to conceive and reported for AI were recorded for calculation of number of services per conception.

Analytical procedures

The proximate analysis of concentrates and forages were done by AOAC (1995). The α-tocopherol in the feed and plasma samples was estimated on HPLC (Chawla and Kaur, 2001). The HPLC system (Waters) consisted of a model 510 pump, uv visible multi wavelength absorbance detector 486 and rheodyne injector with 20 µl loop. A reverse phase Discovery C-18 (15 cm×4.6 mm) column was used. The mobile phase consisted of acetonitrile, tetrahydrofuran and HPLC water in the ratio of 47:42:11. The flow rate was 1.5 ml/minute. 20 µl of standard/sample was injected in HPLC column for chromatographic separation and the run time was 5 minutes per sample. A specific programme was developed for the separation of retinol and α-tocopherol at 325 and 290 nm wavelengths at 1.75 and 3.37 min respectively.

Plasma total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1999). 100 µl of plasma sample was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 minute at 593 nm after vortexing. Thereafter, samples were placed at 37°C in a water bath and absorbance was measured after 4 minutes. Ascorbic acid standards were processed in the same way. Change in absorbance (ΔA_{593} nm) is translated into FRAP value (µM) by comparing the test samples to that of standard solution of known FRAP value. FRAP value of ascorbic acid is 2.

Statistical analysis of experimental data was carried out to find out the effect of supplementation of vitamin E to dairy buffaloes on plasma vitamin E content and antioxidant activity by two way ANOVA and reproductive performance by one way ANOVA (Snedecor and Cochran, 1980) using SPSS package.

RESULTS AND DISCUSSION

Intake of DM, CP, TDN and Vitamin E

The dry matter intake during 60 days dry period averaged 11.23±0.13, 11.32±0.24, 11.52±0.12 and 11.26±0.12 kg/day in four groups respectively (Table 1). The average DMI in the four respective groups during lactation was 11.98±0.16, 12.29±0.14, 12.34±0.09 and 12.20±0.08 which did not vary among different groups (Table 2). Intake of vitamin E during dry period through feeds and fodder was calculated to be 221.23, 232.55, 252.52 and 226.54 mg/day in T_1, T_2, T_3 and T_4 respectively ($p>0.05$). The total intake of vitamin E (including

Table 1. DM, CP, TDN and vitamin E intake in buffaloes during dry period

Particular	Treatments				p value
	T_1	T_2	T_3	T_4	
Dry matter intake					
kg/ d	11.23±0.13	11.32±0.24	11.52±0.12	11.26±0.12	0.83
kg/100 kg BW	1.77±0.04	1.76±0.03	1.83±0.04	1.74±0.06	0.56
CP intake					
g/day	977.56±43.56	995.24±37.29	1,021.32±64.24	984.36±56.6	0.72
g/100 kg BW	157.48±11.26	161.25±9.65	167.46±18.54	159.43±16.5	0.53
TDN intake					
kg/day	5.71±0.28	5.76±0.22	5.82±0.18	5.74±0.23	0.58
kg/100 kg BW	0.89±0.02	0.90±0.03	0.92±0.02	0.88±0.02	0.62
Vitamin E intake					
Feed and fodder (mg/d)	221.23±12.63	232.55±8.25	252.52±5.83	226.54±3.96	
Suppl. vitamin E mg/day	0	1,000	1,500	2,000	
Total intake mg/day	221.23±1.68	1,232.55±11.23	1,752.52±26.48	2,226.54±51.6	
IU/kg DM	19.40±0.23	108.84±2.31	151.14±2.16	1,97.71±3.16	

Table 2. DM, CP, TDN and vitamin E intake in buffaloes during lactation period

Particulars	Treatments				p value
	T_1	T_2	T_3	T_4	
Dry matter intake					
kg/d	11.98±0.16	12.29±0.14	12.34±0.09	12.20±0.08	0.71
kg/100 kg BW	1.89±0.04	1.91±0.03	1.94±0.06	1.89±0.06	0.63
CP intake					
g/day	1,434.56±41.3	1,482.44±35.2	1,524.18±52.3	1,466.52±48.3	0.26
g/100 kg BW	228.48±12.20	231.25±11.35	237.28±16.24	229.28±14.4	0.35
TDN intake					
kg/day	7.73±0.18	7.92±0.22	8.12±0.24	7.84±0.16	0.62
kg/100 kg BW	1.23±0.12	1.27±0.07	1.28±0.09	1.24±0.04	0.55
Vitamin E intake (first month)					
mg/day	246.23±7.63	1,267.55±8.25	1,779.52±5.83	2,255.54±3.96	
IU/kg DM	20.36±0.21	103.20±9.68	143.27±12.46	184.84±17.36	
Second month					
mg/day	238.45±9.21	752.63±6.58	963.21±6.29	1249.56±38	
IU/kg DM	19.56±0.36	61.21±3.46	78.05±6.50	102.42±9.56	

supplemental) during dry period, first and second month of lactation was 221.23, 1,232.55, 1,752.52, 2,226.54; 246.23, 1,267.55, 1,779.52, 2,255.54 and 238.45, 752.63, 963.21, 1,249.56 mg/day, respectively in the four respective groups (Tables 1 and 2). The CP and TDN intakes did not differ in the four groups as envisaged showing that all the buffaloes were in the same plane of energy and protein. The intake of all the nutrients in lactation was greater than dry period due to their higher requirements (Kearl, 1982).

Plasma α-tocopherol

Plasma α-tocopherol level 60 days before parturition averaged 1.10±0.10, 1.18±0.14, 1.11±0.10 and 1.06±0.08 μg/ml in T_1, T_2, T_3 and T_4 respectively (Table 3, $p>0.05$). The plasma α-tocopherol level did not vary between different groups from 60 days to 15 days prepartum. The level decreased sharply from 15 days before parturition in all the groups up to parturition. However, the decrease was less in T_3 and T_4 groups as compared to T_2 and T_1, which was similar to the response observed in cows supplemented with vitamin E (Weiss et al., 1990; Weiss et al., 1997). On the day of parturition, the α-tocopherol level was 0.50, 0.61, 0.75 and 0.78 μg/ml in the four groups respectively. From two way ANOVA (treatment×period) interaction, it was observed that plasma α-tocopherol levels around parturition (7 day prepartum to 15 day post partum) were significantly higher in T_3 and T_4 groups ($p<0.05$) than T_1. Singh et al. (1997) recorded decrease in α-tocopherol concentration in buffaloes from 1.85 to 1.50 μg/ml from 10 days before parturition to parturition. In cows, α-tocopherol concentration of 1.7 μg/ml at 14 days before parturition was found to decrease to 0.8 μg/ml at parturition (Goff and Stabel, 1990).

After calving, plasma α-tocopherol level started to recover in all the groups but the recovery was quicker in the supplemented groups. After 45 days of parturition, the α-

Table 3. Plasma α-tocopherol concentration (μg/ml) in Buffaloes Supplemented with vitamin E

Days	Treatments					CD
	T_1	T_2	T_3	T_4	Mean	
-60	1.10±0.10	1.18±0.14	1.11±0.10	1.06±0.08	1.11^{A}±0.05	NS
-45	1.13±0.06	1.14±0.09	1.16±0.07	1.11±0.04	1.13^{A}±0.03	NS
-30	0.95±0.11	1.10±0.12	0.95±0.06	0.94±0.05	0.98^{BC}±0.04	NS
-15	0.78±0.08	0.84±0.12	0.92±0.04	0.85±0.07	0.85^{DE}±0.04	NS
-7	0.65^{b}±0.05	0.67^{ab}±0.09	0.81^{a}±0.07	0.82^{a}±0.06	0.74^{D}±0.04	0.15
0	0.50^{b}±0.03	0.61^{bc}±0.07	0.75^{ac}±0.06	0.78^{a}±0.08	0.66^{F}±0.03	0.16
7	0.56^{b}±0.07	0.70^{ab}±0.12	0.82^{a}±0.05	0.87^{a}±0.06	0.74^{D}±0.05	0.25
15	0.62^{b}±0.07	0.78^{ab}±0.08	0.86^{a}±0.06	0.93^{a}±0.07	0.80^{DE}±0.04	0.19
30	0.73±0.06	0.99±0.11	0.94±0.05	0.97±0.05	0.91^{BE}±0.07	NS
45	0.86±0.09	1.04±0.08	1.01±0.09	1.05±0.08	0.99^{BC}±0.06	NS
60	0.92±0.10	1.07±0.07	1.10±0.06	1.07±0.07	1.04^{AB}±0.06	NS
Mean	0.80^{b}±0.03	0.92^{a}±0.04	0.95^{a}±0.02	0.95^{a}±0.03		

CD (treatment) = 0.07, CD (Period) = 0.11, CD (Treatment×period) = 0.22.
Values bearing a, b, c, d superscripts in a row differ significantly ($p<0.05$).
Values bearing A, B, C, D, E, F superscripts in a column differ significantly ($p<0.05$).

Table 4. Plasma total antioxidant activity (FRAP value) μmol/L in buffaloes supplemented with vitamin E

Days	T_1	T_2	T_3	T_4	Mean	CD
-60	2,173.70±79.41	2,238.00±104.20	2,144.34±107.72	2,216.40±89.04	$2,193.11^{A}$±45.30	NS
-45	2,217.92±69.58	2,122.03±83.45	2,197.89±54.81	2,169.05±68.81	$2,176.72^{A}$±33.45	NS
-30	2,185.35±62.13	2,113.92±72.52	2,206.79±100.45	2,117.81±54.71	$2,155.97^{A}$±35.77	NS
-15	1,806.76±78.55	1,912.38±.63	2,068.49±.94	2,088.43±28.93	$1,969.01^{B}$±40.45	203.7
-7	$1,627.57^{b}$±62.18	$1,830.73^{ab}$±64.23	$1,922.94^{a}$±57.53	$2,053.67^{a}$±71.40	$1,858.73^{C}$±44.03	221.6
0	$1,306.16^{c}$±62.36	$1,574.28^{b}$±49.67	$1,745.47^{a}$±45.68	$1,847.03^{a}$±52.66	$1,618.23^{E}$±49.34	155.01
7	$1,497.16^{b}$±69.22	$1,775.43^{a}$±43.13	$1,834.07^{a}$±20.20	$1,881.43^{a}$±46.49	$1,747.02^{D}$±38.34	140.52
15	$1,738.27^{b}$±45.05	$1,851.99^{a}$±28.28	$1,920.91^{a}$±32.97	$1,902.15^{a}$±18.52	$1,853.33^{C}$±21.30	95.55
30	2,022.10±89.52	2,282.53±92.78	2,126.79±32.38	2,070.69±46.41	$2,125.53^{A}$±38.66	NS
45	1,986.20±64.14	2,196.78±75.20	2,227.42±84.34	2,285.54±57.01	$2,173.99^{A}$±40.61	NS
60	2,032.82±48.20	2,118.86±107.47	2,172.11±40.94	2,180.03±45.87	$2,125.96^{A}$±33.30	NS
Mean	$1,872.18^{c}$±40.35	$2,001.54^{b}$±34.16	$2,051.57^{ab}$±26.77	$2,073.84^{a}$±22.99		

CD (treatment) $_{(0.05)}$ = 55.85, CD (Period) $_{(0.05)}$ = 92.62, CD (Treatment×period)$_{(0.05)}$ = 226.87.
Values bearing a, b, c superscripts in a row differ significantly ($p<0.05$).
Values bearing A, B, C, D, E in a column differ significantly ($p<0.05$).

tocopherol concentration in T_2, T_3 and T_4 was more than 1.0 μg/ml which was at par with 60 days pre partum status. Overall mean values of α-tocopherol throughout the experimental period in all the supplemented groups were higher ($p<0.05$) than control group.

Plasma total antioxidant activity (TAA)

Plasma total antioxidant activity (FRAP values) did not vary among different treatment groups at 60 days before calving and continued to decline from 30 days prepartum till calving in all the groups (Table 4). The decrease in antioxidant values at calving was 39.91, 29.65, 18.60 and 16.67% in the respective four groups in comparison to 60-day prepartum status showing comparatively less decrease in vitamin E supplemented groups. Brezezinska et al. (1994) also reported decrease in TAA with approaching parturition in cows. The FRAP values started increasing after parturition and reached normal range after 30 days of parturition (Panda and Kaur, 2003). Miller et al. (1993) reported that feeding 0 and 1,000 IU vitamin E $head^{-1}$ d^{-1} during dry period to cows, led to significant increase in the plasma total antioxidant ($p<0.01$) activity at parturition. Plasma antioxidant status of cows 2 weeks before parturition was found to be better in cows that shed placenta normally at parturition than cows that retained placenta ($p<0.01$) Brezezinska et al. (1994) and Chatterjee et al. (2003) also found higher antioxidant status during parturition in the cows supplemented with vitamin E and a significant positive correlation between the antioxidant status and retention of fetal membranes as the animal after parturition recognizes the placenta more quickly and strongly as a foreign body.

Gestation length and birth weight of calves

The gestation length of buffaloes in all the groups was similar. However, buffaloes in vitamin E supplemented

Table 5. Influence of vitamin E supplementation on reproductive performance in buffaloes

Particulars	Lactation	T_1	T_2	T_3	T_4	CD
Gestation length (days)	Current	310.50±2.38	305.33±2.08	304.67±1.20	306.67±1.45	
	Previous	309.00±1.41	306.50±1.20	309.17±17	310.17±1.56	
Birth weight of calves (kg)	Current	33.16^{b}±1.19	35.33^{b}±1.54	35.50^{b}±0.76	37.17^{a}±0.95	2.46
	Previous	32.83±1.35	34.33±1.12	32.82±1.08	33.00±0.93	
Cases of RFM	Current	2	1	0	1	
	Previous	1	0	1	1	
Hours of shedding of FM	Current	8.15±0.81	8.20±0.58	7.22±0.90	7.18±0.70	
	Previous	7.46±0.84	8.08±0.39	8.23±0.72	8.46±0.52	
Cases of Metritis	Current	2	1	0	1	
	Previous	1	0	1	1	
Days to recover from Metritis	Current	18.5	15.0	0	7.0	
Post partum oestrus interval (days)	Current	58.00^{a}±4.31	55.33^{ac}±4.99	51.83^{ac}±2.61	43.00^{bc}±2.63	13.5
	Previous	60.83±3.64	59.67±3.30	63.50±3.05	60.67±3.05	
No of services/conception	Current	2.50±0.22	2.33±0.21	2.17±0.17	2.17±0.26	
	Previous	2.33±0.21	2.67±0.21	2.33±0.21	2.50±0.22	
Days open	Current	146.17^{a}±4.06	138.83^{ac}±6.79	127.54^{bc}±3.09	130.00^{bc}±5.32	15.4
	Previous	150.67±4.16	154.33±5.65	147.00±6.20	148.17±4.93	
Milk yield (kg/d)		8.12^{b}±0.75	9.13^{ab}±61	10.40^{a}±0.40	10.15^{a}±0.35	1.58

Values bearing a, b, c superscripts in a row differ significantly ($p<0.05$).

groups T_2, T_3 and T_4 calved 4-5 days earlier than T_1 group, which might be due to earlier fetal maturity. The average birth weight of calves born from T_1, T_2, T_3 and T_4 was 33.16, 35.33, 35.50 and 37.17 kg respectively (Table 5). The birth weight of T_3 and T_4 was significantly higher than T_1 group ($p<0.05$), whereas the birth weight of the calves born from previous gestation of the corresponding buffaloes did not differ ($p>0.05$).

Retention of fetal membranes (RMF)

The number of animals having retained fetal membrane were 2, 1, 0 and 1 in T_1, T_2, T_3 and T_4 groups respectively, showing reduction in RFM to the extent of 17% in T_2 and T_4 in comparison to T_1 group. Decrease in shedding hours of fetal membrane in T_3 and T_4 groups was also evident than control as well as previous calving data (Table 5). The reduction in the hours of shedding of FM in these groups might be due to quicker recognition of the fetal membrane as a foreign material by the animal immune system, owing to better antioxidant status of these animals (Table 4). Miller et al. (1993) suggested that cows with retained placenta had lower total antioxidant status. Reduction in RFM cases from 33 to 100% in cows has been reported by supplementing vitamin E alone (680 IU to 1,000 IU d^{-1}) and in combination with selenium (at 0.1 mg/kg bw or 15 mg of Se) during dry period in cows (Harrison et al., 1984; Brzezinska et al., 1994; Nicola et al., 1996). Vitamin E injection at 3,000 IU one week before calving or mixed vitamin E and selenium injections reduced the incidence of RFM from 6.4-10.1% to 3-3.9% (Arechiga et al., 1994; Erskine et al., 1997). However, no effect on RFM was recorded in vitamin E supplemented cows by Stowe et al. (1988) and Batra et al. (1992).

Metritis

The number of post partum metritis cases was 2, 1, 0 and 1 in the respective four groups. The reduction of metritis in T_2 and T_4 was 17% in comparison to T_1 and no case of metritis was seen in T_3 group. The number of days required to recover from metritis were 18.5, 15.0 and 7.0 in T_1, T_2 and T_4 groups respectively. Significant effect on the incidence of metritis in cows was reported by giving 3,000 IU vitamin E injections, 8-15 days before parturition (Erskine et al., 1997). Decrease in metritis was seen in Holstein cows fed 5,000 IU vitamin d^{-1} during lactation. However, Stowe et al. (1988) and Harrison et al. (1984) did not find any effect of vitamin E supplementation on metritis in cows.

Post partum reproductive performance and milk yield

The post partum estrus interval averaged 58.00, 55.33, 51.83 and 43.00 days in T_1, T_2, T_3 and T_4 respectively indicating the beneficial effect of supplementing vitamin E during prepartum period (Table 5). As the dose of vitamin E increased, the reduction in first postpartum estrus interval (PPI) was noticed whereas PPI of only group T_4 was significantly lower than group T_1 ($p<0.05$). Both T_3 and T_4 groups had significantly lower PPI in comparison to previous breeding performance. Ezzo (1995) observed significant reduction in first post partum estrus from 63 to 35 days in Egyptian buffaloes by supplementing 4,200 mg of vitamin E with 4.2 mg of Se/d from last one month of pregnancy to 30 days of lactation. Supplementing vitamin E at 3,500 IU and 14 mg Se/week to anoestrus buffalo heifers reduced the number of days for commencement of estrus from 133 to 51 d (Nayyar et al., 2002). Supplementing

vitamin E from 740 IU to 1,000 IU d^{-1} during dry period to dairy cows also reduced PPEI from 61.7-70.4 to 42.5-66.1 days (Harrison et al., 1984; Campbell and Miller, 1998). However supplementing vitamin E at 500 IU after parturition did not reduce the PPI in Holstein cows (Stowe et al., 1988).

There was significant reduction in days open in both T_3 and T_4 in comparison to T_1 group. With respect to previous lactation, there was a significant decrease in days open in all the supplemented groups. A significant decrease in days open has been recorded in Holstein and Jersey cows fed 1,000 IU supplemental vitamin E/d from 42 days prepartum (Campbell and Miller, 1998). The services per conception were also reduced from 2.50 (control) to 2.17 in T_3 and T_4 groups, though the difference was not statistically significant. Injection of vitamin E (640 IU) and selenium (50 mg) to cows 3 weeks prepartum shortened the calving to conception interval (141 vs. 121 d) and reduced the number of services per conception from 2.8 to 2.3 and increased pregnancy rate at first service from 25.3 to 41.2% (Arechiga et al., 1994). In another experiment, Archeiga et al. (1998) injected 500 mg of vitamin E and 50 mg of Se to 30 day post partum cows and found reduction in days open (98.1 vs. 84.6 d, $p<0.05$) and services per conception (2.0 vs. 1.7, $p<0.05$).

The average 60 day milk production was 8.12, 9.13, 10.40 and 10.15 kg in the four groups respectively which was significantly higher in T_3 and T_4 as compared to T_1 and T_2 groups (Table 5; $p<0.05$). The overall increase in milk production was 12.44, 28.07 and 25.00% in T_2, T_3 and T_4 groups over T_1 group, Kaur et al. (2002) recorded around 20% increase in milk production in cows supplemented with 1,000 IU d^{-1} during dry period. Chatterjee (2002) also found 28-40% increase in milk yield in first month of lactation in cows fed vitamin E ar 1,000 IU/d from 45 days of prepartum to 30 days of lactation and attributed this increase due to decreased incidence of mastitis in vitamin E supplemented cows.

It is inferred from the present findings that vitamin E supplementation at1,500 IU/d in Murrah buffaloes might be practiced from 60 days prepartum to 30 days postpartum in order to have higher plasma α-tocopherol level and better total antioxidant status at parturition. Vitamin E supplementation ar 1,000 IU from 30 to 60 days postpartum decreased postpartum estrus interval, days open and services per conception suggesting that the supplemental dose might be reduced from 1,500 IU to 1,000 IU from 30-60 days postpartum in buffaloes.

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