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# Effect of Vitamin D<sub>3</sub> Supplementation on Milk Production Performance and Hormones in Murrah Buffaloes

# Research article

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#### **Abstract**

Present investigation was carried out to find out the effect of vitamin  $D_3$  on milk production, circulatory hormones and plasma minerals in buffaloes. The lactating healthy buffaloes (12) were selected for experiment and divided equally as control and treatment groups. The treatment group buffaloes received vitamin  $D_3$  dietary supplementation @ 20,000IU/day from 30-120 days of lactation. Daily milk yield was recorded during experimental period. Blood samples were collected at fortnightly interval and was analysed for plasma vitamin  $D_3$ , PTH, Osteocalcin and IgG levels. Milk production was significantly increased by vitamin  $D_3$  feeding in the treatment group (p<0.01) in comparison to control group. Milk yield increased by 1.99 kg/day in vitamin  $D_3$  supplemented buffaloes over control group. Plasma vitamin  $D_3$  level and Osteocalcin levels were higher in the treatment group of buffaloes (p<0.01) in comparison to control group. However, plasma PTH levels decreased (p<0.01). Plasma IgG levels were higher (p<0.01) in treatment as compared to control group of buffaloes. Milk yield was positively correlated to circulatory vitamin  $D_3$  (p<0.01) and osteocalcin levels (p<0.05) and negatively correlated with PTH (p<0.01). It was concluded that vitamin  $D_3$  supplementation improves milk production and persistency of lactation due to increased vitamin  $D_3$  and Osteocalcin level in Murrah buffaloes. Supplementation also affects animal health by improving their immunity.

#### **Key words**

vitamin D3; hormones; Milk production; Immunity; Buffaloes

### Introduction

India is the largest milk producer in the world (175 MT) accounting for 13% of the world's total milk production due to intervention in breeding, feeding and management of dairy animals. Livestock sector contributes 4.11% of total GDP and 25.6% of total agriculture GDP in India [1]. During the last one decade, there has been an increase in commercial dairy farms which rears elite dairy animals yielding up to 35 lit/day at peak lactation. In such animals the requirement of calcium is very high to maintain the calcium homeostasis during transition and lactation

periods (Av. 90-100g Ca/d). Such animals as individual in villages or in dairy farms are stall fed and kept in shelters with inadequate exposure to sunlight. Since solar radiations are required for adequate synthesis of vitamin  $D_3$  in the body, such animals may have impaired calcium levels. Vitamin  $D_3$  is known as the 'Sunshine Vitamin', and the animal having direct contact with sunlight should not require supplementation of vitamin D in the diet. If the level of this hormone is compromised in any physiological stage of animal then supplementation of vitamin  $D_3$  is essential. It has been found that in-housed animal stocks in growing,

pregnancy or lactation are at greatest risk of deficiency for not getting the exposure to crucial Ultra Violet rays from the sun. Further, dark pigmented skins and heavy coats/wools skins are less effective at producing vitamin  $D_3$ .

The calcium homeostasis could be either altered by dietary calcium level and by changes in plasma level of vitamin D<sub>3</sub> hormone as 1,25(OH)<sub>2</sub> D is derived from vitamin D [2,3]. The 1,25 (OH)<sub>2</sub> D is derived from vitamin D, which can be produced from 7-dehydrocholesterol in ultraviolet exposed skin and feeding supplements [4,5]. Vitamin D<sub>2</sub> plays an important role in intestinal absorption of calcium and the calcium absorption efficiency falls due to age related decline in serum 1,25(OH)<sub>2</sub> D levels and intestinal resistance to the action of 1,25(OH), D [6,7]. Plasma parathyroid hormone regulate the renal production of 1,25(OH)<sub>2</sub> D [8]. PTH secretion increases within seconds of sensing hypocalcemia while intracellular PTH degradation is suppressed by hypocalcemia suggesting that it promote bone resorption [9-11] and renal calcium reabsorption [12,13]. For normal cellular functions to occur, homeostatic mechanisms maintain total Ca between 8.5-10.0mg/dl in lactating dairy cows [14] and cows suffer from hypocalcemic event like milk fever when Ca level is <5 mg % [15]. To manage the optimum calcium homeostasis the strategies of enrichment of animal feed by adding vitamin D into the livestock feeds could have potential to increase the vitamin D status of humans also [16]. Considering the importance of vitamin  $D_3$  in humans and animals the present study was undertaken to investigate the effect of dietary vitamin  $D_3$  supplementation on plasma minerals, hormones and milk production performance of Murrah buffaloes

#### **Materials and Methods**

Murrah buffaloes (12) producing average milk yield of 9.2 kg/d in early lactation (30 days) were selected from the institute livestock herd after parturition and divided equally into two groups of 6 animals each. The treatment group of buffaloes was supplemented with vitamin  $D_{\rm 3}$  in powder form @ 20,000IU/day from 30 to 120 days of lactation along with 50g of concentrate mixture in the morning at 8:30 a.m. Control group of buffaloes (CG) received 50g of concentrate mixture only. All the buffaloes

**Table 1:** Mean milk yield and hormones in control and treatment groups of buffaloes.

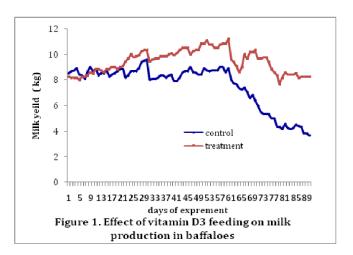
Groups	Fortnights of experiment						
	1	2	3	4	5	6	Overall Av
	Milk yield (kg/d)						
Control	8.25×	8.76×	8.25×	8.74×	6.65×	4.31 <sup>x</sup>	7.50×
Treatment	8.43×	9.56×	9.99 <sup>y</sup>	10.50 <sup>y</sup>	9.63 <sup>y</sup>	8.34 <sup>y</sup>	9.41 <sup>y</sup>
	Vitamin D <sub>3</sub> (ng/ml)						
Control	22.92×	34.84×	32.99×	26.26×	37.11×	23.19×	29.55×
Treatment	26.90×	29.79×	55.96 <sup>y</sup>	42.77 <sup>y</sup>	45.72 <sup>y</sup>	33.29 <sup>y</sup>	39.07 <sup>y</sup>
	Osteocalcin ( ng/ml)						
Control	1.54 ×	1.62×	2.69×	0.78×	1.16 <sup>x</sup>	1.47×	1.54×
Treatment	1.78×	1.48 <sup>y</sup>	4.63 <sup>y</sup>	2.96 <sup>y</sup>	2.52 <sup>y</sup>	1.16 <sup>y</sup>	2.42 <sup>y</sup>
	PTH (ng/ml)						
Control	63.76×	61.25×	54.55×	62.15 <sup>x</sup>	57.27×	65.93×	60.82×
Treatment	63.81 <sup>x</sup>	63.36×	44.50 <sup>y</sup>	55.32 <sup>y</sup>	52.05×	65.51×	57.43 <sup>y</sup>
	Total IgG (mg/ml)						
Control	4.10×	3.96×	4.17×	4.36 ×	4.22×	2.40×	3.87×
Treatment	4.72 <sup>y</sup>	4.64 <sup>y</sup>	4.58 <sup>y</sup>	5.52 <sup>y</sup>	4.79 <sup>y</sup>	2.90 <sup>y</sup>	4.52 <sup>y</sup>

Values with different superscripts  $^{x,y}$  differ (p<0.05) in a column

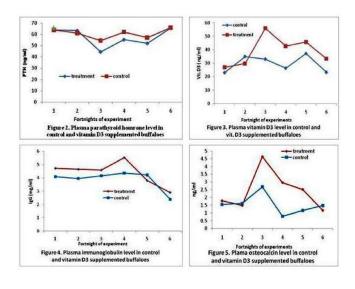
were managed as per the standard routine feeding (ICAR, 2013) and management practices. The buffaloes were offered green fodder maize and/berseem along with wheat straw in the ratio of 60:40. The drinking water was offered ad lib to all buffaloes. Concentrate mixture containing 21% CP and 72% TDN was offered according to their milk production at the time of milking. Buffaloes were hand milked in the morning and evening intervals (6 am and 6 pm) and the milk yield was recorded for a period of 90 days. Blood samples were collected from the experimental buffaloes at fortnightly interval on day 30, 45, 60, 75, 90, 105 days of lactation. Blood samples were collected at 8 a.m. daily in a heparinized Vacutainer tube from the jugular vein and stored frozen at -21°C till analysis of hormones. The analysis of data was carried out by 3-way analysis of variance (ANOVA) using Sigma-stat 3 Software. Mean and standard error was calculated and correlation among the various parameters at 5% and 1% level of significance.

#### **Results**

The milk yield of animals was non-significant between the groups in the beginning of experiment, however milk yield increased significantly (p<0.01) in the treatment group (TG) in comparison to control group (CG). The positive impact of vitamin D<sub>3</sub> on milk production was significant (p<0.01) from day 15 till the end of experiment. Vitamin D<sub>3</sub> supplementation improved the persistency of lactation in the supplemented group, however milk yield declined steadily in control group from the day 30 of experiment. The average increase in milk yield was 21.08% (1.99 kg/d) in the TG over the CG. The variation in milk yield between fortnight and animal was significant (p<0.01). Plasma PTH level was non- significant between the groups during the first fortnight of experiment, however level declined (p<0.05) in the TG over the CG. Plasma vitamin D<sub>3</sub> level significantly increased from  $2^{\mbox{\tiny nd}}$  fortnight of experiment and was consistently higher (p<0.01) till the end of experiment. Plasma vitamin D<sub>2</sub> level were significantly higher in the TG over the CG (p<0.01) and positively correlated with milk yield (r=0.421; p<0.01). The variation in PTH level between the group (p<0.05), fortnight and animal (p<0.01) was significant. In the CG buffaloes the PTH level were consistently maintained during the experiment. Correlation analysis indicated that PTH level was negatively correlated with the plasma IgG (r= -0.206; p<0.05). IgG levels varied significantly between the fortnights of experiment. However between animal variations was non-significant. Plasma osteocalcin level



was not affected by vitamin D<sub>3</sub> supplementation during 1 and 2 fortnight of experiment; however it peaked in 3<sup>rd</sup> fortnight and subsequently declined. The variation in osteocalcin level between fortnight and between animal (p<0.01) was significant. Osteocalcin levels were positively correlated to milk yield (r=0.225; p<0.05) and plasma IgG levels (r= 0.207; p<0.02). Plasma IgG levels had no correlation with the milk yield of buffaloes in both groups. Milk yield was positively correlated with the plasma vitamin D3 level (r=0.421, p<0.01) and Osteocalcin (r=0.225, p<0.01). However, it was negatively correlated to parathyroid hormone (r= -0.281; p< 0.01). Correlation analysis reveal that PTH was negatively correlated with Osteocalcin (r= -0.797; p<0.001) and vitamin D<sub>2</sub> (r= -0.835; p<0.001). A significant positive correlation of osteocalcin was observed with vitamin  $D_3$  level (r= 0.725; p< 0.001). Milk production was positively correlated to osteocalcin



( p< 0.05) and vitamin  $D_3$  (p<0.001) and negatively correlated with PTH ((p<0.01).

#### Discussion

The present investigation revealed that vitamin D<sub>3</sub> in early lactation has galactopoietic effect which resulted in significant increase in milk production by 21% due to elevated plasma vitamin D<sub>3</sub> concentration as evidenced by positive correlation of milk yield with vitamin D<sub>3</sub> levels (p<0.01). However, basal level of vitamin D<sub>3</sub> (21-30ng/ ml) was lower to the values of 60-70 ng/ml reported by Kimura et al., (2006) in the serum of cow. The increase in milk yield was also attributed to higher plasma calcium level as vitamin D<sub>3</sub> feeding @ 50mg/day improves the Calcium levels in cows. The galactopiotic effect of vitamin  $D_3$  in this study further suggests that vitamin  $D_3$  was essential in milk production of dairy animals (NRC, 2001). The decline in PTH concentration was due to higher level of osteocalcin and vitamin D<sub>3</sub> levels which increases the blood calcium concentration. The supplementation of vitamin D<sub>3</sub> could be one of the important tool to improve the circulating level of this hormone as lactating dairy cow in peak lactation secretes higher amounts of calcium (80-100g/d) in milk. It has been reported that osteocalcin serve as bone formation marker [17] in dairy animals and the higher level of osteocalcin further indicated its role in maintaining the calcium homeostasis [18]. The significant increase in plasma IgG level further suggest that vitamin D<sub>3</sub> supplementation @ 20,000IU/day improves the immunity of animals possibly due to co-ordinated effect of plasma vitamin D<sub>3</sub> and osteocalcin through increase in plasma calcium levels, which not only regulate the osmotic pressure of milk but also improves the functioning of body muscles.

# **Conclusion**

Vitamin  $D_3$  feeding @ 20,000IU/day had a galactopietic effect on milk production due to increased circulatory vitamin D3, Osteocalcin level and improves immunity. So vitamin  $D_3$  feeding could be practiced in the organised dairy farms to augment persistency of lactation.

#### **Contributors list**

Dr. Nasir Hussain conducted the experiment and collected the blood samples during the experiment. Dr. Mahendra Singh assisted in planning of experiment, selection of animals, conduct of trial and recording of milk yields. Dr. A. K. Roy assisted in analysis of blood samples

for hormone assays.

#### **Conflicts of Interest**

The authors have no conflict of interest.

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