EFFECT OF ORAL SUPPLEMENTATION OF THE OPTIMIZED DOSE OF VITAMINS ON MILK YIELD, HAEMATOLOGICAL PARAMETERS AND UDDER HEALTH IN DEONI COWS

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A study was conducted to observe the effect of oral supplementation of optimized doses of vitamins on the somatic cell count (SCC), haematology and milk production in Deoni cows. Thirty-two Deoni cows (second parity) in their peak lactation (four weeks post-calving) were randomly divided into four groups (n=8) based on milk production (3.17 ± 0.18 kg/day) and fed with a basal diet (CON) and/or supplemented with optimized doses of water-soluble (WS), fat-soluble (FS) vitamins, or both (WSFS). Oral dose of vitamins were optimized using *in vitro* gas production model and reported earlier. No significant influence of oral vitamin supplementation was observed on the milk yield, composition and haematology. However, the SCC reduced significantly ($P < 0.05$) with supplementation, with highest reduction in the WS group. The SCC values after the trial were higher compared to that before trial only in CON. The results underlined the influence of oral vitamin supplementation on the SCC and thereby udder health.

Key words: Cattle, Mastitis, Milk-composition, Milk-yield, Nutrition

India is the largest producer of milk in the world. This is primarily due to large population of cattle in the country, rather than higher productivity of the animals. Majority of the human population in India are vegetarians and their main source of high quality protein is milk. The major constraint for milk production in India is the unavailability of high quality forages. Any measure to improve the utilization of low quality forages by rumen microbiota can

improve the nutrient status of the cow. Rumen microbes require specific vitamins for their proliferation (Ashwin *et al.*, 2018). In an earlier study conducted in our laboratory, we had optimized the oral requirements of each vitamin for cattle for optimum rumen microbial proliferation through *in vitro* studies, and validated it *in vivo* in Deoni cows (Kandathil and Srinivas, 2019). The present study was conducted to validate the effect of optimized

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oral supplementation of vitamins on milk yield, composition and SCC. Apart from this, the effect of supplementation on haematology was also assessed.

MATERIALS AND METHODS

Animals, experimental design, diets: The study was carried out at Deoni conservation farm, National Dairy Research Institute, Bengaluru, India. Thirty-two milking Deoni cows (second parity) in their peak lactation (four week post-calving) were selected. They were randomly divided into four groups (n=8) based on average milk production at fourth week post-calving (3.17 ± 0.18 kg/day). The treatments were: (i) basal diet with no vitamins (CON), or supplemented with the optimized dose of (ii) water-soluble (WS), (iii) fat-soluble vitamins (FS), or (iv) both (WSFS). Basal diet in the study consisted of concentrate mixture and Finger millet (*Elusine coracana*) straw in 30:70 ratio. The concentrate used for the present study contained 25 parts maize, 25 parts de-oiled rice bran, 15 parts molasses, 12 parts cotton seed cake, 11 parts soya bean meal, 9 parts de-oiled copra cake, 2 part mineral mixture and 1 part common salt. The nutrient composition of concentrate (mean $\pm$ SE, in percentage) were 16.84 ± 0.33 crude protein (CP), 3.48 ± 0.21 ether extract (EE), 8.85 ± 0.09 total ash (TA), 1.38 ± 0.06 acid insoluble ash (AIA), 70.84 ± 0.19 total carbohydrate and 91.15 ± 0.09 organic matter (OM). For finger millet straw the CP, EE, TA, AIA, TCHO and OM were respectively 5.44 ± 0.51 , 1.36 ± 0.09 , 9.08 ± 0.12 , 2.32 ± 0.08 , 84.13 ± 0.54 and 90.92 ± 0.12 percentage. The nutrient requirements of the animals were calculated based on Indian Council of Agricultural Research guidelines (ICAR, 2013).

Dose of vitamins optimized in an earlier study conducted in our laboratory was used in the present study (Kandathil and Srinivas, 2019), which was 3.66, 6.78, 16.3, 41.1, 3.87, 0.323, 4.12 and 0.055 mg/kg DM intake for thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid and B₁₂ respectively. Among FS vitamins, only vitamin K was observed as essential for optimal rumen fermentation at a dose rate of 500 IU/kg DM intake. All the animals were dewormed using fenbendazole before the start of experiment.

Experimental protocol and sampling:

Experimental feeding was carried out for a period of 45 days. Five milliliter of blood was collected in ethylene diamine tetraacetic acid (EDTA) coated vials at the beginning (after the adjustment period), and at the end of the digestibility trial. Feed was weighed and offered individually to cows twice daily at 9 AM and 5 PM. Drinking water was offered *ad libitum*. Daily milk yield (MY) was recorded throughout the experiment duration. Change in the MY with oral vitamin supplementation was calculated by subtracting initial reading from weekly average MY for up to five weeks. Milk samples from cows were collected twice a week in morning and evening, and were pooled for milk fat (MF) and solid non-fat (SNF) estimation. The SCC was estimated after adjustment period, and at the end of the digestibility trial. The difference in SCC before and after experimental feeding was calculated for each treatment.

Chemical analyses: Milk fat (MF; AOAC, 2000; Method 2000.18) and total solids (TS; AOAC, 2000; Method 925.23) were calculated on the same day of collection using standard protocols. The SNF was calculated as the difference between TS and MF.

The somatic cell count (SCC) in the milk before and after the experimental feeding was estimated using digital reader (M/S Porta Check Inc., USA). A drop of well mixed sample of milk was taken using a sterile pipette and was added to the sample well of the Porta-strip. Three drops of activator solution was added to this, and it was kept for 30 to 45 minutes at room temperature for colour development. The strip after incubation was inserted to the digital reader to get the reading.

Haematological parameters were estimated within 2 hour of collection using auto-haematology analyzer (BC-2800 Vet, M/S Mindray Medical International Ltd, Shenzhen, China). Haematological parameters studied were haemoglobin (Hb; g/dL), packed cell volume (PCV; %), erythrocytes (RBC; $\times 10^6/\mu\text{L}$), leukocytes (WBC; $\times 10^3/\mu\text{L}$), lymphocytes ($\times 10^3/\mu\text{L}$), monocytes ($\times 10^3/\mu\text{L}$), granulocytes ($\times 10^3/\mu\text{L}$), platelets ($\times 10^3/\mu\text{L}$), monocyte (%), lymphocyte (%) and granulocyte (%).

Statistical analyses: The results obtained were subjected to analysis of variance and are presented as means with standard error of means (SEMs). Univariate analysis was carried out with treatment and period as fixed factors and respective statistic as dependent variable using SPSS v 20.0. Post hoc comparisons were performed using Tukey test. The SCC before and, after experimental feeding were analyzed using paired T test. The difference between means was considered statistically significant at 5% level of probability ($P \leq 0.05$).

RESULTS

Weekly change in MY, MF and SNF are given in Table 1. There was no significant change in

the milk yield with vitamin supplementation ($P > 0.05$). Temporal changes in MY was also not significant ($P > 0.05$). Solid non-fat (SNF) content of milk was not significantly different between groups. Milk fat content was significantly higher with FS supplementation (5.43%) compared to WS and WSFS (4.36 and 4.61% respectively), whereas it was intermediate in CON (4.90%). This data is not conclusive as the initial MF (before the start of trial) varied between groups.

Temporal variation in PCV, TEC, granulocyte, platelets and lymphocyte were significant ($P < 0.05$; Table 2). However, no effect of vitamin supplementation was observed in haematology ($P > 0.05$).

The initial SCC of different treatment groups varied from 57500 cells/mL in WSFS to 105000 cells/mL in WS (Table 3). Paired T-test was used to compare the effect of supplementations on the SCC. In CON, the SCC showed an increasing trend ($P = 0.08$) after trial compared to the corresponding reading before experimental feeding. In WS group, a decreasing trend with supplementation was obvious (105000 vs 80000 cells/mL; $P = 0.063$). With FS and WSFS supplementation, no such trends were observed on the SCC ($P > 0.1$). As the initial SCC before start of experimental feeding were not uniform among groups, the difference before and after trial was calculated and compared (Fig. 1). There was a significant reduction in the SCC in all treatment groups compared to CON ($P = 0.022$). The highest reduction in SCC was observed with WS supplementation, the reduction was intermediate in FS and WSFS groups, whereas SCC increased only in CON.

Table 1. Weekly changes in the milk yield (MY), milk fat (MF) and solid-non-fat (SNF) in lactating Deoni cows supplemented orally with optimized doses of water-soluble (WS), fat-soluble (FS) or both (WSFS) vitamins vis-à-vis control (CON)

Attribute	Treatment	Period						Treatment mean	SEM	P value
		0wk	1wk	2wk	3wk	4wk	5wk			
Change in milk yield	CON	0.00	0.06	0.10	0.32	0.41	0.26	0.19	T=0.061	T=0.393
	WS	0.00	0.04	0.06	0.11	0.11	0.17	0.08	P=0.074	P=0.384
	FS	0.00	0.08	0.18	0.06	0.09	-0.09	0.05	T×P=0.148	T×P=0.986
	WSFS	0.00	0.03	0.13	0.19	0.24	0.19	0.13		
	Period mean	0.00	0.05	0.11	0.17	0.21	0.13	10.37		
Milk SNF	CON	10.37	10.42	9.81	9.50	9.35	9.97		T=0.180	T=0.089
	WS	10.41	9.88	9.31	8.99	9.18	9.34	9.52	P=0.221	P=0.008
	FS	10.68	10.52	10.25	9.71	9.52	9.90	10.10	T×P=0.442	T×P=0.998
	WSFS	10.11	9.78	9.75	9.48	9.36	9.38	9.64		
	Period mean	10.39 ^b	10.14 ^{ab}	9.93 ^{ab}	9.50 ^{ab}	9.39 ^a	9.49 ^{ab}			
Milk fat	CON	4.45	4.74	4.81	4.91	5.15	5.35	4.90 ^{ab}	T=0.215	T=0.005
	WS	4.35	4.42	4.38	4.29	4.20	4.50	4.36 ^a	P=0.263	P=0.696
	FS	5.02	5.16	5.22	5.59	5.73	5.87	5.43 ^b	T×P=0.527	T×P=1
	WSFS	4.34	4.46	4.64	4.73	4.74	4.75	4.61 ^a		
	Period mean	4.54	4.69	4.76	4.88	4.96	5.12			

^{ab}Means bearing different superscripts differ significantly according to Tukey test (P<0.05)

Table 2. Haematological values in lactating Deoni cows supplemented orally with optimized doses of water-soluble (WS), fat-soluble (FS) or both (WSFS) vitamins

Attributes		Period		Treatment mean	SEM	P value
		0d	45d			
Hb (g/dL)	CON	12.18	11.33	11.75	T=0.248	T=0.138
	WS	11.60	10.95	11.28	P=0.175	P=0.164
	FS	11.90	12.00	11.95	T×P=0.351	T×P=0.167
	WSFS	11.25	11.23	11.24		
	Period mean	11.73	11.38			

Cont. Table- 2.

Attributes		Period		Treatment mean	SEM	P value
		0d	45d			
PCV (%)	CON	34.60	36.05	35.33	T=0.254	T=0.154
	WS	33.00	35.30	34.15	P=0.179	P=0.001
	FS	34.13	39.30	36.71	T×P=1.24	T×P=0.446
	WSFS	32.15	36.20	34.18		
	Period mean	33.47 ^a	36.71 ^b			
TEC (×10 ⁶ /μL)	CON	6.78	7.93	7.35	T=0.254	T=0.52
	WS	6.45	7.20	6.83	P=0.179	P<0.001
	FS	6.48	7.88	7.18	T×P=0.360	T×P=0.841
	WSFS	6.65	7.78	7.21		
	Period mean	6.59 ^a	7.69 ^b			
TLC (×10 ³ /μL)	CON	10.63	9.95	10.29	T=0.742	T=0.901
	WS	11.45	10.58	11.01	P=0.525	P=0.752
	FS	10.08	11.25	10.66	T×P=1.049	T×P=0.747
	WSFS	10.68	10.10	10.39		
	Period mean	10.71	10.47			
Lymphocyte (×10 ³ /μL)	CON	4.53	5.25	4.89	T=0.519	T=0.745
	WS	5.30	5.68	5.49	P=0.367	P=0.078
	FS	4.60	5.60	5.10	T×P=0.734	T×P=0.822
	WSFS	3.85	5.58	4.71		
	Period mean	4.57	5.53			
Monocyte (×10 ³ /μL)	CON	0.95	0.80	0.88	T=0.107	T=0.778
	WS	0.88	0.78	0.83	P=0.076	P=0.390
	FS	0.80	1.10	0.95	T×P=0.152	T×P=0.149
	WSFS	1.18	0.75	0.96		
	Period mean	0.95	0.86			
Granulocyte (×10 ³ /μL)	CON	5.15	3.90	4.53	T=0.443	T=0.99
	WS	5.28	4.13	4.70	P=0.313	P=0.02
	FS	4.68	4.55	4.61	T×P=0.626	T×P=0.579
	WSFS	5.65	3.78	4.71		
	Period mean	5.19 ^a	4.09 ^b			

Cont. Table- 2.

Attributes		Period		Treatment mean	SEM	P value
		0d	45d			
Platelets ($\times 10^3/\mu\text{L}$)	CON	552.5	309.0	430.75	T=63.58	T=0.138
	WS	526.3	237.3	381.75	P=44.96	P<0.001
	FS	766.5	327.8	547.13	T $\times$ P=89.92	T $\times$ P=0.728
	WSFS	720.3	417.8	569.00		
	Period mean	641.4 ^a	322.9 ^b			
Lymphocyte (%)	CON	42.050	52.075	47.063	T=3.268	T=0.847
	WS	45.375	53.375	49.375	P=2.311	P=0.004
	FS	45.900	50.800	48.350	T $\times$ P=4.622	T $\times$ P=0.476
	WSFS	35.950	54.950	45.450		
	Period mean	42.319 ^a	52.80 ^b			
Monocyte (%)	CON	8.950	8.325	8.638	T=0.635	T=0.283
	WS	7.750	7.600	7.675	P=0.449	P=0.321
	FS	8.200	9.725	8.963	T $\times$ P=0.898	T $\times$ P=0.083
	WSFS	11.075	7.750	9.413		
	Period mean	8.994	8.350			
Granulocyte (%)	CON	49.000	39.600	44.300	T=3.017	T=0.932
	WS	46.875	39.050	42.963	P=2.133	P=0.003
	FS	45.900	39.475	42.688	T $\times$ P=4.267	T $\times$ P=0.715
	WSFS	52.975	37.300	45.138		
	Period mean	48.688 ^b	38.856 ^a			

^{ab}Means with different superscripts differ significantly according to Tukey test (P<0.05)

Table 3. Somatic cell count (SCC, cells/mL) of milk in lactating Deoni cows before (0 day) vs. after (45 day) supplementation with water-soluble (WS), fat-soluble (FS) or both vitamins (WSFS) vis-à-vis control (CON)

Treatments	Period	Mean SCC	SEM	P value
CON	0d	60000	10000	0.08
	45d	72500	13150	
WS	0d	105000	19365	0.063
	45d	80000	10801	
FS	0d	77500	10308	0.252
	45d	67500	10308	
WSFS	0d	57500	7500	0.391
	45d	50000	0	

^{ab}Means bearing different superscripts differ significantly between groups according to Tukey test

Fig. 1. Difference in the somatic cell count (SCC) of milk (mean±SEM) in lactating Deoni cows after supplementation with water-soluble (WS), fat-soluble (FS) or both vitamins (WSFS) vis-à-vis control (CON)

DISCUSSION

Oral supplementation of an optimized dose of vitamins showed higher rumen microbial protein synthesis in an earlier study conducted in our laboratory (Kandathil and Srinivas, 2019). Hence, we hypothesized that an increase in microbial protein supply would improve the nutrient status of the animal and ultimately result in higher MY. However, in the present study, we could not observe any effect of oral supplementation on the MY. The primary reason for the absence of supplementation effects may be the inherent low productivity of Deoni cows. Studies conducted in high producing cows showed increase in milk yield with vitamin A (Oldham *et al.*, 1991; De-

Ondarza *et al.*, 2009), vitamin D (Hibbs and Conrad, 1983), thiamin (Bal *et al.*, 2000); niacin (Tamizrad and Karkoodi, 2009); folic acid (Girard and Matte, 1998); vitamin B₁₂ (Graulet *et al.*, 2007) and biotin (Midla *et al.*, 1998; Bergsten *et al.*, 2003) supplementations. On the contrary, Rahmani *et al.* (2014) and Fitzgerald *et al.* (2000) failed to observe any effect of oral supplementation of vitamin E and biotin respectively in the MY. The results from most of the experiments were inconsistent and varying in nature. However, higher productivity was observed in most cases supplemented with folic acid, vitamin B₁₂ and choline. This may be mainly attributed to the methyl group

donating property of these compounds. Often, methionine is the limiting amino acid for the synthesis of microbial protein in the rumen. Supply of methyl group may improve the quality of microbial protein synthesized, eventually resulting in higher nutritional status of the animal and higher productivity.

Somatic cell count is widely used to monitor udder health worldwide (Sharif and Muhammad, 2008). The SCC mainly consists of milk secreting epithelial cells and leukocytes that shed/enter the mammary gland following infections (Jones, 2006; Sharma *et al.*, 2011). The Deoni cows selected for the present study showed an initial SCC reading ranging from 57500 to 105000 cells/mL. The normal SCC values of Deoni breed of cows have not been published yet at best of our knowledge. Earlier reports indicated increase in the SCC with age (Blackburn, 1966) which was mainly due to increased prevalence of infections in older cows (Sharma *et al.*, 2011). In our present study we had selected only cows of similar parity to reduce such an error. The normal SCC in high producing cattle breeds like Brown Swiss and Holstein Friesian were reported as 423×10^3 and 310×10^3 cells/mL respectively in an earlier study (Sharma *et al.*, 2011). Among Indian breeds, the average SCC ($\times 10^3$ cells/mL) of studied herds of Tharparker, Sahiwal, Karan Fries and Karan Swiss were 1.26, 1.31, 1.61 and 1.54 respectively (Singh, 2002). The initial average SCC in the Deoni cows selected for the study was 75000 cells/mL. In the present study, the highest reduction in the SCC post-treatment was observed in WS group, whereas reduction was intermediate in FS and WSFS groups. Absence of additive effect of WS and FS supplementation in the reduction of SCC

cannot be concluded from the present study as the SCC reduced to minimum detectable levels (50000 cells/mL) with WSFS supplementation. In the present experiment, none of the groups had SCC required for concluding clinical mastitis (< 200000 cells/mL). A reduction in stress with vitamin supplementation was assumed as the possible reason for the reduction in milk SCC.

Haematological parameters are generally used to evaluate the nutritional status of the animals. This is also used as an indicator to study the effect of nutritional supplementation (Cherdthong *et al.*, 2014). We could not find any study correlating B complex vitamin supplementation with haematological parameters in dairy cows. In neonatal calves, supplementation of vitamin A for first five days significantly improved Hb concentration by affecting iron metabolism (Moosavian *et al.*, 2010). On the contrary, no such effect was observed by Kume and Toharmat (2001). Vitamin E supplementation improved blood leukocyte concentration in many studies (Bednarek *et al.*, 1996; Mohri *et al.*, 2005). No reports correlating vitamin K supplementation with haematology in dairy cows were available. However, in our present study we supplemented only vitamin K among FS vitamins. In the present study, no influence of vitamin supplementation was observed on the haematology parameters. The temporal change in certain haematological parameters may be due to deprivation of green fodder and thereby certain micronutrients during the entire experimental feeding period.

The study concluded that the supplementation of optimized doses of WS vitamins alone or in

combination with FS (WSFS) had a beneficial role in promoting udder health as evident from the extent of reduction in the SCC. Further experiments with high producing animals are required to validate the effect of oral supplementation of vitamins on the yield of milk and its constituents.

Conflict of interest: The authors declare that

there is no conflict of interest regarding the present research work.

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