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Hemato-biochemical and electrolytes profiling of Deoni cows supplemented with dietary betaine

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Abstract
A study was conducted to assess effects of dietary betaine supplementation on haemato-biochemical profile and electrolytes characteristics in lactating Deoni cows. Twelve lactating cows were randomly divided into control and treatment group with six in each group. Control group was fed basal diet alone whereas treatment group was supplemented with anhydrous betaine hydrochloride @ 15 g/day in the diet during the study period of 8 week in addition to basal diet. There were no differences between two groups in TEC, Hb, PCV and TLC. Percentages of lymphocytes were significantly higher and that of neutrophils were lower in treatment group as compared to control. The concentration of cholesterol, triglycerides and BUN were significantly (p<0.05) decreased from 4th week and total proteins increased from 2nd week of betaine supplementation in lactating Deoni cows. There were no significant differences in plasma electrolytes in betaine supplemented groups compared to control, although plasma sodium and potassium were numerically higher and chloride levels were numerically lower when compared to control group. It can be concluded that detailed study regarding dietary betaine supplementation on haemato-biochemical profile and electrolytes characteristics in lactating Deoni cows and other dairy breeds of cattle has to be done.

Keywords: Betaine, electrolytes, haemoglobin, lactating and Deoni

1. Introduction
The Deoni is an important dual-purpose cattle breed in India. These animals are quite popular in Bidar district of Karnataka as well as adjoining districts of Maharashtra and Telangana. Deoni cows have a greater potential for producing more quantity of milk under better feeding and management practices. Betaine, naturally present in wheat and sugar beets, is an oxidative product of choline and a trimethylated derivative of glycine (de Zwart *et al*., 2003) [4]. Commercially available betaine is a coproduct of the sugar beet industry (Lever and Slow, 2010) [10] and is extracted from molasses by water-based chromatographic separation and crystallization (Craig, 2004) [3]. It has been demonstrated that betaine has many physiological functions in an animal's body. Chudak (2020) [2] found that there was an increase in the concentration of erythrocytes by 5.61%, in betaine supplemented piglets compared with the control indicators. It serves as an organic osmolyte, helping to reduce dehydration, stabilize protein structure, and preserve enzyme function when a cell is under osmotic stress. By providing the methyl group to the universal methyl donor, S-adenosylmethionine, through methionine, betaine influences many key functions in the body, such as growth, liver health, and lactation (Ratriyanto *et al*., 2009) [13]. In nonruminants, betaine has been used for several decades as a nutritional supplement to improve animal performance. Previous studies suggest that betaine supplementation significantly improved body weight gain and feed conversion in chickens (Zhan *et al*., 2006) [19], ducks (Wang *et al*., 2004) [17], and pigs (Campbell *et al*., 1995) [1]. Moreover, betaine is involved in enhancing immune response (Klasing *et al*., 2002) [9]. Betaine could also alter ruminal fermentation by serving as a source of either ruminally available nitrogen or methyl groups (Wang *et al*., 2010) [16]. Therefore, the aim of the present work was to study the effects of dietary betaine supplementation on hemato-biochemical profile, and plasma biochemical characteristics in lactating Deoni cows.

2. Materials and Methods

In the current study, 12 apparently healthy, lactating Deoni cows free from any anatomical, physiological and infectious disease/disorders within age group of 3-8 years and body weight of around 325-350 kgs were selected from Livestock Research and Information Centre (Deoni), Halliked of KVAFSU, Bidar. All the Cows were fed a ration consisting of concentrate mixture and roughages, green fodder and maize or jowar (sorghum) stovers as per the availability at the farm. The concentrate mixture was offered in the morning whereas, the chaffed green fodder was offered at 11:00 am and stovers for remaining part of the day. Fresh tap water for drinking was available throughout the day. The lactating cows under study were divided into 2 group *viz*. Control (C) and Betaine supplemented group (T) consisting of 6 animals each based on parity and body weight. Control group was fed basal di*et al*one and Betaine supplemented group was fed Betaine (Tri methyl glycine) @ 15 g/ day during the two months of study period. Blood samples were collected from jugular vein at an interval of 15 days from all the experimental animals for estimation haematological and biochemical parameters. From each animal, 5-7 mL of blood was collected with anticoagulant EDTA (2.7%). Haematological parameters *viz*., total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV) and total leukocyte count (TLC) were analysed using veterinary auto-haematology analyser (ERMA INC, PCE-210VET make from by PVT.LTD). Differential leukocyte count (DLC) was done manually as described by Weiss and Wardrop (2010) [18]. The blood biochemical parameters *viz*., glucose, total protein, blood urea nitrogen, cholesterol and triglycerides were estimated using semi-automated biochemistry analyzer (ARTOS®, Swemed Biomedicals) using SWEMED® diagnostic kits, following the instruction and procedure supplied with the diagnostic kits. The data was analysed using TWO WAY ANOVA using Graph Pad Prism version 5.0 statistical software ways as per methods described by Snedecor and Cochran (1989) [15].

3. Results and Discussion

3.1 Haematological Parameters

In the present study, the erythrocyte counts, haematocrit levels, haemoglobin concentrations in the treatment group were numerically higher but the differences were statistically non-significant compared to that of control at 2 nd, 4th,6th and 8th week of study period (Table 1). The information about the effect of betaine supplementation on total erythrocyte counts in lactating cows is scare. However, Chudak (2020) [2]. Has reported an increase in the concentration of erythrocytes by 5.61%, in crossbred piglets fed by natural betaine as compared to the control indicator. Park and Kim (2017) [12], have reported a significantly higher haemoglobin concentration in betaine supplemented ducks compared to heat stress control ducks. The significant differences in haemoglobin concentration might be due to heat stress which lowered RBC counts in heat stressed control ducks whereas betaine being a proven alleviator of heat stress in various domestic species might have improved the haemoglobin concentrations and haematocrit levels towards normal in betaine supplemented groups.

The total leucocyte counts in the treatment groups were numerically lower but the differences were statistically non-significant compared to that of control at 2nd, 4th,6th and 8th week of study period (Table 2). The results were in contrary with El-Moniem *et al*. (2016) [5] and Mendoza (2016) [11] study in heat stressed animals which caused a lower WBC counts, the betaine supplementation improved the WBC counts towards normal in these treatment groups. the Lymphocyte (%), neutrophil (%) in the treatment groups were significantly higher and lower respectively when compared to that of control at 4th, 6th and 8th week of study period which is in agreement with the studies of Mendoza (2016) [11] who reported lymphocytes increased and neutrophils decreased on day 3, upon dietary supplementation of betaine to swine exposed to heat stress. There were no significant differences in among the Monocyte (%), Eosinophil (%), and Basophil (%) in treatment group and that of control throughout the study period.

3.2 Biochemical Parameters

The plasma glucose levels in the betaine supplemented lactating cows did not differ significantly compared to that of control at 2nd, 4th,6th and 8th week of study period (Table 3). The results are in agreement with the studies of El-Shinnawy (2015) [6] in broiler chicken who reported no significant effect of betaine supplementation on plasma glucose levels. In betaine supplemented group, along the study period, plasma total protein levels were significantly (p<0.05) higher at 2nd, 4th, 6th and 8th week of study period compared to 0 day. The results obtained in the present study are in agreement with the studies of Hassan *et al*. (2011) [7] in rabbits, Chudak (2020) [2] in growing piglets and El-Shinnawy (2015) [6] in broiler chicken, who reported dietary supplementation of betaine significantly, increased plasma total protein levels compared to those of control cows. Betaine is an osmolyte in intestinal cells and probably affects nutrient digestibility and partitioning; increasing the availability of methionine has been invoked to explain increased protein production (Lever and Slow, 2010) [10]. The positive effect of betaine on serum total protein also suggests the improvement in humoral and cellular immunity. The increase in serum total protein due to betaine supplementation could be associated with its ability as a methyl group donor which is fairly consistent in protein metabolism (Kidd *et al*., 1997) [8]. The BUN, plasma triglycerides and plasma cholesterol levels were significantly lower at 4th, 6th and 8th week of study period compared to 0 day. The results obtained in the present study are in agreement with the studies of Zhang (2014) [20] in lactating cows, who reported dietary supplementation of betaine declined BUN, triglycerides and plasma cholesterol levels compared to those of control cows. However, our results are in disagreement with the studies of Chudak (2020) [2] who reported increased plasma urea levels in growing piglets supplemented with betaine in diet. The decrease in plasma triglycerides in betaine supplemented cows might be attributed by the fact that betaine decreases fatty acid synthesis by decreasing the expression of the enzymes involved in lipogenesis.

3.3 Plasma Electrolytes

Betaine being an osmolyte has the capacity to influence the properties of biological fluids. Their primary role is to maintain the integrity of cells by affecting the viscosity, melting point, and ionic strength of the aqueous solution. Betaine as a major osmolyte is accumulated in most tissues to assist cell volume regulation (Schliess and Häussinger, 2002) [14]. The plasma sodium and plasma potassium levels in the

betaine supplemented lactating Deoni cows were numerically higher whereas the plasma chloride levels were numerically lower but did not differ significantly compared to that of control during the entire study period (Table 4). However, Park and Kim (2017) [12] reported that in meat-type ducks the concentration of Na^+, K^+ and Cl^- levels in the betaine supplemented groups were significantly higher than the control group during heat stress.

Table 1: Erythrogram in lactating Deoni cows (n=6) supplemented with betaine

Parameter	Week	Control	Treatment
Total Erythrocyte Count (x 10^6/µl)	0 day	6.78 ± 0.21	6.77 ± 0.28
	2nd week	6.55 ± 0.32	7.15 ± 0.35
	4th week	6.85 ± 0.31	7.53 ± 0.31
	6th week	6.90 ± 0.30	7.22 ± 0.32
	8th week	6.82 ± 0.21	7.01 ± 0.25
Haemoglobin (g/dl)	0 day	9.37 ± 0.17	9.40 ± 0.22
	2nd week	9.20 ± 0.27	9.72 ± 0.19
	4th week	9.25 ± 0.34	9.92 ± 0.30
	6th week	9.53 ± 0.15	9.95 ± 0.28
	8th week	9.45 ± 0.18	9.65 ± 0.26
Packed Cell Volume (%)	0 day	33.93 ± 0.70	33.73 ± 1.10
	2nd week	33.73 ± 1.10	34.77 ± 0.86
	4th week	33.43 ± 0.53	34.73 ± 1.02
	6th week	34.03 ± 1.07	34.68 ± 0.84
	8th week	33.28 ± 1.22	34.73 ± 1.49

Table 2: Leucogram in lactating Deoni cows (n=6) supplemented with betaine

Parameter	Week	Control	Treatment
Total Leukocyte Count (x 10^3/µl)	0 day	8.00 ± 0.41	7.72 ± 0.55
	2nd week	8.53 ± 0.57	7.60 ± 0.52
	4th week	8.48 ± 0.45	7.87 ± 0.65
	6th week	9.20 ± 0.70	7.87 ± 0.65
	8th week	8.00 ± 0.33	7.05 ± 0.29
Lymphocyte (%)	0 day	53.47 ± 1.87 ^{x}	53.50 ± 1.30 ^{x}
	2nd week	52.30 ± 0.79 ^{x}	56.83 ± 1.43 ^{x}
	4th week	50.37 ± 1.38 ^{x}	56.18 ± 0.81 ^{y}
	6th week	50.73 ± 0.94 ^{x}	56.17 ± 1.19 ^{y}
	8th week	52.33 ± 0.84 ^{x}	58.17 ± 1.30 ^{y}
Neutrophil (%)	0 day	34.50 ± 1.03 ^{x}	33.67 ± 0.84 ^{x}
	2nd week	35.00 ± 1.23 ^{x}	32.67 ± 1.12 ^{x}
	4th week	35.67 ± 2.27 ^{y}	30.50 ± 0.62 ^{x}
	6th week	33.33 ± 1.09 ^{y}	28.67 ± 0.88 ^{x}
	8th week	34.00 ± 1.16 ^{y}	29.17 ± 1.20 ^{x}
Means with different superscripts within a row (x, y) for a particular parameter differ significantly (p<0.05).			

Table 3: Mean Plasma Biochemical parameters in lactating Deoni cows (n=6) supplemented with betaine

Parameter	Week	Control	Treatment
Glucose (mg/dL)	0 day	55.63 ± 1.23	56.22 ± 1.72
	2nd week	55.60 ± 1.23	52.88 ± 1.87
	4th week	55.62 ± 1.00	51.70 ± 2.06
	6th week	57.12 ± 1.85	54.33 ± 1.19
	8th week	57.23 ± 1.25	56.25 ± 1.10
Total protein (g/dL)	0 day	7.10±0.19 $^{x\,a}$	7.07±0.08 $^{x\,a}$
	2nd week	7.08±0.24 $^{x\,a}$	7.77±0.12 $^{y\,b}$
	4th week	7.10±0.23 $^{x\,a}$	7.80±0.16 $^{y\,b}$
	6th week	7.05±0.18 $^{x\,a}$	7.75±0.12 $^{y\,b}$
	8th week	6.98±0.13 $^{x\,a}$	7.68±0.14 $^{y\,b}$
Urea nitrogen (mg/dL)	0 day	15.14 ± 1.07$^{x\,a}$	15.01 ± 1.29$^{x\,b}$
	2nd week	15.36 ± 1.00$^{x\,a}$	13.51 ± 0.70$^{x\,ab}$
	4th week	16.51 ± 1.18$^{y\,a}$	11.57 ± 0.76$^{x\,a}$
	6th week	15.90 ± 0.67$^{y\,a}$	11.26 ± 0.43$^{x\,a}$
	8th week	16.05 ± 1.13$^{y\,a}$	11.26 ± 0.74$^{x\,a}$
Cholesterol (mg/dL)	0 day	113.17 ± 5.65 $^{x\,a}$	112.50 ± 5.54 $^{x\,b}$
	2nd week	108.83 ± 3.93 $^{x\,a}$	102.17 ± 2.66 $^{x\,ab}$
	4th week	116.50 ± 3.78 $^{y\,a}$	95.67 ± 3.63 $^{x\,a}$
	6th week	116.67 ± 5.11 $^{y\,a}$	93.67 ± 2.75 $^{x\,a}$
	8th week	111.67 ± 3.02 $^{y\,a}$	94.33 ± 3.37 $^{x\,a}$
Triglycerides	0 day	28.17 ± 1.62 $^{x\,a}$	28.50 ± 1.82 $^{x\,b}$

(mg/dL)	2nd week	29.00 ± 2.65 x a	25.17 ± 1.14 x ab
	4th week	29.83 ± 1.19 y a	23.00 ± 1.18 x ab
	6th week	30.00 ± 1.29 y a	23.67 ± 0.95 x ab
	8th week	29.00 ± 2.13 y a	22.67 ± 1.54 x a
Means with different superscripts within a row(x,y) and within a column (a,b) for a particular parameter differ significantly (p<0.05).			

Table 4: Mean Plasma electrolyte levels in lactating Deoni cows (n=6) supplemented with betaine

Parameter	Week	Control	Treatment
Sodium (mmol/L)	0 day	142.09 ± 2.16	144.42 ± 2.95
	2nd week	141.14 ± 2.63	148.25 ± 2.37
	4th week	140.22 ± 3.01	148.13 ± 3.45
	6th week	143.17 ± 3.09	148.39 ± 3.60
	8th week	140.26 ± 5.11	147.53 ± 2.77
Potassium (mmol/L)	0 day	4.67 ± 0.16	4.96 ± 0.12
	2nd week	4.95 ± 0.09	5.33 ± 0.12
	4th week	4.97 ± 0.19	5.51 ± 0.18
	6th week	4.79 ± 0.15	5.28 ± 0.26
	8th week	4.61 ± 0.18	5.32 ± 0.40
Chloride (mmol/L)	0 day	101.00 ± 3.26	102.85 ± 2.84
	2nd week	103.67 ± 3.47	99.30 ± 6.13
	4th week	102.83 ± 3.63	100.73 ± 3.54
	6th week	103.90 ± 2.90	98.00 ± 2.53
	8th week	102.40 ± 73.62	98.33 9.28

4. Conclusions

Betaine supplementation did not alter the major hematological parameters in lactating Deoni cows except for increasing the percentage of lymphocytes and decreasing the percentage of neutrophils. Betaine supplementation significantly (p<0.05) decreased the concentration of cholesterol, triglycerides and BUN and increased the concentration of total proteins. There was no effect of betaine supplementation on plasma glucose concentration in lactating Deoni cows. Betaine supplementation did not alter the levels of plasma electrolytes in lactating Deoni cows.

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