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Blood profile of early lactating Murrah buffaloes supplemented with bypass fatty acids and *Tinospora cordifolia*

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Abstract
The study was conducted with the objective of evaluating the effect of bypass fatty acid and *Tinospora cordifolia* supplementation on blood profile of early lactating Murrah buffaloes. The lactating buffaloes were distributed into four groups, each having five buffaloes depending on previous milk yield, parity and body weight. T1, T2 and T3 group buffaloes were supplemented for 90 days with 150g bypass fatty acids, 150g Tinospora stem powder and a combination of both 150g bypass fatty acids and 150g Tinospora stem powder respectively while the control group (T0) given only standard feeding without any supplementations. Blood sampling was done at fortnightly intervals and results showed that among hematological parameters TLC, neutrophil count, N:L and platelet counts were significantly higher in Tinospora supplemented groups (T2 and T3) whereas values of RBC, Hb, PCV, lymphocytes and mix cells were not affected significantly. The blood indices were also not changed significantly on supplementation. From results, it was concluded that change in blood profiling was there due to the immunomodulatory effect if *Tinospora cordifolia*.

Keywords: Buffaloes, blood, bypass fatty acid, supplementation, tinospora

Introduction
The success of dairy farming depends on the production and health of animal during lactation. Transition period is most crucial from production and health point of view. It begins three weeks before parturition and remains up to three weeks after parturition. During this period, severe negative energy balance and low serum or plasma concentrations of several minerals and vitamins are experienced by animal which is an indicative of lowered health status [1]. During the early stage of lactation, the health and immune system of animals gets compromised [2]. The main reason for this is negative energy balance (NEB), which is due to the difference between the energy requirements and availability [3]. The maintenance and production requirements of high producing animals are not fulfilled with the regular dietary sources; therefore, extra energy supplementation is required to fulfil the maintenance and production demand of the lactating animals. Supplementation of bypass fat ensures the availability of high energy [3-6], whereas, *Tinospora cordifolia* can serve as immunity booster for animals [7, 8].
Blood profiles of animals are used to evaluate the health status and ongoing changes inside the animal's body [9]. Analysis of specific blood metabolites and their values allows the determination of accuracy of different metabolic pathways associated with energy, protein, and minerals and showed the true picture of animal health and this can be utilized to optimize the production and health of dairy animals [10, 11]. Considering this the study was conducted to analyze the blood profiles of lactating Murrah buffaloes on supplementation with bypass fatty acid and *Tinospora cordifolia*.

Material and methods
Description of the study area
The following experiment was conducted in the Livestock Research Centre of National Dairy Research Institute (N.D.R.I.), Karnal, Haryana, India which is located 29°42**"** North and 79°54**"** East longitudes at an altitude of 245 meters above the mean sea level in the beds of Indo-Gangetic alluvial plain. The area has a minimum temperature that falls near to freezing point in winter and goes maximum to 45 ºC in months of May/June in summer.

The annual rainfall received is close to 700 mm, most of which is received from July to September. The prevailing climate of the region is subtropical.

Selection of animals and design of experiment

The study was designed for twenty freshly calved Murrah buffaloes that were selected from Livestock Research Center of NDRI, Karnal and were further grouped on the basis of previous milk yield, parity, and body weight in four groups with each group compromising of five buffaloes. Animals with any anatomical, physiological and infectious disorders were debarred from getting included for the experimental trial. It was ensured that the planned experiment was conducted in accordance to the guidelines issued by the institutional ethical committee for animal welfare. After the sought for final approval from the animal welfare committee the animals were assigned their groups where T0 was taken as control and was given standard feed (ICAR-2013 standards) without any supplementation whereas in others groups T1, T2 and T3 different supplements were provided. In group T1 150gm of bypass fatty acids was provided per animal per day; in group T2 150gm of Tinospora along with 150gm bypass fatty acids was provided per animal per day and in group T3150gm Tinospora was supplemented per animal per day, respectively. The animals were housed in loose housing system meeting all their daily requirements as provided by Bureau of Indian Standard (BIS) and throughtout the trial were tied for 1 hour in a day for individual feeding of listed supplements. The period of supplementation for each buffalo was 90 days after calving with each buffalo being kept under observation throughout the trial. The daily feeds that were fed to the buffaloes included green fodders like oats, maize, jowar, sugar graze and berseem depending on their availability and mixture of maize silage and wheat straw with provision of clean and fresh drinking water.

Collection of blood

Blood samples were drawn from individual buffalo in sterile heparinized vacutainer tubes from jugular vein posing minimum disturbances to the animal. Blood samples were collected on day 15th, 30th, 45th, 60th, 75th, 90th and 105th postpartum. Samples were taken to the laboratory in chilled iceboxes soon after collection and small amount was taken in eppendorf for hematological profiling.

Hematological profile

An aliquot of blood was taken at the earliest after collection and analyzed for blood parameters viz. TLC, RBC, DLC, Hb, PCV, MCV, MCH, MCHC, RDW, MPV, PDW and platelet counts by using BC- 2800 auto hematological blood analyzer (Mindray). N:L ratio was also calculated using neutrophils and lymphocyte count.

Statistical Analysis

Data was analysed using the statistical software IBM(r) SPSS(r) Statistics (version 22, IBM SPSS Inc., Chicago, USA) by applying one way ANOVA. The pairwise comparison between treatments was performed using duncan test with a significance level of 95% (α<0.05).

Results

Hematological parameters

Analysis of blood samples at fortnightly interval revealed that there is no significant (P>0.05) effect of supplementation on RBC (red blood cell) count, hemoglobin and PCV (packed cell volume). The values did not differ significantly between different groups during and after supplementation (Table 1). However, a significant (P<0.05) change was observed in TLC (total leuckocyte count) values of different groups. Mean TLC values were on the higher side in T2 (Tinospora) and T3 (Mix) group in comparison to T0 (control) and T1 (Fatty acids) presented graphically in Figure 1. But when the supplementations were withdrawl after 90 days and analysis of blood was done on day 105 no significant (P>0.05) difference was found between groups. Among leukocytes the value of neutrophils was found significantly (P<0.05) increased in T2 (Tinospora) and T3 (mix) as compared to T0 (control) and T1 (fatty acids) (Figure 2) whereas the values of lymphocytes and mix cells (monocytes, eosinophils and basophils) were not affected significantly (P>0.05) by supplementations. The platelet count was also significantly (P<0.05) higher in T2 (Tinospora) and T3 (mix) group then T0 (control) and T1 (fatty acids) groups (Figure 3). The values of different haematological parameters were presented in Table 1.

Table 1: Effect of bypass fatty acid and *Tinospora* supplementation on Hematological parameters

Parameters	Control (T0)	Fatty Acids (T1)	Tinospora (T2)	Mix (T3)	P value
During supplementation (Day 1-90)					
RBC (M/mm^3)	6.87 ± 0.13	7.05 ± 0.21	7.01 ± 0.37	6.97 ± 0.38	0.978
Hb (g/dl)	12.52 ± 0.15	12.62 ± 0.48	12.47 ± 0.37	12.52 ± 0.53	0.994
PCV (%)	37.73 ± 0.70	38.56 ± 1.76	37.54 ± 1.04	40.54 ± 2.28	0.529
TLC (M/mm^3)	11.46^{ax} ± 0.25	12.35^{abx} ± 0.48	14.09^{bcxy} ± 0.60	15.02^{cxy} ± 1.10	0.012
Lymphocyte (M/mm^3)	6.97 ± 0.26	7.72 ± 0.50	7.78 ± 0.29	7.96 ± 0.45	0.313
Neutrophil (M/mm^3)	3.94^{ayz} ± 0.08	4.06^{ay} ± 0.19	5.60^{bzu} ± 0.27	6.27^{byzu} ± 0.51	0.000
Mix cell (Monocytes, Eosinophils and Basophils) (M/mm^3)	0.55 ± 0.05	0.57 ± 0.06	0.67 ± 0.04	0.70 ± 0.08	0.113
Platelet count (M/mm^3)	162.20^{ax} ± 7.39	171.54^{ax} ± 16.44	219.20^{by} ± 8.39	233.00^{by} ± 16.29	0.005
When supplementation was withdrawal (Day 105)					
RBC (M/mm^3)	7.59 ± 0.15	7.31 ± 0.15	7.13 ± 0.46	7.51 ± 0.30	0.691
Hb (g/dl)	12.92 ± 0.51	13.52 ± 0.40	13.48 ± 0.41	12.80 ± 0.25	0.480
PCV (%)	37.10 ± 0.65	38.00 ± 1.65	38.26 ± 2.25	38.48 ± 1.63	0.937
TLC (M/mm^3)	11.02^{ax} ± 0.15	12.55^{ax} ± 1.60	11.34^{ax} ± 0.78	10.8^{ax} ± 0.97	0.639
Lymphocyte (M/mm^3)	7.52 ± 0.14	8.53 ± 0.90	7.67 ± 0.48	7.54 ± 0.66	0.608
Neutrophil (M/mm^3)	2.94^{ax} ± 0.12	3.38^{axy} ± 0.44	3.15^{ax} ± 0.22	2.75^{ax} ± 0.19	0.408
Mix cell (Monocytes, Eosinophils and Basophils) (M/mm^3)	0.56 ± 0.06	0.63 ± 0.04	0.52 ± 0.06	0.52 ± 0.04	0.416
Platelet count (M/mm^3)	141.75^{ax} ± 18.90	142.50^{ax} ± 16.86	160.75^{ax} ± 3.64	153.50^{ax} ± 16.50	0.782

- The values are Mean ± SE of observations on five animals in each group.
- Values with different superscripts a,b,c and x,y differs significantly (P<0.05) in a row and column(between same parameter), respectively.

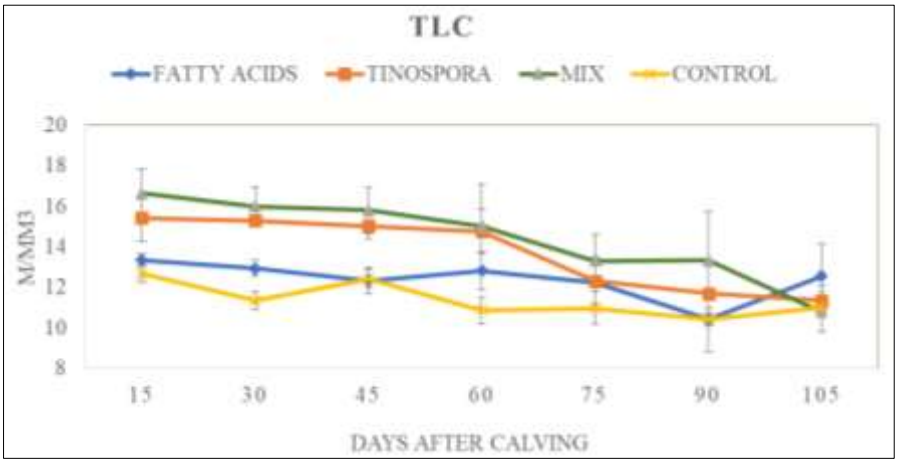

Fig 1: Mean TLC (M/mm^3) during different fortnights of experimental period in buffaloes

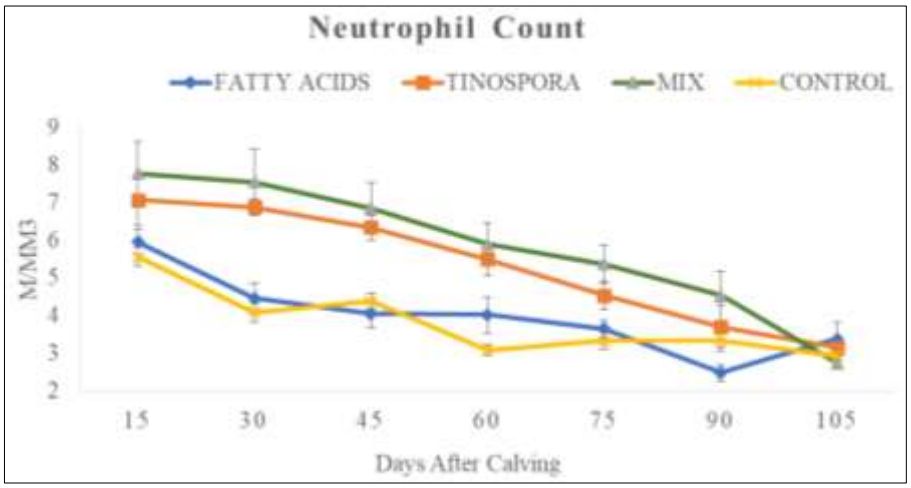

Fig 2: Mean neutrophil count (M/mm^3) during different fortnights of experimental period in buffaloes

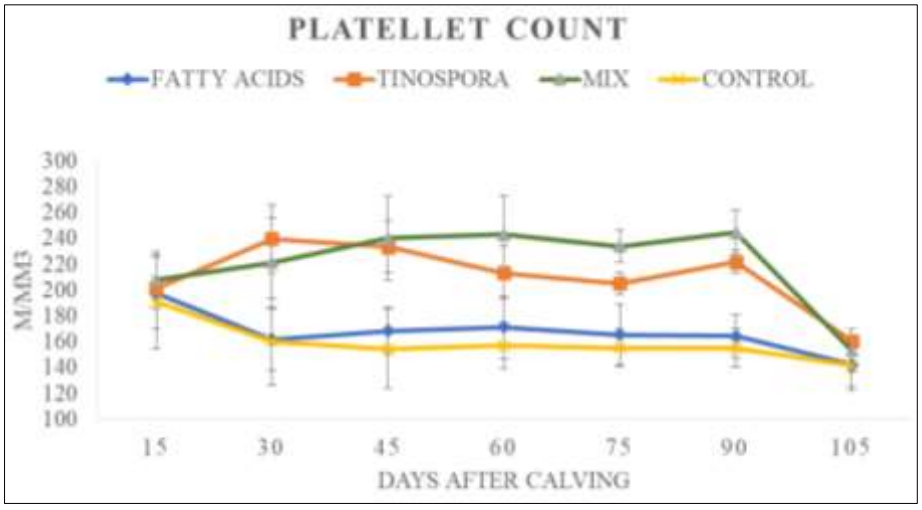

Fig 3: Mean platelet count (M/mm^3) during different fortnights of experimental period in buffaloes

Neutrophil lymphocyte ratio (N:L)

The mean N:L at fortnightly intervals for T0 (control), T1 (fatty acid), T2 (Tinospora) and T3 (mix) groups during experimental period are shown in Table 2. A significant difference was found in N:L between groups (P<0.05) and between days (P<0.01) throughout the experimental period. Highest N:L was observed in the beginning of experiment i.e. after parturition in all the groups and it goes on decreasing with the advancement in lactation. However, overall N:L ratio was significantly higher in T2 (Tinospora) and T3 (mix) as compared to T0 (control) and T1 (fatty acids). After the withdrawl of supplements there was no significant (P>0.05) difference observed between different groups.

Table 2: Effect of bypass fatty acid and *Tinospora* supplementation on N:L (neutrophil lymphocytic ratio)

Days	Control (T0)	Fatty Acids (T1)	Tinospora (T2)	Mix (T3)	P Value
15	$0.84^{au} \pm 0.06$	$0.89^{az} \pm 0.09$	$0.91^{av} \pm 0.07$	$1.00^{au} \pm 0.14$	0.709
30	$0.61^{az} \pm 0.06$	$0.59^{ay} \pm 0.11$	$0.90^{buv} \pm 0.06$	$1.00^{bu} \pm 0.13$	0.014
45	$0.59^{az} \pm 0.03$	$0.55^{axy} \pm 0.08$	$0.81^{bzuv} \pm 0.05$	$0.85^{bzu} \pm 0.07$	0.007
60	$0.44^{axy} \pm 0.04$	$0.51^{abxy} \pm 0.08$	$0.67^{bcyz} \pm 0.09$	$0.73^{cyz} \pm 0.05$	0.026
75	$0.49^{axyz} \pm 0.06$	$0.48^{axy} \pm 0.05$	$0.66^{byz} \pm 0.07$	$0.75^{byzu} \pm 0.05$	0.008
90	$0.53^{bxyz} \pm 0.06$	$0.35^{ax} \pm 0.03$	$0.50^{bxy} \pm 0.05$	$0.56^{bxy} \pm 0.04$	0.023
105	$0.39^{ax} \pm 0.02$	$0.40^{axy} \pm 0.04$	$0.41^{ax} \pm 0.03$	$0.37^{ax} \pm 0.03$	0.830
Mean ± SE	$0.57^{ayz} \pm 0.03$	$0.54^{axy} \pm 0.06$	$0.72^{bzu} \pm 0.03$	$0.79^{byzu} \pm 0.05$	0.004
P Value	0.000	0.001	0.000	0.000	

- The values are Mean ± SE of observations on five animals in each group.
- Values with different superscripts a,b and u,v,x,y,z differ significantly ($P<0.05$) in a row and column, respectively.

DLC (Differential leuckocyte count)

The DLC values of blood samples showed a significant ($P<0.05$) increase in percentage of neutrophils and significant ($P<0.05$) decrease in lymphocyte percent in T2 (Tinospora) and T3 (mix) group in comparison to T0 (control) and T1 (fatty acids). The values of DLC were presented in Table 3.

Table 3: Effect of bypass fatty acid and *Tinospora* supplementation on DLC (Differential leuckocyte count)

Parameters	Control (T0)	Fatty Acids (T1)	Tinospora (T2)	Mix (T3)	P value
Lymphocyte %	$60.75^{a} \pm 1.27$	$62.22^{a} \pm 2.29$	$55.23^{b} \pm 0.87$	$53.13^{b} \pm 1.81$	.006
Neutrophil %	$34.43^{a} \pm 1.28$	$33.14^{a} \pm 2.38$	$39.68^{b} \pm 1.03$	$41.67^{b} \pm 1.67$	.003
Mix cell (Monocytes, Eosinophils and Basophils) %	6.02 ± 0.33	4.91 ± 0.62	5.70 ± 0.83	5.47 ± 0.69	.840

- The values are Mean ± SE of observations on five animals in each group.
- Values with different superscripts a,b differs significantly ($P<0.05$) in a row.

Blood indices

Different blood indices like MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), RDW (red cell distribution width), MPV (mean platelet volume) and PDW (platelet distribution width) were measured along with hematological parameters at fortnightly intervals and the mean values were presented in Table 4. Most of the blood indices were not affected by the supplementations and the values did not vary significantly ($P<0.05$) between different groups. The value of MPV was found significantly increased in T2 (Tinospora) and T3 (mix) groups in comparison to T0 (control) and T1 (fatty acids).

Table 4: Effect of bypass fatty acid and *Tinospora* supplementation on blood indices

Parameters	Control (T0)	Fatty Acids (T1)	Tinospora (T2)	Mix (T3)	P value
MCV (fl)	51.44 ± 0.34	52.71 ± 1.14	53.98 ± 1.12	51.81 ± 1.17	0.319
MCH (pg)	17.71 ± 0.28	17.50 ± 0.37	18.52 ± 0.32	18.31 ± 0.74	0.390
MCHC (g/dl)	33.75 ± 0.40	33.66 ± 0.35	32.92 ±0.37	33.79 ± 0.84	0.621
RDW	13.01 ± 0.07	13.12 ± 0.26	12.83 ± 0.15	13.12 ± 0.46	0.866
MPV (fl)	$6.86^{a} \pm 0.06$	$6.91^{a} \pm 0.10$	$7.12^{ab} \pm 0.08$	$7.23^{b} \pm 0.11$	0.030
PDW	7.78 ± 0.26	7.68 ± 0.22	7.02 ± 0.16	7.30 ± 0.31	0.139

The values are Mean ± SE of observations on five animals in each group.

Discussion

Hematological parameters, Neutrophil lymphocyte ratio (N:L) and DLC (Differential leuckocyte count)

Any effect on the health of individual can be depicted by the change in blood profile. In the present study results showed a significant increase in TLC of T2 (Tinospora) and T3 (mix) group over T0 (control) and T1 (fatty acids group). In both of these groups i.e. T2 and T3 supplementation of Tinospora was done. On DLC, the values of neutrophil count were found significantly elevated in Tinospora supplemented groups (T2 and T3). Numerical change was also found in the values of lymphocyte count but the difference is non-significant between different groups. Similarly, non-significant difference was found in the values of mixed cells. Due to increase in values of neutrophil count the N:L ratio of the Tinospora supplemented groups were also raised and this increased N:L is a sign of enhanced immune response. These change in the blood profiling of different groups may be due to the immunomodulatory [12, 13] and antioxidant [14, 15] properties of *Tinospora cordifolia*. Other parameters like RBC, Hb and PCV were not affected by the supplementations. Similar results were reported by Mallick and Prakash [16], who showed a significant increase in TLC, neutrophil count, lymphocytic count and N:L on supplementation of *Tinospora cordifolia* in Karan fries cows. Savsani [17] also reported no change in white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb) and pack cell volume (PCV) on supplementation of bypass fat in Jaffrabadi buffaloes. Results of the present investigation showed a significant increase in platelet counts in Tinospora supplemented groups (T2 and T3). Till now, no study reported effect of Tinospora supplementation on platelet counts in bovines but the results of our study were supported by the increase in platelet count on *Tinospora cordifolia* supplementation in rabbits [18].

Blood indices

Blood indices show the size and volume of different blood cells. In the present study almost all the blood indices were not affected by supplementation. This showed that buffaloes

are in healthy condition and they are free from disease conditions. Significant difference was found in the values of MPV in Tinospora supplemented groups (T2 and T3) over control (T0) and fatty acid (T1) supplemented groups and it may be due to the increased production of platelets in the bone marrow. No reports of blood indices on supplementation of Tinospora in bovines were found but our results were supported by the similar reports of no change in MCH, MCV, MCHC in mice on giving Tinospora [19].

Conclusions

Blood profiling of early lactating Murrah buffaloes showed that supplementation of bypass fatty acid and Tinospora cordifolia elicit immune response which leads to change in TLC, neutrophil count, N:L and platelet counts without any alteration of RBC, Hb, PCV, lymphocytes and mix cells and blood indices.

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