RESEARCH ARTICLE

# Modulatory Effects of *Tinospora cordifolia* Supplementation on Haematological and Biochemical Indices in Periparturient Crossbred Cows

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## Abstract

The present study evaluated the effects of dietary supplementation with *Tinospora cordifolia* stem powder on the haematological and biochemical indices of periparturient crossbred cows. Twelve clinically healthy advanced pregnant crossbred (HF × K) cows were randomly allocated into two groups (n=6 each). The control group received the routine farm ration, whereas the treatment group received *Tinospora cordifolia* stem powder at 6% of the total concentrate mixture in addition to the routine farm ration, from 30 days prepartum to 30 days postpartum. Blood samples were collected at predetermined intervals on days -30, -20, -10, -5 prepartum, on the day of calving (day 0) and days +5, +10, +20 and +30 postpartum for haematological and biochemical analyses. In the treatment group, supplementation significantly ($p<0.05$) improved erythrocytic indices, including total erythrocyte count, haemoglobin concentration and packed cell volume and enhanced leukocyte responses, particularly total leukocyte count and lymphocyte percentage, indicating improved immune competence. Biochemically, treatment group exhibited significantly ($p<0.05$) lower blood glucose and non-esterified fatty acid concentrations, suggesting improved metabolic adaptation and energy balance. Furthermore, serum total protein and albumin concentrations were significantly ($p<0.05$) higher in the treatment group, whereas globulin concentration remained comparable between two groups. These findings demonstrate that dietary *Tinospora cordifolia* supplementation may promote haematological stability, metabolic homeostasis and immune function, highlighting its potential as a natural phytogenic feed supplement for improving the health and physiological status of periparturient dairy cows.

**Key words:** Biochemical indices, Crossbred cows, Haematological indices, Periparturient period, *Tinospora cordifolia*.

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## Introduction

The peripartum period, encompassing approximately three weeks before and three weeks after calving, is recognized as one of the most physiologically demanding and metabolically challenging phases in the productive life of dairy animals (Praveen and Dharani, 2018). The peripartum period is characterized by a pronounced negative energy balance (NEB), primarily resulting from a marked increase in nutrient and energy demands associated with the onset of lactation, coupled with a transient decline in dry matter intake. Consequently, this metabolic imbalance adversely affects the overall physiological status of dairy animals (Goff, 2006). During this critical phase, animals commonly experience compromised health, impaired immune competence and increased susceptibility to metabolic and infectious disorders, which collectively may reduce productive and reproductive performance (Sordillo, 2016). In high-producing dairy animals, the energy and nutrient supply derived from conventional feeding practices is frequently inadequate to meet the substantial demands of maintenance, fetal growth and subsequent milk synthesis. Therefore, strategic nutritional supplementation becomes essential to alleviate metabolic stress, maintain physiological homeostasis and support optimal health and lactational performance during the peripartum period (Mudgal *et al*., 2012).

In recent years, there has been an increasing interest in the use of plant-derived feed supplements as natural alternatives for improving livestock health and productivity owing to their diverse bioactive constituents and pharmacological properties (Wang *et al*., 2024). Among

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the numerous medicinal plants indigenous to India, *Tinospora cordifolia* (Giloy) has attracted considerable attention because of its broad spectrum of therapeutic and adaptogenic activities. The plant is a rich source of biologically active compounds, including alkaloids, diterpenoid lactones, glycosides, steroids and other phytochemicals, which collectively contribute to its potent immunomodulatory and disease-resistance properties (Mittal *et al*., 2014). Despite its well-documented therapeutic efficacy in human medicine and encouraging findings from poultry studies, scientific evidence regarding its application in dairy animals remains scarce. Moreover, information on its potential to mitigate the physiological and metabolic challenges encountered during the peripartum period is still limited, highlighting the need for further investigation (Saeed *et al*., 2020).

To elucidate the physiological effects of such nutritional interventions, assessment of haematological and biochemical biomarkers offers a reliable approach for evaluating the health and metabolic status of dairy animals during the peripartum period (Payne *et al*., 1970). Evaluation of specific blood metabolites further provides valuable insights into the functional status of metabolic pathways associated with energy and protein metabolism (Kida, 2003). Collectively, these biomarkers facilitate the assessment of nutritional status, early detection of metabolic disturbances and optimization of health, productivity and overall herd performance in dairy animals (Macrae *et al*., 2006). *Tinospora cordifolia* is a well-recognized medicinal plant possessing hematopoietic, immunomodulatory and metabolic regulatory properties. It has been reported to support normal erythropoiesis, improve haemoglobin concentration and erythrocyte integrity and modulate leukocyte populations by enhancing the functional activity of neutrophils and lymphocytes (Ghatpande *et al*., 2019). Further, its bioactive constituents stimulate cellular immune responses by promoting phagocytosis, cytokine regulation and lymphocyte proliferation. These effects contribute to improved innate and adaptive immunity, particularly during the periparturient period (Thatte *et al*., 1994). In addition, its bioactive constituents may influence carbohydrate, protein and lipid metabolism, thereby contributing to improved glucose homeostasis, protein status and energy balance (Upadhyay *et al*., 2010). Therefore, the present study was undertaken to assess the effects of dietary supplementation with *Tinospora cordifolia* on the haematological and biochemical indices of periparturient crossbred cows.

## Materials and Methods

### Experimental Animals and Management

A total of 12 clinically healthy advanced pregnant crossbred (HF × K) cows maintained at the Livestock Research Station, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India, were selected for the study. Animals were selected based on their clinical health status and stage of pregnancy and were randomly allocated into two experimental groups, each comprising six animals (n=6). Cows in control group received the routine farm ration formulated according to the standard feeding practices of the farm. Cows in treatment group received the same routine farm ration supplemented with *Tinospora cordifolia* stem powder at 6% of the total concentrate mixture. Dietary supplementation was initiated 30 days before the expected calving date and continued up to 30 days postpartum, thereby encompassing the entire periparturient period. Throughout the experimental period, all animals were maintained under identical housing, feeding and management conditions to minimize the influence of environmental and managerial variations.

### Blood Collection and Sample Processing

Whole blood samples were collected aseptically from each experimental animal at predetermined intervals on days −30, −20, −10 and −5 before calving, on the day of calving (day 0) and on days +5, +10, +20 and +30 postpartum. The prepartum sampling schedule was determined based on the expected calving date calculated from the date of service, which varied ±5 days from expected date of calving. Approximately 2 mL of blood was collected from the jugular vein into EDTA-coated vacutainers for haematological analysis, while an additional 4 mL was collected into clot activator vacutainers for serum separation. The blood samples were allowed to clot at room temperature and were subsequently centrifuged at 3,000 rpm for 15 min to obtain serum. The separated serum was aliquoted into sterile microcentrifuge tubes and stored at −20°C until biochemical and ELISA analyses.

Haematological parameters, including total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), granulocyte percentage and lymphocyte percentage, were determined using an automated veterinary haematology analyser (BC-2800 Vet, Mindray) following the manufacturer's instructions and standard laboratory procedures. Serum biochemical analyses were performed to evaluate key indicators of energy metabolism, protein metabolism and lipid metabolism, including glucose, total protein, albumin, globulin and non-esterified fatty acids. All biochemical parameters, except non-esterified fatty acids, were estimated using commercially available diagnostic reagent

kits according to the manufacturers' protocols and analysed using an automated serum analyser (AutoQuant 200*i* VET, Meril). In addition, serum non-esterified fatty acid (NEFA) concentration was determined using a commercially available Puregene™ enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

### Statistical Analysis

The experimental data were analysed using statistical software. Between group comparisons at each sampling interval were performed using the independent samples *t*-test, while changes across different sampling periods within each experimental group were analysed by one-way analysis of variance. All statistical analyses were performed according to the procedures described by Snedecor and Cochran (2014).

## Results and Discussion

### Haematological Findings

The values of total erythrocyte count, haemoglobin concentration and packed cell volume exhibited a progressive increase from the prepartum period, reached at peak levels on the day of calving, followed by a gradual decline during the postpartum period up to day 30 after calving in both groups. The total erythrocyte count, haemoglobin concentration and packed cell volume values exhibited a highly significant ($p<0.01$) difference between the two groups throughout the experimental period, with consistently higher values recorded in treatment group compared with control group. The observed enhancement in total erythrocyte count, haemoglobin concentration and packed cell volume in treatment group in the present study may be attributed to the haematopoietic properties of *Tinospora cordifolia*, which might have supported erythropoiesis and improved overall haematological status during the experimental period. The findings of the present study were in agreement with Gupta *et al*. (2026), who reported a non-significant but numerically higher total erythrocyte count in *Tinospora cordifolia*-supplemented non-descript cattle. Similarly, the present findings corroborated the observations of Mallick and Prakash (2011), who demonstrated a non-significant but numerically higher haemoglobin concentration following *Tinospora cordifolia* supplementation in crossbred cows. Furthermore, the present findings agreed with Rajneesh *et al*. (2020), who reported higher PCV values in *Tinospora cordifolia* plus fatty acid-supplemented Murrah buffaloes than in controls (Table 1).

The total leukocyte count and granulocyte percentage exhibited a similar temporal pattern in both experimental groups. Both parameters increased progressively during the prepartum period, attained peak values on the day of calving and subsequently declined gradually throughout the postpartum period up to day 30 after calving. Between group comparison revealed a highly significant ($p<0.01$) variation in total leukocyte count on days −30, +10 and +20, whereas significant ($p<0.05$) differences were observed on days −5 and +30. The granulocyte percentage exhibited a significant ($p<0.05$) difference between groups only at calving, while comparable values were recorded throughout the remaining sampling intervals. The elevated total leukocyte count and granulocyte percentage observed in treatment group may be attributed to immunostimulatory properties of *Tinospora cordifolia*, which could enhance leukopoiesis and improve immune preparedness during the periparturient period. The findings of the present investigation were in close agreement with Rajneesh *et al*. (2020), who reported a significant ($p<0.05$) increase in total leukocyte count following *Tinospora cordifolia* supplementation in early lactating Murrah buffaloes. Additionally, the present findings partially agreed with Baghbadorani *et al*. (2022), who reported non-significant variations but numerically higher granulocyte counts in Immunofin®-supplemented postpartum Holstein cows than in controls.

Lymphocyte percentage exhibited distinct temporal patterns in the two experimental groups. In control group, lymphocyte percentage increased slightly during the prepartum period, declined markedly on the day of calving and subsequently increased progressively throughout the postpartum period up to day 30 after calving. In contrast, treatment group maintained relatively stable lymphocyte percentages throughout the experimental period. Lymphocyte percentage showed a highly significant ($p<0.01$) intergroup difference at calving, with consistently higher values observed in the treatment group compared with the control group. A significant ($p<0.05$) difference was observed on days −30, −10, −5, +5, +10 and +20, whereas a highly significant ($p<0.01$) difference was observed on the day of calving. No significant intergroup differences were observed on days -20 and +30. The significant differences observed at most sampling intervals in treatment cows may be attributed to the immunomodulatory and antioxidant properties of *Tinospora cordifolia*, which may help maintain physiological homeostasis and mitigate periparturient stress. The present findings concurred with Mallick and Prakash (2011), who documented a significant elevation in lymphocyte counts at selected sampling intervals in *Tinospora cordifolia*-supplemented crossbred cows.

### Blood Biochemical Findings

Blood glucose concentration exhibited distinct temporal patterns in the two experimental groups. In the control group, glucose concentration increased gradually during

**Table 1:** Mean ± SE values of haematological and biochemical indices in periparturient crossbred cows of control and treatment (*Tinospora cordifolia*-supplemented) groups

| Parameters | Group | -30 | -20 | -10 | -5 | 0 | 5 | 10 | 20 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|
| TEC ($10^6$/µL) | Control | 7.31±0.06 | 7.46 ±0.13 | 7.70±0.15 | 7.71±0.12 | 7.74±0.13 | 7.65±0.21 | 7.49±0.07 | 7.48± 0.13 | 7.50± 0.12 |
| | Treatment | 9.08±0.38** | 9.16±0.35** | 9.19±0.32** | 9.28±0.28** | 9.35±0.14** | 9.15±0.26** | 9.12±0.11** | 9.08±0.19** | 9.03± 0.14** |
| Hb (g/dL) | Control | 11.63±0.31 | 11.95±0.23 | 11.98±0.37 | 12.22±0.14 | 12.33±0.21 | 11.92±0.44 | 11.87±0.34 | 11.55±0.36 | 11.28±0.38 |
| | Treatment | 13.92$^{ab}$±0.29** | 14.55$^{ab}$±0.34** | 14.68$^{ab}$±0.31** | 14.93$^{ab}$±0.42** | 15.16$^{b}$±0.45** | 14.60$^{ab}$±0.39** | 14.38$^{ab}$±0.37** | 14.17$^{ab}$±0.27** | 13.77$^{a}$±0.41** |
| PCV (%) | Control | 37.32$^{abc}$±0.72 | 38.43$^{c}$±0.46 | 38.60$^{c}$±0.54 | 38.83$^{c}$±0.38 | 39.28$^{c}$±0.70 | 37.93$^{bc}$±1.52 | 35.57$^{ab}$±0.78 | 35.02$^{a}$±0.70 | 34.97$^{a}$±0.67 |
| | Treatment | 45.15$^{ab}$±0.79** | 46.27$^{ab}$±0.79** | 46.30$^{ab}$±0.72** | 46.70$^{ab}$±0.70** | 47.15$^{b}$±0.52** | 46.87$^{b}$±0.38** | 45.70$^{ab}$±0.89** | 45.27$^{ab}$±0.29** | 44.42$^{a}$±0.58** |
| TLC ($10^3$/µL) | Control | 8.63$^{a}$±0.25 | 10.05$^{a}$±1.11 | 10.22$^{a}$±0.66 | 10.70$^{a}$±0.44 | 13.12$^{b}$±0.85 | 10.43$^{a}$±0.77 | 10.13$^{a}$±0.48 | 10.03$^{a}$±0.53 | 9.98$^{a}$±0.69 |
| | Treatment | 11.73$^{a}$±0.26** | 11.75$^{a}$±0.30 | 12.18$^{a}$±0.69 | 12.30$^{a}$±0.26* | 14.07$^{b}$±1.22 | 12.32$^{a}$±0.36 | 12.12$^{a}$±0.16** | 12.05$^{a}$±0.17** | 11.97$^{a}$±0.18* |
| Granulocytes (%) | Control | 44.17$^{a}$±1.14 | 45.35$^{a}$±3.04 | 47.40$^{a}$±0.96 | 50.27$^{a}$±1.93 | 66.23$^{b}$±4.13 | 50.95$^{a}$±1.90 | 50.68$^{a}$±1.98 | 45.43$^{a}$±1.83 | 45.07$^{a}$±1.60 |
| | Treatment | 44.25$^{a}$±1.07 | 46.63$^{ab}$±1.51 | 47.88$^{ab}$±1.70 | 51.28$^{bc}$±1.73 | 54.75$^{c}$±1.36* | 54.18$^{c}$±1.05 | 52.37$^{bc}$±2.59 | 50.07$^{bc}$±2.36 | 46.83$^{ab}$±1.12 |
| Lymphocytes (%) | Control | 42.32$^{cd}$±0.47 | 42.88$^{cd}$±0.60 | 43.09$^{cd}$±0.38 | 43.22$^{d}$±1.08 | 25.25$^{a}$±2.83 | 38.39$^{b}$±2.29 | 38.95$^{bc}$±1.81 | 40.97$^{bcd}$±1.92 | 43.02$^{bcd}$±0.54 |
| | Treatment | 44.65±1.01* | 44.80±1.18 | 45.03±0.65* | 46.63±1.21* | 44.60±1.66** | 44.40±1.33* | 43.50±1.50* | 43.13±1.04* | 44.37±2.01 |
| Glucose (mg/dL) | Control | 61.51$^{a}$±3.26 | 65.92$^{a}$±2.11 | 66.06$^{a}$±3.92 | 66.39$^{a}$±4.01 | 84.10$^{b}$±7.50 | 55.19$^{a}$±3.35 | 54.48$^{a}$±1.48 | 54.11$^{a}$±4.02 | 52.53$^{a}$±3.85 |
| | Treatment | 52.35$^{ab}$±4.70 | 52.05$^{ab}$±4.89$^{*}$ | 43.09$^{ab}$± 0.38$^{**}$ | 47.92$^{ab}$±1.34$^{**}$ | 53.86$^{b}$±4.23$^{**}$ | 44.51$^{ab}$±2.72$^{*}$ | 43.26$^{a}$±1.78$^{**}$ | 42.41$^{a}$±0.52$^{*}$ | 42.02$^{a}$±0.36 |
| Total protein (g/dL) | Control | 6.34±0.41 | 6.22±0.27 | 6.25±0.17 | 6.19±0.15 | 6.04±0.13 | 6.06±0.24 | 6.23±0.17 | 6.30±0.30 | 6.45±0.40 |
| | Treatment | 6.97±0.22 | 6.90±0.18 | 6.80±0.28 | 6.77±0.13$^{*}$ | 6.75±0.22$^{*}$ | 7.05±0.10$^{*}$ | 7.16±0.15$^{**}$ | 7.25±0.25$^{*}$ | 7.28±0.33 |
| Albumin (g/dL) | Control | 4.07±0.24 | 4.16±0.19 | 4.50±0.11 | 4.53±0.20 | 4.56±0.19 | 4.14±0.18 | 4.13±0.12 | 4.11±0.14 | 4.05±0.17 |
| | Treatment | 4.25±0.10 | 4.27±0.12 | 4.36±0.14 | 4.39±0.18 | 4.67±0.14 | 4.69±0.18 | 4.70±0.26 | 4.72±0.06$^{**}$ | 4.77±0.11$^{**}$ |
| Globulin (g/dL) | Control | 2.28±0.35 | 2.06±0.27 | 1.75±0.26 | 1.66±0.26 | 1.48±0.23 | 1.92±0.23 | 2.11±0.27 | 2.20±0.29 | 2.40±0.28 |
| | Treatment | 2.72±0.25 | 2.64±0.16 | 2.44±0.34 | 2.38±0.21 | 2.08±0.19 | 2.36±0.22 | 2.46±0.40 | 2.53±0.24 | 2.51±0.38 |
| NEFA (µmol/L) | Control | 187.01$^{a}$±3.86 | 225.85$^{b}$±6.42 | 283.56$^{c}$±7.30 | 372.58$^{d}$±5.13 | 567.58$^{f}$±8.71 | 710.90$^{h}$±23.06 | 609.71$^{g}$±20.64 | 422.92$^{e}$±10.86 | 310.52$^{c}$±5.52 |
| | Treatment | 176.32$^{a}$±2.90 | 204.31$^{b}$±5.41$^{*}$ | 249.65$^{c}$±5.91$^{*}$ | 324.29$^{d}$±8.79$^{**}$ | 480.72$^{f}$±6.90$^{**}$ | 560.28$^{h}$±8.30 | 519.64$^{g}$±7.47$^{**}$ | 369.14$^{e}$±4.48$^{**}$ | 296.21$^{e}$±7.16$^{**}$ |

Mean values with different superscript letters (a, b, c, d, e, f, g, h) within a row indicates statistically significant ($p<0.05$) differences between sampling days within a group. Asterisks (* and **) indicate significant differences between two groups within the same sampling day (same column) at $p<0.05$ and $p<0.01$, respectively.

the prepartum period, reached a peak on the day of calving and subsequently declined progressively throughout the postpartum period up to day 30 after calving. In contrast, treatment group exhibited relatively stable glucose concentrations during the prepartum period, followed by a moderate increase at calving and a gradual decline thereafter until day 30 postpartum. Comparison between the two groups revealed significant ($p<0.05$) variations in blood glucose concentration on days −20, +5 and +20, whereas highly significant ($p<0.01$) differences were recorded on days −10, −5, 0 and +10. The treatment group consistently exhibited lower blood glucose concentrations than the control group throughout the study period, which may be attributed to metabolic regulatory properties of *Tinospora cordifolia*, which could help maintain glucose homeostasis and attenuate stress-induced metabolic fluctuations associated with parturition. The present findings were consistent with Sharma *et al*. (2018), who reported significantly ($p<0.05$) lower serum glucose concentrations in *Tinospora cordifolia* stem powder-supplemented Jersey crossbred cows than in controls.

Total protein and globulin concentrations exhibited similar temporal trends in both groups, declined gradually until calving and increased progressively thereafter to reach peak values on day 30 postpartum. Total protein concentration was significantly ($p<0.05$) higher in treatment group on day −5, 0, +5 and +20, with a highly significant ($p<0.01$) inter-group difference recorded on day +10. In contrast, early prepartum and final postpartum observations showed no significant intergroup variation. Serum globulin concentration did not differ significantly between the two groups at any sampling interval, despite consistently higher numerical values in the treatment group. The higher total protein concentration in treatment group may reflect improved protein metabolism and hepatic synthetic activity, whereas the numerically higher globulin concentrations suggest a potential immunomodulatory effect of *Tinospora cordifolia* that did not reach statistical significance. The present findings concurred with Ran *et al*. (2022), who observed numerically higher serum total protein concentrations in herbal medicine-supplemented Holstein cows than in the control group. Moreover, the findings of the present study are closely aligning with Gupta *et al*. (2026), who investigated the impact of Giloy (*Tinospora cordifolia*) supplementation on serum globulin concentration in lactating cows.

Serum albumin concentration exhibited distinct temporal patterns between the two groups. The control group showed an increase up to calving followed by a gradual decline, whereas the treatment group exhibited a progressive increase throughout the experimental period, reaching peak values on day 30 postpartum. Comparison between the two groups demonstrated significantly ($p<0.01$) higher serum albumin concentrations in the treatment group on days +20 and +30 postpartum. However, no significant differences were detected during the other sampling periods. The higher serum albumin concentrations observed during the late postpartum period may reflect the hepatoprotective and metabolic regulatory properties of *Tinospora cordifolia*, which could enhance hepatic protein synthesis and improve protein metabolism during recovery from parturition. The results of the present study are consistent with those reported by Sharma *et al*. (2018), who investigated the impact of Giloy (*Tinospora cordifolia*) and Fenugreek (*Trigonella foenum-graecum*) supplementation on blood biochemical parameters in lactating cows.

Serum NEFA concentration exhibited a similar temporal profile in both experimental groups, with values increased progressively before calving, reached a peak on day +5 and subsequently declined throughout the postpartum period. The treatment group showed highly significant ($p<0.01$) lower values than the control group on days −5, 0, +5, +10, +20 and +30, while a significant ($p<0.05$) difference was observed on days −20 and -10; day −30 remained statistically comparable between the groups. A consistently lower serum NEFA concentrations observed in the treatment group may indicate improved energy balance and reduced adipose tissue mobilization during the periparturient period, suggesting alleviation of negative energy balance. The observations of the present investigation are consistent with the findings of Mir *et al*. (2014), who studied the influence of *Tinospora cordifolia* stem powder supplementation in early lactating Murrah buffaloes.

## Conclusion

In conclusion, dietary supplementation with *Tinospora cordifolia* stem powder at 6% of the concentrate mixture during the periparturient period exerted beneficial effects on the haematological and biochemical indices of crossbred cows. Treatment group exhibited significantly improved erythrocytic indices, including total erythrocyte count, haemoglobin concentration and packed cell volume, indicating enhanced haematopoietic status. The higher total leukocyte count and lymphocyte percentage observed around calving further suggest improved immune competence during this physiologically stressful period. Biochemically, *Tinospora cordifolia* supplementation was associated with lower serum NEFA and blood glucose concentrations, reflecting improved metabolic adaptation and energy homeostasis. In addition, higher serum total protein and albumin concentrations indicated enhanced protein metabolism and hepatic synthetic function,

while serum globulin concentration remained unaffected. Collectively, these findings demonstrate that *Tinospora cordifolia* supplementation may support haematological stability, immune function and metabolic adaptation during the periparturient period in crossbred cows, thereby contributing to improved physiological resilience. Therefore, *Tinospora cordifolia* may be considered a promising natural phytogenic feed supplement for promoting the health and metabolic well-being of dairy cows during the periparturient period.

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