Effect of Herbal Feed Additive Supplementation Along with High Plane of Nutrition on Productive and Reproductive Performance of Post-Partum Tharparkar Cows

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Research Article

Keywords: Asparagus racemosus, Milk yield, Reproduction, Tharparkar, Tinospora cordifolia, Withania somnifera

Posted Date: July 14th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6983300/v1

Abstract

This study investigated the effects of a high plane of nutrition combined with a herbal feed additive on feed intake, antioxidant status, metabolic and hormonal profiles, immunity, and productive and reproductive performance in postpartum Tharparkar cows. Eighteen cows (30–60 days postpartum) were randomly assigned to three groups: a control group (CON) fed green fodder, wheat straw, and concentrate mixture; T1, receiving a 10% higher concentrate mixture; and T2, receiving the T1 diet plus a herbal supplement containing *Tinospora cordifolia*, *Withania somnifera*, and *Asparagus racemosus* (2:1:1) at 150 mg/kg BW for 90 days. T1 and T2 cows had significantly higher dry and organic matter intake ($P < 0.01$). T2 cows exhibited reduced serum NEFA levels ($P < 0.01$), enhanced antioxidant enzyme activities (GPx, CAT, SOD, TAOC), higher serum T3 and T4, lower cortisol and lipid peroxidation, and improved cell-mediated immunity. Both T1 and T2 groups had greater daily milk yield, 4% FCM and ECM ($P < 0.01$), while T2 showed higher DPPH activity in milk ($P \leq 0.001$). Reproductively, T2 cows showed earlier estrus ($P < 0.05$), higher conception rates ($P \leq 0.001$), and required fewer AIs. These findings suggest that integrating a herbal additive with enhanced nutrition can improve health and performance in postpartum indigenous cows.

Introduction

The livestock sector plays a pivotal role in India's economy, contributing 5.50% to the national Gross Value Added (GVA) and 30.23% to the Agricultural and Allied Sector GVA in 2022–23 (DADF, 2023). Enhancing the productive and reproductive performance of ruminants, particularly dairy cattle, is essential for sustaining this contribution. Proper nutritional management during the postpartum period is critical for maintaining regular estrous cycles, supporting ovarian function, and achieving optimal conception rates. Early lactation often presents a mismatch between the cow's energy demands and dietary intake, leading to negative energy balance (NEB), which can delay the resumption of ovarian cyclicity, increase stress, and impair immune function, ultimately affecting productivity (Robinson et al., 2006; Rajneesh et al., 2020).

Indigenous cattle breeds like Tharparkar are renowned for their resilience to low-quality forages, disease resistance, and heat tolerance but typically exhibit lower milk yields and shorter lactation periods compared to improved breeds (Yadav et al., 2020). Addressing these limitations necessitates targeted nutritional strategies during the periparturient and early postpartum phases, which are marked by hormonal changes, metabolic stress, reduced dry matter intake, and energy deficits. These conditions lead to body fat mobilization and elevated plasma non-esterified fatty acids (NEFA). Enhancing dietary energy density during this critical window can improve voluntary feed intake, energy balance, body weight gain, and reduce plasma NEFA levels (VandeHaar et al., 1999).

Beyond essential macronutrients, optimal animal nutrition encompasses adequate supplementation of minerals, vitamins, and strategic use of feed additives. Herbal feed additives have garnered attention due to their natural, non-hormonal, and eco-friendly properties. When integrated with effective feed management, these additives can enhance animal performance by modulating physiological functions and bolstering immune responses through their antimicrobial and immunostimulatory properties (Afzal et al., 2021). Studies have reported galactopoietic and reproductive benefits associated with herbal supplementation, including enhanced estrus expression, improved first-service conception rates, and reduced services per conception (Singhal, 1995; Kuri et al., 2019).

In light of this evidence, the present study aims to evaluate the effect of supplementing a high plane of nutrition with a herbal feed additive on the productive and reproductive performance of postpartum Tharparkar cows. This research seeks to provide insights into integrated nutritional approaches that can support better health, fertility, and milk production in indigenous dairy breeds managed under tropical conditions.

Materials and Methods

Study Area

The study was conducted at the Cattle and Buffalo Farm of ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, India, located at 28.20°N latitude, 79.24°E longitude, and 172 m above sea level. The region practices traditional mixed farming, where crop-livestock integration has been an age-old system. However, productivity on smallholder farms remains limited, largely due to the use of non-descript breeds and inadequate nutrition. The dominant crops in this region include wheat, rice, and sugarcane, with cereal straws (wheat/rice) forming the bulk of ruminant feed. All experimental procedures were approved by the Institutional Animal Ethics Committee and conducted in accordance with guidelines issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Preparation of Herbal Feed Additive

Fresh stems of *Tinospora cordifolia*, roots of *Withania somnifera*, and *Asparagus racemosus* were procured from the ICAR-IVRI campus and local markets. The plant materials were shade-dried, ground into fine powder, and stored in airtight containers at room temperature until further use.

Experimental Design and Feeding Regime

Tharparkar is a dual-purpose zebu (Bos indicus) cattle breed native to the Thar Desert of western Rajasthan and Gujarat. Adapted to arid climates, it is valued for both milk production (1800–2600 liters/lactation, 4.5–5% fat) and draught power. The breed is medium to large, with a white or light grey coat and a prominent hump in males. Cows have a straight back, long body, and well-formed udders. Known for heat and disease resistance, Tharparkar thrives on sparse forage. The average age at first calving is 36–42 months, with a calving interval of 14–16 months (DAHD 2019; ICAR-NBAGR 2021).

Eighteen postpartum Tharparkar cows (30–60 days postpartum), ranging from second to fifth parity, with an average body weight of 340.3 ± 5.5 kg and mean milk yield of 6.38 ± 0.32 kg/d, were randomly assigned to three treatment groups (n = 6 each) in a completely randomized design.

- **CON (Control)**: Fed green fodder, wheat straw, and concentrate mixture as per ICAR (2013) feeding standards.
- **T1**: Received the CON diet with 10% additional concentrate mixture.
- **T2**: Received the T1 diet along with herbal feed additive (*T. cordifolia*, *W. somnifera*, A. *racemosus* in 2:1:1 ratio) at 150 mg/kg body weight.

The concentrate mixture and herbal additive dosages were adjusted fortnightly based on body weight and milk yield.

Milk Production, Composition, and Quality Assessment

Cows were milked twice daily at 06:00 and 16:00 hours. Pre-milking hygiene protocols included washing and disinfection of udders. Daily milk yield was recorded throughout the 90-day trial. Fortnightly, 100 mL milk samples were collected in sterilized bottles for compositional analysis using an ultrasonic milk analyzer. Milk acidity (titrable acidity) was determined following Wilkowske (1956). Milk energy values were calculated as 4% fat-corrected milk (FCM) using Gaines (1928) and energy-corrected milk (ECM) by Tyrrell and Reid (1965). Antioxidant activity in milk was assessed via the DPPH assay as per Adesegun et al. (2008).

Blood Collection and Serum Preparation

Blood samples (~ 10 mL) were collected via jugular venipuncture before feeding and watering on days 0, 45, and 90. Half the volume was transferred to serum vacutainers and centrifuged at 2,000 × g for 10 minutes to separate serum. The remaining blood was collected in heparinized vacutainers for isolation of mononuclear cells for lymphocyte transformation testing. Serum levels of non-esterified fatty acids (NEFA), urea, and total cholesterol were analyzed spectrophotometrically using a Multiskan™ FC Microplate Photometer (Thermo Scientific, USA) and diagnostic kits from Coral Clinical Systems (Goa, India).

Feed, Fodder and Herb Analysis

Representative samples of feed, fodder, herbal powders, refusals, and feces were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and total ash (TA) as per AOAC (2012). Fiber fractions neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following Van Soest et al. (1991). Herbal powders were also evaluated for total phenolic content (TPC) using the Folin–Ciocalteu method (Singleton et al. 1999), total flavonoid content (TFC) via the aluminum chloride method (Ordonez et al. 2006), and antioxidant capacity by DPPH assay (Brand-Williams et al. 1995). Results were expressed as mg/g tannic acid equivalents (TAE), mg/g quercetin equivalents (QE), and percentage inhibition.

Reproductive Performance

Estrus detection was conducted twice daily using a trained teaser bull. Cows observed in estrus were artificially inseminated using frozen semen. Pregnancy was diagnosed by transrectal palpation 60 days post-insemination in non-return cows. Reproductive parameters such as onset of estrus, services per conception, and conception rate were recorded. Cows were also monitored for reproductive disorders such as mastitis and metritis.

Statistical Analysis

All data were statistically analyzed using IBM SPSS Statistics version 26.0, following the procedures described by Snedecor and Cochran (2004). One-way ANOVA was applied for comparison among treatment groups. Duncan's Multiple Range Test (DMRT) was used for post hoc comparisons of means where applicable. Repeated-measures ANOVA were employed for parameters recorded over time using the General Linear Model (GLM) framework. Differences were considered statistically significant at $P < 0.05$. Results are presented as mean ± standard error of the mean (SEM).

Results

Chemical composition of feeds offered

The chemical composition and fibre fractions of wheat straw, green maize fodder and concentrate mixture are presented in Table 1. The chemical composition and fibre fractions of *T. cordifolia*, *W. somnifera* and *A. racemosus* are presented in the Table 2, where the CP content of *T. cordifolia* was higher ($P \leq 0.001$) than *W. somnifera* and *A. racemosus*. However, EE, TA, NDF and ADF values of *A. racemosus* were lower ($P \leq 0.001$) than *T. cordifolia* and *W. somnifera*.

Table 1
Chemical composition of feeds and herbs (% DM basis)

Attributes (%)	Concentrate mixture	Wheat straw	fodder (maize
DM	88.02	92.14	23.00
OM	92.53	91.29	86.87
CP	18.23	3.28	8.02
EE	3.59	1.07	1.27
TA	7.47	8.71	13.13
NDF	41.95	83.30	66.51
ADF	13.31	53.60	37.73

DM- Dry matter, OM- Organic matter, CP- Crude protein, EE- Ether extract, TA- Total ash, NDF- Neutral detergent fibre, ADF- Acid detergent fibre

Table 2
Total phenolic, flavonoid and IC_{50} values of *T. cordifolia*, *W. somnifera* and *A. racemosus*

Attributes	*T. cordifolia*	*W. somnifera*	*A. racemosus*	SEM	P-value
DM (%)	94.35	91.38	89.70	0.98	0.139
OM (%)	91.67	92.18	95.31	0.87	0.184
CP (%)	7.47^{b}	5.39^{a}	5.07^{a}	0.38	≤ 0.001
EE (%)	1.53^{b}	2.55^{c}	0.82^{a}	0.26	≤ 0.001
TA (%)	8.33^{b}	7.82^{b}	4.69^{a}	1.75	≤ 0.001
NDF (%)	38.83^{b}	39.86^{b}	22.55^{a}	2.83	≤ 0.001
ADF (%)	23.15^{b}	26.54^{c}	13.1^{a}	2.04	≤ 0.001
TPC (mgg^{-1}TAE)	7.42^{c}	5.05^{b}	3.11^{a}	0.53	≤ 0.001
TFC (mgg^{-1} QE)	3.83^{c}	2.21^{b}	1.46^{a}	0.30	≤ 0.001
IC_{50}(μgmL^{-1})	45.39^{a}	55.05^{b}	60.13^{c}	2.16	≤ 0.001

^{abc} Means bearing different superscript in a row differ significantly

TPC-Total phenolic content, TFC-Total flavanoid content, TAE-Tannic acid equivalent, QE- Quercetin equivalent, IC_{50}- Inhibitory concentration$_{50}$

Phytochemical analysis of herbs

The TPC, TFC and IC_{50} values of *T. cordifolia*, *W. somnifera* and *A. racemosus* are mentioned in Table 2. The TPC and TFC values were significantly higher ($P \leq 0.001$) in *T. cordifolia* followed by *W. somnifera* and *A. racemosus*, respectively, whereas, IC_{50} values of *A. racemosus* were higher ($P \leq 0.001$) followed by *W. somnifera* and *T. cordifolia*, respectively.

Feed intake

The effect of supplementation on feed intake in Tharparkar cows is presented in the Table3. Intake of concentrate mixture (kgd^{-1}) was significantly ($P < 0.05$) higher in T-1 and T- 2 groups relative to CON; however, wheat straw and green fodder (maize) intake was similar among the treatment groups. The DM and OM intake ($gkg^{-1}W^{0.75}$) was significantly ($P < 0.01$) higher in T-1 and T-2 groups relative to CON.

Table 3
Effect of supplementation on feed intake in post-partum Tharparkar cows

Attributes	Treatments			SEM	P-value
	CON	T-1	T-2		
Intake, kgd^{-1}					
Concentrate mixture	4.37^{a}	4.76^{b}	4.76^{b}	0.10	0.027
Wheat straw	1.83	1.90	1.91	0.02	0.394
Green fodder	2.53	2.63	2.64	0.03	0.394
Total roughage	4.37	4.54	4.55	0.05	0.394
Intake, $gkg^{-1}W^{0.75}$					
DM	109.38^{a}	113.06^{b}	112.74^{b}	0.58	0.007
OM	99.12^{a}	104.94^{b}	104.64^{b}	0.76	≤ 0.001

^{ab} Means bearing different superscript in a row differ significantly

DM- Dry matter, OM- Organic matter

Serum biochemical parameters

Serum NEFA, urea and total cholesterol levels in post-partum Tharparkar cows are illustrated in Table 4. There was no effect of dietary treatments on serum urea and total cholesterol levels, however, serum NEFA values were significantly ($P < 0.01$) lower in T-2 group and showed a noteworthy decline ($P \leq 0.001$) from 45 d onwards.

Table 4
Effect of supplementation on serum biochemical parameters in post-partum Tharparkar cows

Periods	Treatment			Period mean	SEM	P- value		
	CON	T-1	T-2			T	P	T*P
NEFA, pgmL^{-1}								
0	139.85	141.91	143.90	142.07^{z}	5.08	0.002	≤ 0.001	0.003
45	95.74	90.00	79.49	88.78^{y}				
90	80.83	79.49	71.91	77.10^{x}				
Treatment mean	105.47^{b}	103.80^{b}	99.39^{a}					
Serum urea, mgdL^{-1}								
0	28.17	28.61	28.47	28.42	0.35	0.92	0.24	0.87
45	29.79	30.68	29.20	29.89				
90	29.35	29.06	29.94	29.45				
Treatment mean	29.11	29.45	29.20					
Total cholesterol, mgdL^{-1}								
0	130.14	129.43	130.00	129.86	0.36	0.83	0.58	0.16
45	128.29	131.57	131.29	130.38				
90	131.71	130.71	129.86	130.76				
Treatment mean	130.05	130.57	130.38					

^{ab} and ^{xy} Means bearing different superscript in a row and column differ significantly

NEFA- Non-esterified fatty acids.

Serum hormonal profile

The results on serum T_3, T_4, cortisol and progesterone are summarized in Table 5. T-2 group cows had higher ($P < 0.05$) T_3, T_4 and progesterone levels. Nevertheless, serum cortisol level was lower ($P < 0.01$) in T-2 group and started to decrease pointedly ($P \le 0.001$) from 45 d onwards.

Table 5
Effect of supplementation on serum hormonal profile in post-partum Tharparkar cows

Periods	Treatment			Period mean	SEM	P- value		
	CON	T-1	T-2			T	P	T*P
T_3, $ngmL^{-1}$								
0	2.57	2.63	2.79	2.67^x	0.05	≤ 0.001	≤ 0.001	≤ 0.001
45	2.58	2.63	3.00	2.74^x				
90	2.61	2.64	3.51	2.92^y				
Treatment mean	2.59^a	2.63^a	3.10^b					
T_4, $ngmL^{-1}$								
0	44.00	45.50	44.63	44.77^x	1.50	≤ 0.001	≤ 0.001	0.001
45	49.50	52.63	57.63	53.59^y				
90	55.83	57.63	70.25	61.73^z				
Treatment mean	49.78^a	51.92^a	57.50^b					
Cortisol, $ngmL^{-1}$								
0	8.60	8.63	8.91	8.71^z	0.16	0.002	≤ 0.001	0.002
45	7.27	7.14	6.58	7.00^y				
90	6.78	6.77	5.54	6.36^x				
Treatment mean	7.55^b	7.51^b	7.01^a					
Progesterone, $ngmL^{-1}$								
0	1.11	1.08	1.27	1.16^x	0.42	0.026	≤ 0.001	0.320
45	1.45	1.39	2.68	1.84^x				
^{ab} and ^{xy}Means bearing different superscript in a row and column differ significantly								
T_3-Triiodothyronine; T_4- Thyroxine								

Periods	Treatment			Period mean	SEM	P- value		
	CON	T-1	T-2			T	P	T*P
90	3.94	3.71	6.13	4.59^{y}				
Treatment mean	2.17^{a}	2.06^{a}	3.36^{b}					
^{ab} and ^{xy}Means bearing different superscript in a row and column differ significantly								
T_3-Triiodothyronine; T_4- Thyroxine								

Serum antioxidant profile and cell mediated immune response

Figure 1 reveals the substantial improvement ($P \leq 0.001$) in antioxidant indices like GPx, SOD, CAT and TAOC in T-2 group with significantly ($P < 0.05$) elevated values at 90 d feeding and lowered ($P < 0.05$) LPO levels in T-2 group amongst the groups. The CMI response assessed by LTT is depicted in Fig. 2 that was found to be noticeably ($P \leq 0.001$) higher in T-2 group.

Lactation performance

The results on milk yield and composition are illustrated in Table 6. The daily and total milk yield (kg), 4% FCM and ECM (kgd^{-1}), fat and protein yield (gd^{-1}) were considerably ($P \leq 0.001$) higher in T-1 and T-2 groups without any effect on milk composition (fat, SNF, protein and total solids), whereas, the DPPH activity of milk was prominently ($P \leq 0.001$) higher in T-2 group. The effect of dietary treatments on milk yield and DPPH activity of milk in different groups at fortnight intervals are depicted in Fig. 3.

Table 6
Effect of supplementation on lactation performance in post-partum Tharparkar cows [ab] Means bearing different superscript in a row differ significantly

Attributes	Dietary treatments			SEM	P- value
	CON	T-1	T-2		
Milk yield					
Daily milk yield, kgd^{-1}	4.99^a	6.01^b	6.16^b	0.02	0.001
4% FCM, kgd^{-1}	5.45^a	6.59^b	6.76^b	0.15	0.001
ECM, kgd^{-1}	6.40^a	8.08^b	8.46^b	0.25	0.001
Total milk yield, kg	462.27^a	556.96^b	571.48^b	13.28	0.001
Fat yield, gd^{-1}	230.26^a	278.95^b	286.32^b	6.54	0.001
Protein yield, gd^{-1}	145.05^a	174.98^b	177.93^b	4.13	0.001
Milk pH	6.60	6.58	6.59	0.01	0.754
Milk composition (%)					
Fat	4.62	4.66	4.67	0.03	0.695
SNF	9.00	8.97	8.99	0.015	0.592
Total solids	13.62	13.63	13.67	0.032	0.823
Protein	2.91	2.91	2.89	0.01	0.275
Lactose	4.51	4.47	4.44	0.021	0.454
Titrable acidity	0.17	0.17	0.18	0.009	0.488
DPPH	56.18^a	57.30^a	59.2^b	0.33	0.001

Reproductive performance

The post-partum Tharparkar cows of T-2 group exhibited estrus earlier ($P < 0.05$) relative to T-1 and CON groups. The conception at first insemination was higher in T-2 than T-1 and CON groups, while at subsequent insemination was also higher in T-2 group. The overall conception rate was higher ($P \le 0.001$) in T-2 group when compared to CON and T-1 groups. However, 16.7% of cows from each group showed no estrus signs, and were non-responsive. The cows in T-2 group require more ($P < 0.05$) number of AIs per conception relative to CON and T-1 groups (Fig. 4)

Discussion

Chemical composition of feeds and herb

The chemical composition of wheat straw, concentrate mixture and green maize fodder provided to Tharpakar cows was in accordance with previous reports (Thanh and Suksombat 2015; Raj and Bharadwaj 2021; Chaudhary et al. 2021). Chemical composition of *T. cordifolia*, *W. somnifera* and *A. racemosus* was within the normal range as reported by earlier workers (Rajalakshmi et al. 2015; Kumari and Gupta 2016; Verma and Mishra 2021; Chavan et al. 2021; Verma et al. 2024).

Phytochemical analysis of herbs

The TPC and TFC were significantly higher in *T. cordifolia*, followed by *W. somnifera* and *A. racemosus*. These values remained within the previously reported standard ranges (Garg and Garg 2018; Polumackanycz et al. 2023; Royani et al. 2023; Oviya 2023). In contrary, Shwetha et al. (2016) reported lower levels of TPC and TFC in *T. cordifolia*, while Udayakumar et al. (2010) observed higher TPC and TFC in *W. somnifera* and Behera et al. (2013) reported elevated TFC in *A. racemosus*. Such discrepancies in phyto-constituent levels may be attributed to variations in extraction protocols as well as ecological factors, including soil type, climate, sunlight exposure, geographical distribution and differences in grazing (Hussain et al. 2009; Nobosse et al. 2018).

The IC_{50} values were significantly higher ($P \leq 0.001$) in *A. racemosus*, followed by *W. somnifera* and *T. cordifolia*, indicating that *T. cordifolia* exhibited the strongest antioxidant potential among the three, as lower IC_{50} values signify stronger antioxidant activity (Olugbami et al. 2014). DPPH assay is widely performed to evaluate the antioxidant capacity by assessing the ability of antioxidants to donate hydrogen atoms to neutralize DPPH free radicals, forming stable non-radical DPPH-H (Praveen et al. 2012). The present results are in agreement with earlier studies by Jain et al. (2008) and Oviya (2023), who reported higher IC_{50} values for *A. racemosus* than *W. somnifera*. The observed results support the established relationship between phenolic and flavonoid contents and antioxidant capacity, wherein TPC and TFC are positively correlated with DPPH scavenging activity and negatively correlated with IC_{50} values (Farasat et al. 2014). Thus, the higher TPC and TFC levels in *T. cordifolia* likely contributed to its comparatively lower IC_{50} value and greater antioxidant potential.

Feed intake

DM and OM intake was significantly ($P < 0.01$) higher in T-1 and T-2 groups than CON. Our findings are compatible with the previous reports (Patton et al. 2006; Drackley et al. 2003) who demonstrated that increasing the energy density of rations enhanced DM intake in lactating animals. Similarly, Hailu et al. (2011) and Tufarelli et al. (2011) observed higher DM intake in sheep fed higher level of concentrate mixture. Moreover, supplementation of *A. racemosus* @ 125 and 150 $mgkg^{-1}$BW led to higher DM intake in crossbred cows and Sahiwal heifers, respectively (Kumavat et al. 2017; Jamara et al. 2014). Mir et al. (2015) reported significant increase in DMI of early lactating Murrah buffaloes supplemented with *T. cordifolia* @ $120gd^{-1}$. The observed increase in DMI can be attributed to a significant rise in concentrate intake which improved the availability of fermentable carbohydrates and proteins, creating a more favorable rumen environment for microbial growth and enhances rumen fermentation leading to optimized rumen environment.

Serum biochemical profile

The serum biochemical parameters reflect the general health of animals. Early post-partum cows often experience negative energy balance due to increased energy demands and reduced dry matter intake (Grummer 1995; Bradford et al. 2015). Our findings of lowered ($P < 0.01$) NEFA levels in T-2 group aligned with that of Sharma et al. (2014), who reported decreased NEFA levels in Karan Fries cows supplemented with *W. somnifera*, *A. racemosus*, and *T. cordifolia* (200–250 $mgkg^{-1}$BW) based polyherbal mixture. Herbs could inhibit lipolysis, enhance lipid metabolism and improve metabolic status of cows (Cigari et al. 2015). Serum urea and cholesterol levels were unaffected in our study and consistent with that of Sharma et al. (2018) by *T. cordifolia* supplementation ($150gd^{-1}$) in crossbred cows. However, Kumar et al. (2014) observed reduced cholesterol in Karan Fries cows with *A. racemosus* supplementation ($100mgkg^{-1}$BW). The comparable serum urea and cholesterol levels suggest that feeding 10% higher concentrate mixture and herbal feed additive do not disrupt the animal's homeostasis.

Serum hormonal profile

With respect to hormones, there was a significant ($P < 0.05$) increase in serum T_3, T_4 and progesterone hormones and substantially ($P < 0.01$) decreased serum cortisol in T-2 group. Some previous reports (Patel et al. 2016; Rajneesh et al. 2020) showed that supplementation of *T. cordifolia* based herbal mixture positively affected serum T_4 level in Murrah buffalo calves. The observed positive effect of addition of herbal feed additive on hormonal profile could be explained by thyroid regulating and stimulating effect of *T. cordifolia* and Withaferin A in *W. somnifera* (Tewari and Tewari 2020; Jatwa and Kar 2009) and the higher conception rate in T-2 group. The reduced serum cortisol level was likely due to reduction in adrenocorticotropic hormone caused by *W. somnifera* and *A. racemosus* (Sengupta et al. 2018; Singh et al. 2023).

Serum antioxidant profile

The present study showed significantly ($P \leq 0.001$) higher levels of GPx, CAT, SOD, and TAOC, alongside a lower ($P < 0.05$) LPO in T-2 group. These findings are in agreement with previous reports of Ingale et al. (2017) who observed enhanced SOD, CAT and GPx, as well as decreased LPO levels in Jamunapari kids supplemented with *W. somnifera* at 1.5 and 2.5% of DM intake. Similarly, Vaswani et al. (2022) reported that dietary supplementation of *T. cordifolia* at 1% of DMI significantly elevated SOD levels while reduced LPO levels in Sahiwal heifers. Furthermore, Oviya (2023) reported increased SOD, GST and GPx levels in goat kids fed herbal mixture (*W. somnifera* and *A. racemosus*; 2:1) @150 $mgkg^{-1}$BW.

The antioxidant and anti-peroxidative effects of *W. somnifera* are attributed to its bioactive constituents, notably withanolides (Sitoindosides VII–X) and withaferin A, which have been shown to enhance endogenous SOD, CAT and ascorbic acid levels while suppressing LPO (Ojha and Arya 2009; Dhuley 1998). Likewise, the antioxidant potential of *A. racemosus* is linked to phytochemicals including racemofuran, asparagamine A and racemosol (Wiboonpun et al. 2004). *T. cordifolia* has been shown to elevate GSH levels, upregulate gamma-glutamylcysteine ligase gene expression and exhibit free radical scavenging activities (Rawal et al. 2004). Moreover, the flavonoids present in these herbs are known to modulate the expression of gamma-glutamylcysteine synthetase, a key rate-limiting enzyme in GSH biosynthesis (Moskaug et al. 2005). Therefore, the elevated antioxidant indices observed in T-2 group can be attributed to the synergistic effects of the phytoconstituents present in the herbal mixture of *T. cordifolia*, *W. somnifera* and *A. racemosus*.

Cell mediated immune response

The CMI response involves the activation of phagocytic cells and antigen-specific cytotoxic T-lymphocytes with release of various cytokines (Russell et al. 2018). In the present study, Tharparkar cows in T-2 group exhibited a markedly improved ($P \leq 0.001$) CMI response compared to those in T-1 and CON groups. Consistent with our results, Mallick and Prakash (2011) reported significantly higher lymphocyte counts in Karan Fries cows supplemented with *T. cordifolia* @ $60gd^{-1}$ for 45 d before parturition and @ $120gd^{-1}$ for 45 d post-calving. The α-d-glucan of *T. cordifolia* activates lymphocytes, especially NK cells and influences Th1 cytokine profile that produce IFN-γ, IL-2 and TNF-β which are crucial for cell-mediated immunity and complement activation (Nair et al. 2004).

The presence of Withaferin A and 3-β-hydroxy-2, 3-dihydro withanolide F in *W. somnifera* and steroidal saponins- Shatavaroside A and Shatavaroside B in *A. racemosus* are responsible for their immunomodulatory potential (Budhiraja and Sudhir 1987; Thakur et al. 2021). Furthermore, carotenoids present in both *A. racemosus* and *W. somnifera* have been shown to enhance T and B-lymphocytes proliferation, stimulate macrophage activity and promote cytokine production (Milani et al. 2007). Thus, it can be inferred that the mutual effect of phytoconstituents present in herbal feed additive likely contributed to the significantly enhanced CMI response in T-2 group compared to other groups.

Lactation performance

The results revealed substantially ($P \leq 0.001$) higher daily and total milk yield, 4% FCM, ECM, fat and protein yield in T-1 and T-2 groups without alterations in milk composition. This was supported by the findings of Nielson et al. (2007) and Mallick and Prakash (2012), who reported increased milk yield and comparable milk composition in early lactating HF cows and crossbred cows by feeding high energy diet and *T. cordifolia* supplementation, respectively. Additionally, supplementation of A. racemosus @ 200 $mgkg^{-1}$BW improved milk yield and 4% FCM (Choudhary et al. 2024). In contrary, Sharma et al. (2017) observed similar milk yield in crossbred cows by supplementing *T. cordifolia* stem powder to crossbred cows. It is well established that dietary energy and proteins are critical for optimal lactation performance in dairy animals (Wang et al. 2014). Enhanced milk production is closely associated with a continuous supply of rumen-fermentable carbohydrates, readily degradable proteins and microbial protein synthesis in the rumen (Zhu et al. 2013; Wang et al. 2014; Zhou et al. 2015). This resulted in increased milk yield by feeding higher concentrate diet in T-1 and T-2 groups.

Furthermore, the enhanced milk DPPH activity in T-2 group was supported by Stobiecka et al. (2023), who observed enhanced antioxidant potential and bioactive compounds in milk, including β-lactoglobulin, lactoferrin and vitamins A and E following herbal supplementation. Higher antioxidant levels in milk are associated with better spoilage prevention and an extended shelf life (Aguiar et al. 2014). Lactoferrin, a functional protein in milk, has antioxidant properties that protect cells from oxidative stress by inhibiting the reaction of free ferric ions with superoxide molecules, thereby reducing the formation of ferrous salts and ground state oxygen (Bielecka et al. 2022; Superti 2020). Herbal feed additives are rich in phenolic and flavonoids compounds that are positively correlated with DPPH radical scavenging activity (Aryal et al. 2019; Farasat et al. 2014; Jan et al. 2013). Thus, the observed enhanced DPPH activity of milk in T-2 group might be attributed to the higher concentration of phenols and flavonoids present in the herbal feed additive.

Reproduction performance

The post-partum Tharparkar cows of T-2 group came to estrus earlier ($P < 0.05$) than T-1 and CON groups with higher conception rate ($P \leq 0.001$) and lower number ($P < 0.05$) of AIs per conception. These findings are consistent with previous reports of Kumar et al. (2012) and Kumavat et al. (2017), who documented earlier onset of estrus and improved conception rates in post-partum cows supplemented with *A. racemosus* @ 125-200mgkg^{-1} BW. Similarly, Kumavat et al. (2017), Barjibhe et al. (2019) and Muwal et al. (2020) reported reduced service periods, services per conception, and earlier estrus induction in dairy cows supplemented with *A. racemosus*. Supplementation of A. racemosus @ 100gd^{-1} to early post-partum crossbred cows resulted in 100% estrus induction and 75% conception within three months of calving (Berhane and Singh 2022). Likewise, Mallick and Prakash (2011) reported earlier onset of estrus in post-partum Karan Fries cows following dietary supplementation of *T. cordifolia* stem powder @ 60 gd^{-1}for 45 d. Honnegowda (2000) reported that dietary addition of polyherbal mixture containing *T. cordifolia* increased conception rates in cows and buffaloes, while Chaudhiry et al. (2019) observed that *T. cordifolia* combined with mineral mixture achieved 62.5 and 80.0% estrus induction and conception rates in anestrus buffalo heifers, respectively. Singh et al. (2022) reported reduced service periods and improved conception rates in Sahiwal cows with supplementation of polyherbal mixture containing *W. somnifera* @ 20gd^{-1}from 3 days pre-partum to 21 days post-partum

A. racemosus has been shown to support uterine involution and enhance reproductive health by stimulating epithelial cell proliferation and ovarian activity (Frawley and Lad 2006; Sattar et al. 2007; Amer and Badr 2008). Furthermore, *T. cordifolia* has immunomodulatory effects that promote early resumption of ovarian cyclicity (Mallick and Prakash 2011). Thus, the presence of antioxidants, phenolics, flavonoids and saponins in *T. cordifolia*, *W. somnifera* and *A. racemosus* could have enhanced the reproductive performance by supporting follicular development and hormonal balance in Tharparkar cows of T-2 group (Dhama et al. 2014; Aghsaghali 2012; Perumal et al. 2013; Kumar et al. 2014).

Conclusion

Supplementation of herbal feed additive (*T. cordifolia*, *W. somnifera* and *A. racemosus*; 2:1:1) @ 150 $mgkg^{-1}$ BW along with high plane of nutrition (through 10% higher concentrate) to early post-partum Tharparkar cows significantly improved the lactation and reproductive performance. Besides this the supplementation had positive influence on feed intake, antioxidant status, hormonal profile, and cell mediated immune response with decreased serum NEFA and cortisol levels. These findings demonstrate that providing high plane of nutrition with herbal feed additive has potential for improving the general health as well as performance of post-partum indigenous cattle breeds.

Declarations

Acknowledgments

The authors would like to thank Director, ICAR-IVRI, Izatnagar, UP-243122 for providing the facilities to conduct the research.

The study was approved by Institutional Animals Ethics Committee under the supervision of CPCSEA, India (IAEC/20.06.2024/L2).

Conflict of interest

The authors declare no conflict of interest.

Funding

The study was financially supported by Indian Council of Agricultural Research (AICRP), New Delhi, India.

Author’s contributions

K. G: experimental trial, formal and laboratory analysis and writing of manuscript. Narayan Dutta: methodology, supervision and conceptualization.

S. N. and N.K.: Laboratory analysis and editing.

N.D., H.O.P., S.E.J: Formulation the research hypothesis and constructed the technical programme for the trial. They provided guidance and oversight throughout the experiment.

G.S. and S.K.S.: Provided assistance for hormonal and reproduction work.

Availability of data and material

Data will be made available on request.

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Figures

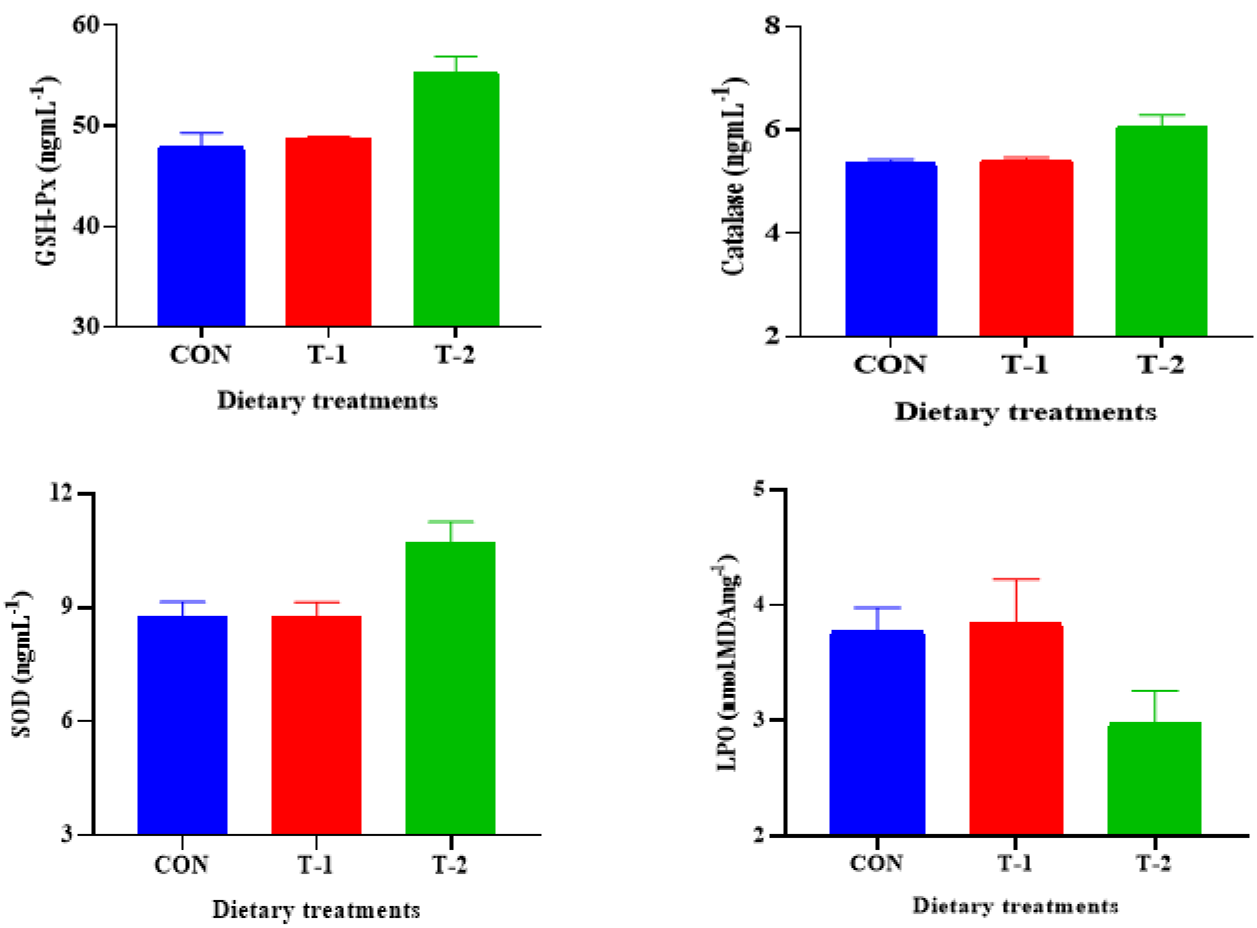

Figure 1

Effect of supplementation on antioxidant indices (GPx, Catalase, SOD and LPO) in post-partum Tharparkar cows

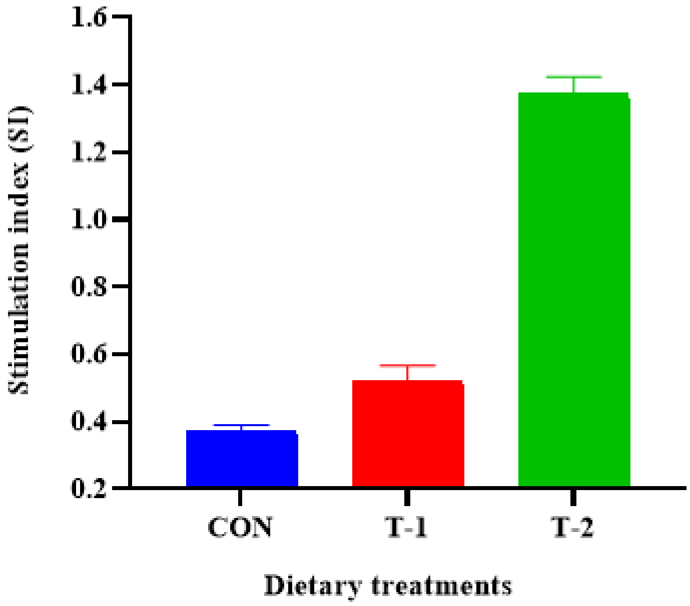

Figure 2

Effect of supplementation on CMI response in post-partum Tharparkar cows.

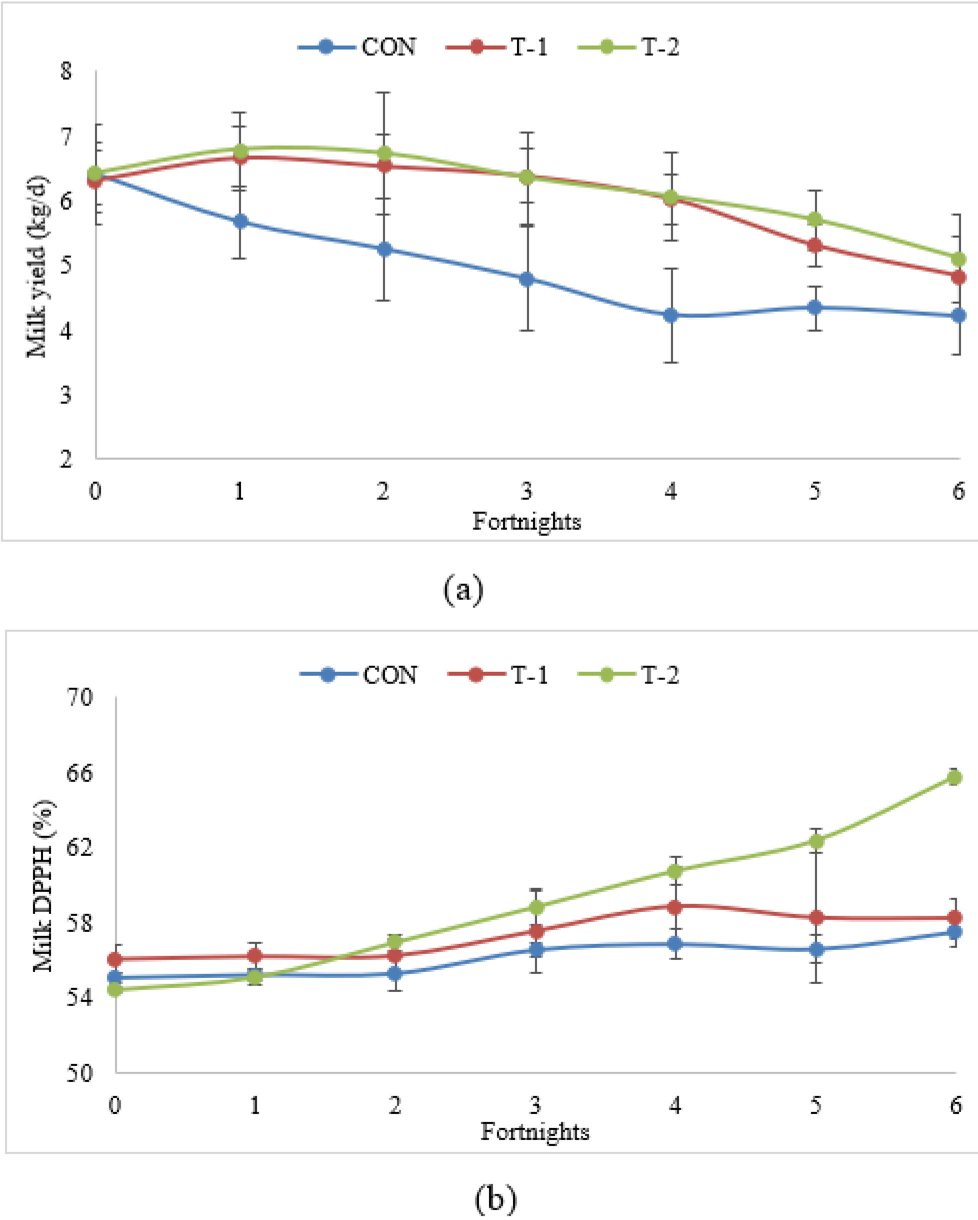

Figure 3

Effect of supplementation on (a) fortnightly milk yield and (b) DPPH activity in milk in post-partum Tharparkar cows

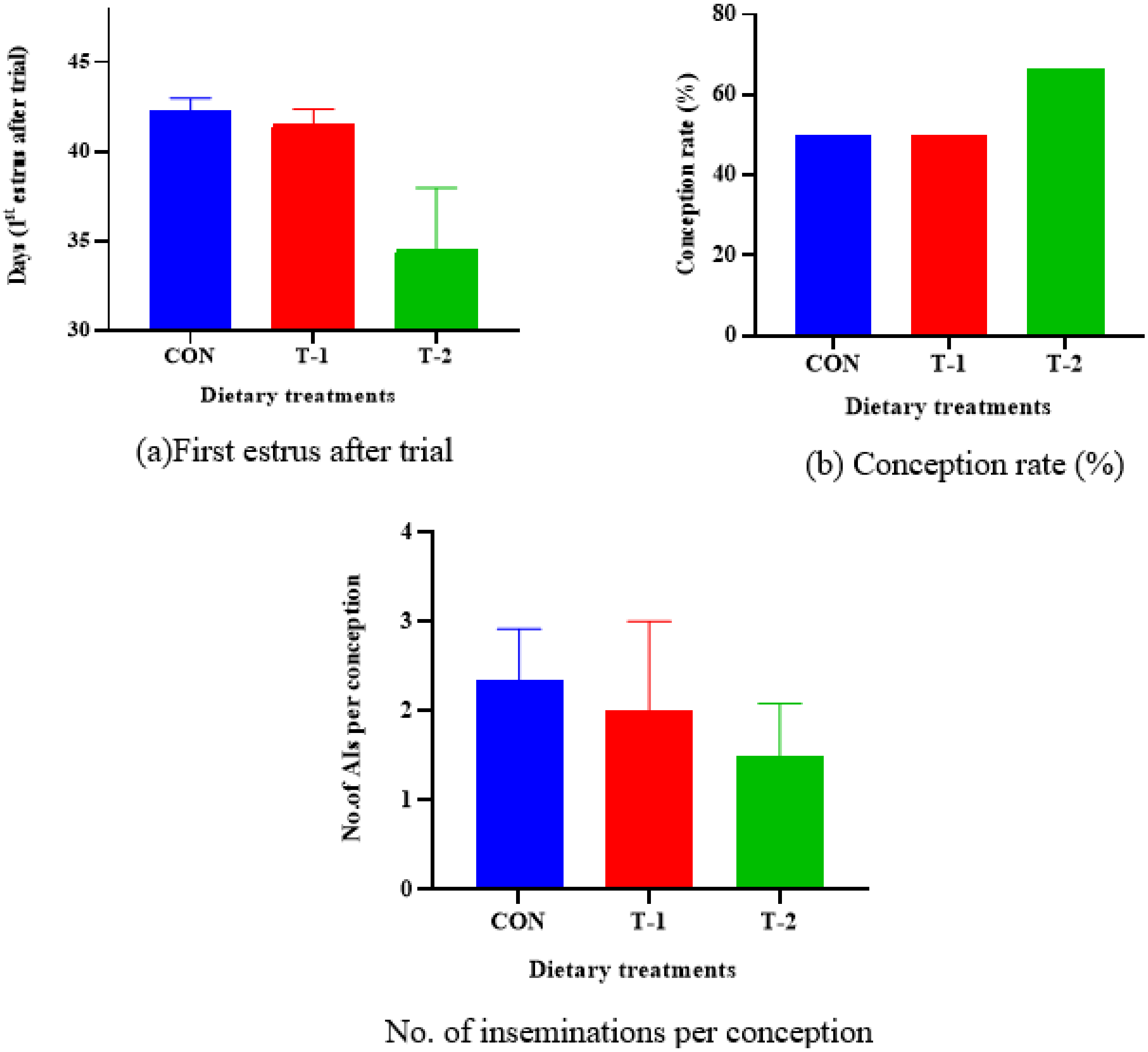

Figure 4

Effect of supplementation on reproductive performance in post-partum Tharparkar cows-