



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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ABSTRACT

Background: The exploratory trial explored the effects of a high plane of nutrition in combination with herbal feed additive on feed intake, metabolic and hormonal profiles, antioxidant potential, immunity and productive and reproductive efficiency in postpartum Tharparkar cows.

Methods: The experiment was carried out on eighteen cows (30-60 d post calving), randomly allocated to three groups: A control group (CON) fed fresh green fodder, wheat straw and concentrate feed; T1 receives a 10% higher concentrate feed and T2 receiving the T1 diet plus a herbal supplement.

Result: The cows of T1 as well as T2 groups had substantially elevated DM and OM intake. The cows of T2 group exhibited reduced serum NEFA levels (99.39 vs. 103.80), enhanced antioxidant enzyme activities including GPx, CAT, SOD and total antioxidant potential, higher serum T3 as well as T4, lower cortisol and LPO and enhanced delayed type immunity. Both T1 and T2 groups had more daily milk yield, 4% FCM and ECM, while T2 exhibited higher DPPH action in milk. The cows in T2 group showed earlier estrus, higher conception rates and required fewer artificial inseminations.

Key words: *A. racemosus*, Milk production, Reproduction, Tharparkar, *T. cordifolia*, *W. somnifera*.

INTRODUCTION

Native cattle breeds such as Tharparkar are well adapted to harsh environmental conditions, possessing traits such as tolerance to low-quality feed, resistance to diseases and heat resilience, although they generally produce less milk and have shorter lactation durations (260 d). Overcoming these challenges requires targeted nutritional interventions during the transition period, particularly throughout the peri-parturient and early postpartum stages. These phases are characterized by profound hormonal alterations, metabolic stress, reduced dry matter intake and a negative energy balance. Consequently, cows mobilize body fat reserves, resulting in elevated plasma non-esterified fatty acid (NEFA) concentrations. Increasing the energy density of the diet during this critical period can enhance voluntary feed intake, improve energy status and body weight recovery and reduce circulating NEFA levels. Feeding high energy diet to fresh cows promoted early onset of estrus, increased ovulation and conception rate and lowered the number of inseminations (Ciccioli *et al.*, 2003). Correspondingly, Kang *et al.* (2015) reported increased digestibility of CP, NDF and ADF in buffaloes fed high protein diet. Beyond critical macronutrients, optimal animal nutrition comprises of suitable supplementation of energy, protein, vitamins, minerals and tactical use of feed additives. Herbal feed additives have garnered attention due to their natural, non-hormonal and eco-friendly properties and with effective feed management,

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these additives containing *Withania somnifera* and *Asparagus racemosus* can enhance animal performance by modulating physiological functions, bolster immune responses through their antimicrobial and immune-activating properties, show galactopoietic and reproductive benefits including enhanced estrus expression, improved first-service conception rates and reduced services per conception (Afzal *et al.*, 2021). Hence, access to high plane of nutrition with herbal feed additives is believed to enhance the overall performance of the indigenous cows. In view of the foregoing, the present study was conducted to assess the impact of supplementing a high plane of nutrition with herbal feed additive on the intake, metabolic profile, immunity and antioxidant

potential, lactation and reproductive efficiency of Tharparkar cows.

MATERIALS AND METHODS

Study location

The experiment was carried out at the C&B Farm of ICAR-Indian Veterinary Research Institute, Izatnagar, India, located at 28.20°N latitude, 79.24°E longitude and 172 m above sea level. All experimental procedures were approved by IAEC (approval no. IAEC/20.06.2024/L2) and conducted in accordance CPCSEA.

Formulation of herbal feed additive

Fresh stems of *Tinospora cordifolia*, roots of *Withania somnifera* and *Asparagus racemosus* were procured from the ICAR-Indian Veterinary Research Institute (IVRI) campus and nearby local markets. The plant materials were shade-dried, pulverized into a fine powder and stored in airtight vessels until use.

Experimental design and management

Tharparkar is an indigenous dual-purpose *Bos indicus* breed originating from the arid regions of the Thar desert in Rajasthan and Gujarat, India. Owing to its excellent adaptation to hot and dry environments, the breed is widely recognized for its utility in both milk production and draught work. Under suitable management conditions, cows typically produce 1,800-2,600 L of milk per lactation with a milk fat content 4.5-5.0%. Eighteen Tharparkar cows (30-60 d postpartum), with an mean BW of 340.3±5.5 kg and avg. milk yield of 6.4±0.3 kg/d, were allotted to 3 treatment groups in a completely randomized design. The CON fed fresh green fodder, wheat straw and concentrate mixture as per ICAR (2013); T1 receives a 10% higher concentrate mixture and T2 receiving the T1 diet along with herbal feed additive (*T. cordifolia*, *W. somnifera*, *A. racemosus* in 2:1:1 ratio) at 150 mgkg⁻¹ BW. All the cows were fed individually and dosages of herbal additive were attuned fortnightly based on BW. The herbal feed additive dosage was decided based on the previous studies in our laboratory.

Milk production and milk quality

Milking was performed twice daily and pre-milking hygiene procedures were followed. Daily milk production was noted during the 90-d trial. Fortnightly, 100 mL milk samples were drawn for investigation using an ultrasonic analyzer. Milk energy values were calculated as 4% FCM using Gaines (1928) and ECM by Tyrrell and Reid (1965).

Blood collection

Blood samples (~10 mL) from all the cows were withdrawal on 0, 45 and 90 d before feeding and watering via jugular venipuncture. Half volume was taken in serum vacutainers and rest of the blood was transferred in heparinized vacutainers for LTT. Serum levels of NEFA, urea and cholesterol were investigated with Coral Clinical Systems diagnostic kits.

Feeds and herb analysis

The samples of feed, fodder, herbal powders, leftovers and dung samples were investigated for OM, EE, CP and total ash (TA) as per AOAC (2012). ADF and NDF were determined as per Van Soest *et al.* (1991). Herbal powders were 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 total antioxidant capacity (TAOC) by DPPH assay.

Hormonal, antioxidant and immune profile

Serum tri-iodothyronine (T3) and thyroxine (T4) were quantified using BIOGENIX ELISA kits, while progesterone levels were measured using Bovine Progesterone ELISA kits. Serum cortisol, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were estimated using ELISA kits. Lipid peroxidation (LPO) was assessed by the TBARS method and Cell-mediated immunity (CMI) was measured via the LTT.

Reproductive performance

Estrus detection was performed twice daily using a trained vasectomized bull. Animals exhibiting estrus were inseminated artificially and pregnancy was confirmed by trans-rectal palpation 60 d after insemination. Reproductive indices, including time to onset of estrus, services per conception and conception rate, were subsequently recorded.

Statistical analysis

All data were statistically analyzed using IBM SPSS Statistics version 26.0, following the procedures described by Snedecor and Cochran (1994). One-way ANOVA was performed to determine the effect of treatments on the measured variables. Significant differences among treatment means were further assessed using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Chemical composition of feeds and herb

The chemical composition of wheat straw, concentrate mixture and green maize fodder (Table 1) was in accordance with Chaudhary *et al.* (2021). Chemical composition of *T. cordifolia*, *W. somnifera* and *A. racemosus* was within the normal range as reported by earlier workers (Chavan *et al.*, 2021; Verma *et al.*, 2024).

Phytochemicals in herbs

The substantially higher TPC and TFC values were found in *T. cordifolia* relative to *W. somnifera* and *A. racemosus* (Table 2). These values were within the reported standard ranges (Royani *et al.*, 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*. Such discrepancies in phyto-

constituent levels may be ascribed to variations in extraction protocols as well as ecological factors, including soil type, climate, sunlight exposure and geographical variation (Nobossé *et al.*, 2018). The IC_{50} values were 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 enhanced antioxidant activity (Olugbami *et al.*, 2014).

Feed intake

DM and OM intake were higher ($P < 0.01$) in T1 and T2 groups, consistent with earlier reports that increased energy density of rations or concentrate levels enhanced DM intake in ruminants (Hailu *et al.*, 2011; Tufarelli *et al.*, 2011; Chaudhary *et al.*, 2020). Moreover, supplementation of *A. racemosus* @ 125 and 150 mg kg^{-1} BW led to higher DM intake in Sahiwal heifers (Kumawat *et al.*, 2017). Mir *et al.* (2015) reported significant increase in DMI in lactating Murrah buffaloes fed with *T. cordifolia* @ 120 gd^{-1} . The elevated DMI was likely associated with increased concentrate intake, leading to greater availability of fermentable carbohydrates and protein that promoted more efficient rumen fermentation.

Metabolic profile

Early post-partum cows frequently suffer with negative energy balance due to enhanced energy requirements and

reduced DMI (Bradford *et al.*, 2015). The noteworthy decline of NEFA levels in T2 group ($P = 0.002$) aligned with that of Sharma *et al.* (2014), who reported decreased NEFA levels in Karan Fries cows given *W. somnifera*, *A. racemosus* and *T. cordifolia* mixture (200-250 mg kg^{-1} BW). These herbs could inhibit lipolysis, enhance lipid metabolism and improve metabolic status of cows (Hashemzadeh-Cigari *et al.*, 2015). Serum urea and cholesterol levels were uninfluenced and were coherent with Sharma *et al.* (2018). However, Kumar *et al.* (2014) observed reduced cholesterol in Karan Fries cows with *A. racemosus* (100 mg kg^{-1} BW). Overall, the herbal feed additive with high plane of nutrition maintained stable homeostasis without altering the blood biochemical profile.

Serum hormonal profile

There was a substantial ($P < 0.05$) rise in serum T3, T4 and progesterone (Table 3). Previous reports (Patel *et al.*, 2016; Rajneesh *et al.*, 2020) showed that supplementation of *T. cordifolia* based herbal mixture positively affected serum T4 level in buffalo calves. The positive effect of addition of herbal feed additive could be explained by thyroid regulating and stimulating effect of *T. cordifolia* and Withaferin A in *W. somnifera* (Tewari and Tewari 2020) and the higher conception rate in T2 group. The reduced serum cortisol level was likely due to reduction in adrenocorticotrophic hormone caused by *W. somnifera* and *A. racemosus* (Singh *et al.*, 2023).

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

Table 2: Total phenolic, flavonoid and IC_{50} values of herbs.

Attributes (%)	<i>T. cordifolia</i>	<i>W. somnifera</i>	<i>A. racemosus</i>	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 (mg g^{-1} TAE)	7.42 ^c	5.05 ^b	3.11 ^a	0.53	≤ 0.001
TFC (mg g^{-1} QE)	3.83 ^c	2.21 ^b	1.46 ^a	0.30	≤ 0.001
IC_{50} ($\mu g mL^{-1}$)	45.39 ^a	55.05 ^b	60.13 ^c	2.16	≤ 0.001

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

Antioxidant profile

The present results exhibited higher ($P \leq 0.001$) SOD, CAT, GPx and TAOC, along with a reduced ($P = 0.04$) LPO in T2 group (Fig 1). These findings are compatible with Vaswani *et al.* (2022) and Oviya (2023) who reported elevated SOD activity while reduced LPO levels in Sahiwal heifers and increased SOD, GST and GPx activity in goat kids by supplementation of *T. cordifolia* at 1% of DMI and herbal mixture (*W. somnifera* and *A. racemosus*; 2:1) @150 mg kg⁻¹ BW, respectively.

The antioxidant consequences of *W. somnifera* and *A. racemosus* are attributed to its bioactive constituents, notably withanolides (Sitoinosides VII-X), withaferin A, racemofuran, asparagine A and racemosol (Ojha and Arya, 2009; Wiboonpun *et al.*, 2004). Moreover, the flavonoids present in these herbs regulate the expression of gamma-glutamylcysteine synthetase (Moskaug *et al.*, 2005). Therefore, the beneficial effects may result from the synergistic action of phytoconstituents in the herbal mixture.

CMI response

The cows in T2 group showed a noticeably enhanced ($P \leq 0.001$) CMI response (Fig 2). This suggests a potent immunomodulatory effect of the herbal feed additive which is in line with Mallick and Prakash (2011) who stated considerably greater lymphocyte counts in *T. cordifolia* fed (@ 60 g d⁻¹ for 45 d before parturition and @ 120 g d⁻¹ for 45 d post-calving) Karan Fries cows. The immunomodulatory action of herbal feed additive is primarily endorsed to its active components like α -D-glucan, Withaferin A, 3- β -hydroxy-2, 3-dihydro withanolide F, Shatavaroside A and Shatavaroside B (Thakur *et al.*, 2021). Moreover, carotenoids existing in both *A. racemosus* and *W. somnifera* have been stated to enhance the proliferation of T and B lymphocytes, stimulate macrophage activity and promote the production of pro-inflammatory cytokines (Milani *et al.*, 2007). Thus, the synergistic action of these phytoconstituents likely contributed to the substantially elevated CMI response.

Table 3: Effect on serum biochemical and hormonal profile.

Periods	Treatment			SEM	P value
	CON	T1	T2		
NEFA, pg mL ⁻¹	105.47 ^b	103.80 ^b	99.39 ^a	5.08	0.002
Serum urea, mg dL ⁻¹	29.11	29.45	29.20	0.35	0.92
Cholesterol, mg dL ⁻¹	130.05	130.57	130.38	0.36	0.83
T ₃ , ng mL ⁻¹	2.59 ^a	2.63 ^a	3.10 ^b	0.05	≤ 0.001
T ₄ , ng mL ⁻¹	49.78 ^a	51.92 ^a	57.50 ^b	1.50	≤ 0.001
Cortisol, ng mL ⁻¹	7.55 ^b	7.51 ^b	7.01 ^a	0.16	0.002
Progesterone, ng mL ⁻¹	2.17 ^a	2.06 ^a	3.36 ^b	0.42	0.026

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

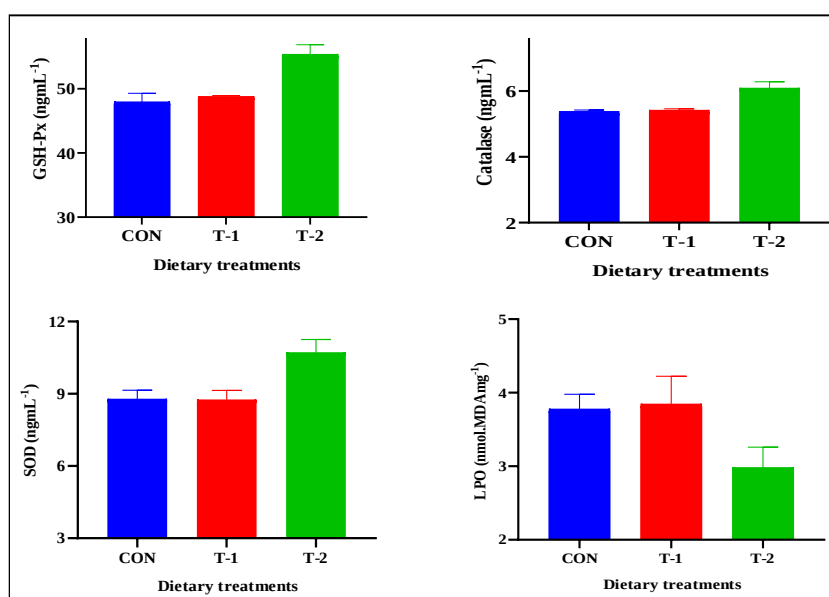


Fig 1: Antioxidant indices in Tharparkar cows.

Lactation performance

Supplementation in the T1 and T2 groups significantly ($P \leq 0.001$) increased daily and total milk yield (Table 4), 4% FCM, ECM (kg day^{-1}) and fat and protein yields (g day^{-1}), without affecting milk composition. Our results line up with Choudhary *et al.* (2024) who reported increased milk production, similar milk composition and improved 4% FCM in milching cows with *T. cordifolia* and *A. racemosus* feeding. Dori *et al.* (2026) and Prasanna *et al.* (2023) also reported similar milk composition in cows fed dried moringa stem. In contrary, Sharma *et al.* (2017) observed similar milk yield in crossbred cows fed *T. cordifolia* stem powder. The role of dietary energy and protein in supporting lactation is well established with enhanced production linked to increased availability of rumen-fermentable carbohydrates, degradable N and efficiency of microbial protein synthesis (Zhou *et al.*, 2015). Thus, the improved milk yield was likely due to higher concentrate feeding.

Furthermore, the enhanced milk DPPH activity in T2 group was supported by Stobiecka *et al.* (2023), who witnessed enhanced antioxidant potential following herbal administration. Herbal feed additives are positively correlated with DPPH radical scavenging activity (Aryal *et al.*, 2019).

Reproduction performance

The cows in the T2 group exhibited an earlier onset of estrus ($P=0.043$), a higher conception rate ($P \leq 0.01$) and required significantly ($P=0.01$) less artificial inseminations per conception (Fig 3). These findings align with Kumar *et al.* (2012) who documented earlier onset of estrus and improved conception rates in post-partum cows provided *A. racemosus* @ $125\text{--}200 \text{ mg kg}^{-1} \text{ BW}$. Similarly, Muwal *et al.* (2020) reported reduced service periods, services

per conception and earlier estrus induction in dairy cows. Likewise, Mallick and Prakash (2011) reported earlier onset of estrus in post-partum Karan Fries cows by dietary inclusion of *T. cordifolia* stem powder @ 60 g d^{-1} which was mainly due to its immunomodulatory properties that promote early resumption of ovarian cyclicity. Singh *et al.* (2022) reported reduced service periods and improved conception rates in Sahiwal cows given polyherbal mixture containing *W. somnifera* @ 20 g d^{-1} from 3 d pre-partum to 21 d post-partum. Previous studies have confirmed that *A. racemosus* supports postpartum uterine involution and reproductive health through stimulation of epithelial cell growth and ovarian function. Thus, the presence of bioactives in herbal feed additive could have enhanced the reproductive performance by supporting follicular development and hormonal balance (Kumar *et al.*, 2014).

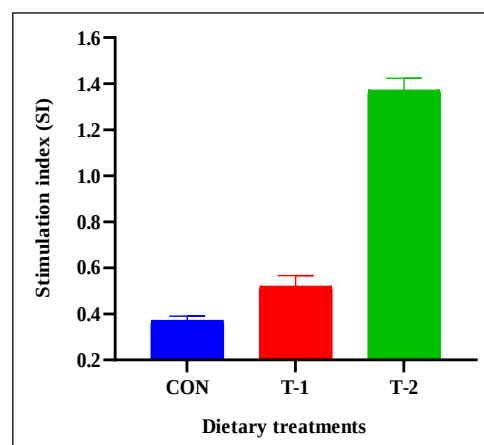


Fig 2: CMI response in Tharparkar cows.

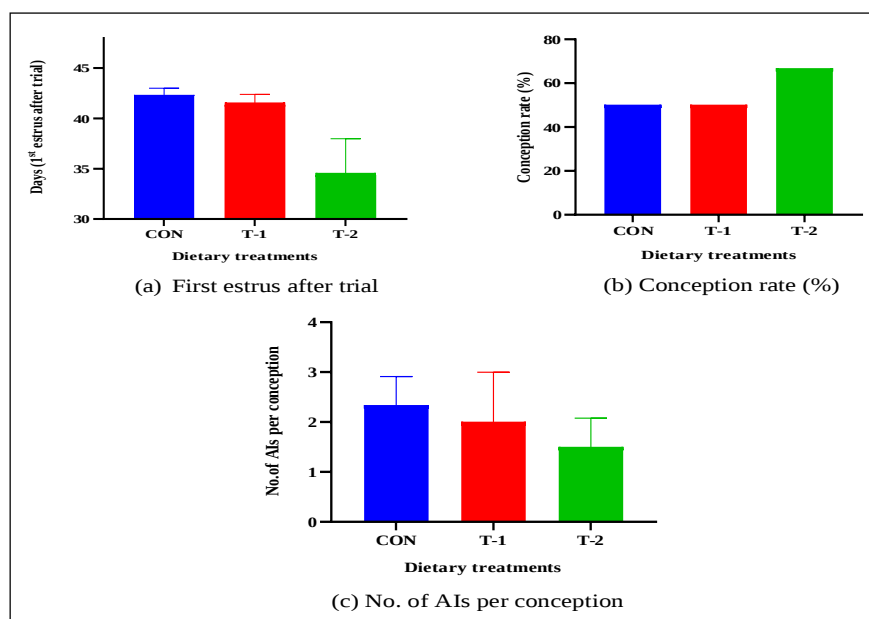


Fig 3: Effect on reproductive performance.

Table 4: Effect on intake and lactation performance.

Attributes	Treatment			SEM	P value
	CON	T1	T2		
Intake, g kg⁻¹ W^{0.75}					
DM	109.38 ^a	113.06 ^b	112.74 ^b	0.58	0.007
DM	99.12 ^a	104.94 ^b	104.64 ^b	0.76	≤0.001
Daily milk yield, kg d ⁻¹	4.99 ^a	6.01 ^b	6.16 ^b	0.02	0.001
4% FCM, kg d ⁻¹	5.45 ^a	6.59 ^b	6.76 ^b	0.15	0.001
ECM, kg d ⁻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, g d ⁻¹	230.26 ^a	278.95 ^b	286.32 ^b	6.54	0.001
Protein yield, g d ⁻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
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

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

CONCLUSION

Supplementation of a polyherbal feed additive containing *Tinospora cordifolia*, *Withania somnifera* and *Asparagus racemosus* (2:1:1) at 150 mg kg⁻¹ body weight, in combination with a high plane of nutrition achieved through a 10% increase in concentrate feed, significantly enhanced lactation and reproductive performance in early postpartum Tharparkar cows. The dietary intervention also improved feed intake, antioxidant status, hormonal profile and cell-mediated immune response, while reducing serum cortisol and NEFA levels. These findings indicate that integrating a high plane of nutrition with a polyherbal feed additive is an effective nutritional strategy for improving the health, metabolic status and productive and reproductive performance of postpartum native dairy cows.

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Conflict of interest

The authors declare no conflict of interest.

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