



Effect of *Asparagus racemosus* Root Powder Supplementation on Oxidative Stress and Hepatic Enzyme Markers in Jersey Crossbred Cows During Summer

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ABSTRACT: Summer heat stress induces lipid peroxidation and challenges hepatic homeostasis in crossbred dairy cows, thereby reducing productive performance. *Asparagus racemosus* (Shatavari) root has documented antioxidant and hepatoprotective activity, yet controlled summer-trial data in Jersey crossbreds are sparse. The present study evaluated whether oral *A. racemosus* root powder at 200 mg/kg body weight/day attenuates plasma malondialdehyde (MDA) and stabilises gamma-glutamyltransferase (GGT) and aspartate aminotransferase (AST) in lactating Jersey crossbred cows under Indian summer. Twelve healthy lactating cows balanced for parity, body weight and lactation stage were randomly allocated to control (C, n = 6) and experimental (E, n = 6) groups. The E group received the supplement for four consecutive weeks during April–May 2020. Jugular blood was sampled weekly at 06:00 h. Plasma GGT, MDA and AST were quantified. Data were analysed by Student's t-test and one-way ANOVA. All markers remained within reference ranges. Plasma MDA and AST showed non-significant numerical reductions in Experiment versus Control during weeks 3 and 4; GGT did not differ between groups. *A. racemosus* directionally attenuated lipid peroxidation and AST without disturbing GGT, supporting *A. racemosus* as a herbal antioxidant adjunct for heat-stressed Jersey crossbreds.

Keywords: Shatavari, GGT, oxidative stress, malondialdehyde, hepatic enzymes, heat stress.

INTRODUCTION

Crossbred cattle contribute approximately 16 per cent of the total cattle milk production in India and yield an average of 6.75 litres per day, substantially higher than the 1.98 litres per day produced by indigenous cattle (Hegde, 2006; Singh *et al.*, 2008). Despite this productivity advantage, crossbred cows are more susceptible to environmental stress, disease, infertility and sub-fertility, with prolonged postpartum gap days and elevated service per conception remaining persistent field constraints (Muwal *et al.*, 2020a; Rao *et al.*, 1995). Even modest gains in per-animal milk yield translate to a meaningful national-level addition, emphasizing the importance of management interventions that preserve homeostasis during stressful production phases.

Heat stress imposes a considerable economic burden on dairy enterprises in tropical and sub-tropical regions. It depresses feed intake, rumination and nutrient absorption while elevating maintenance requirements (Beede & Collier 1986); driving heat-stressed lactating cows into a negative energy balance (Moore *et al.*, 2005; Shwartz *et al.*, 2009). Lipid peroxidation, indexed by plasma malondialdehyde (MDA), and perturbations in

hepatic enzymes such as gamma-glutamyltransferase (GGT) and aspartate aminotransferase (AST) together provide an accessible window onto this oxidative–hepatic axis (Castillo *et al.*, 2006; Hoffmann & Solter 2008; Janero, 1990; Ayemele *et al.*, 2021).

Asparagus racemosus is the widely used in traditional Indian medicine, owing to the presence steroidal saponins and sapogenins concentrated in the root (Bopana & Saxena 2007; Krishana *et al.*, 2005; Kumar *et al.*, 2014). Therapeutic applications of *A. racemosus* have been documented in the Indian and British Pharmacopoeias and in indigenous medical systems. Reported pharmacological actions include galactagogue and mammogenic activities (Behera *et al.*, 2013; Pandey *et al.*, 2005), antioxidant activity (Visavadiya & Narasimhacharya 2005; Wiboonpun *et al.*, 2004; Banerjee *et al.*, 2025), immunomodulation (Rege *et al.*, 1999), antistress effects (Kamat *et al.*, 2000) and digestive support (Dalvi *et al.*, 1990; Nair & Chanda 2006). *A. racemosus* has also been described as an aphrodisiac and galactopoeitic agent in dairy animals (Kumar *et al.*, 2010; Santosh *et al.*, 2011) and as a uterine

relaxant of value during late pregnancy (Kumar *et al.*, 2014).

Indiscriminate use of synthetic feed additives, hormones and chemicals to enhance milk production carries cost, residue and welfare concerns, motivating the exploration of safer herbal alternatives (Krishana *et al.*, 2005). Although several studies have documented gains in milk yield and reproductive performance in dairy cows supplemented with *A. racemosus* (Kumar *et al.*, 2010; Muwal *et al.*, 2020b; Santosh *et al.*, 2011), a systematic evaluation of its effects on plasma oxidative stress and hepatic enzyme markers in Jersey crossbreds exposed to Indian summer heat stress conditions is lacking. To address this gap, the present study evaluated oral *A. racemosus* root powder on plasma MDA, GGT and AST in lactating Jersey crossbred cows during peak summer. A systematic evaluation of its effects on plasma oxidative stress and hepatic enzyme markers in Jersey crossbreds exposed to documented Indian summer heat stress conditions is lacking.

MATERIAL AND METHODS

A. Experimental site and climate

Experimental animals were maintained at the institutional Livestock Farm Complex, Veterinary College and Research Institute (VCRI), Orathanadu, TANUVAS, situated in the Cauvery delta of Tamil Nadu, India between April and May 2020 under routine animal husbandry management. *A. racemosus* is listed in the Indian Pharmacopoeia as a herbal supplement; the study involved only dietary inclusion of the root powder, with no surgical or pharmacological intervention. The trial was conducted at the Livestock Farm Complex, Ambient minimum and maximum temperatures during the trial were 24°C and 41°C, respectively, with relative humidity ranging from 60 to 78 per cent. The Temperature Humidity Index (THI) was calculated as: $THI = (0.8 \times \text{ambient temperature}) + [(\text{per cent relative humidity} \div 100) \times (\text{ambient temperature} - 14.4)] + 46.4$ (Mader *et al.*, 2006). Weekly THI values ranged from 84.08 ± 0.29 to 84.63 ± 0.29 , confirming sustained heat-stress conditions throughout the trial (Du Preez *et al.*, 1990; Table 1).

Table 1: Weekly mean Temperature Humidity Index (THI) during the experimental period.

Sr. No.	Week	THI (Mean $\pm$ SE)
1	1	84.08 ± 0.29
2	2	84.63 ± 0.29
3	3	84.24 ± 0.32
4	4	84.08 ± 0.35

B. Experimental animals and design

Twelve healthy lactating Jersey crossbred cows in the third to fourth month of lactation were selected from the institutional herd. Animals were allocated to two groups of six animals each by simple random allocation, balanced for parity (Control = 3.33; Experimental = 3.00) and body weight (Control = 332.33 kg; Experimental = 334.50 kg). Sample size was determined based on the availability of homogeneous animals at the institutional farm. The experimental (E) group received

A. racemosus root powder at 200 mg/kg body weight/day, mixed with concentrate at 12:00 h, for four consecutive weeks, while the control (C) group received the same basal diet without supplementation. The dose was selected following Kumar *et al.* (2010). The design is summarised in Table 2.

Table 2: Experimental design and dose of *Asparagus racemosus* root powder.

Stage	Group	<i>A. racemosus</i> dose	Sample collection
Summer heat stress	Experimental (n = 6)	200 mg/kg BW/day	Weekly for 4 weeks

C. Housing and feeding

Animals were housed in well-ventilated sheds with asbestos roofing and a cement concrete floor sloped towards a drain to prevent dampness. Prophylactic measures against viral, bacterial, endoparasitic and ectoparasitic agents were implemented according to the farm health calendar. Feeding followed ICAR (2010) recommendations: 4–5 kg concentrate per day in two split feeds before milking; 3–4 kg dry fodder per day; and 20 kg green fodder per day. Animals consumed approximately 10–12 kg dry matter and 650 g digestible crude protein daily. Clean drinking water was provided ad libitum.

D. Plant material

Fresh roots of *A. racemosus* were procured from the Herbal Garden, Ethno-Veterinary Herbal Product Research and Development Centre, TANUVAS, Orathanadu, Thanjavur District, Tamil Nadu. The roots were harvested in August 2019 to ensure batch homogeneity across the entire trial; they were cleaned, shade-dried, powdered and stored in airtight containers until use.

E. Blood sampling

Approximately 6 mL of jugular blood was drawn aseptically by venipuncture at 06:00 h once weekly from each cow into vacutainers coated with 10 per cent ethylene diamine tetra acetic acid (EDTA). Plasma was separated by centrifugation at 3,500 rpm for 20 minutes and used for analysis.

F. Biochemical assays

Plasma gamma-glutamyltransferase (GGT) was estimated by the IFCC kinetic method using a commercial diagnostic kit (BioSystems, Spain) on an Eppendorf biospectrometer at 410 nm, following the prescribed assay protocol (IFCC, 2002). Plasma malondialdehyde (MDA), a marker of lipid peroxidation, was determined by the thiobarbituric acid (TBA) method of Yagi (1976). Lipid peroxidation was expressed as nmol/mL. Plasma aspartate aminotransferase (AST) was estimated by a kinetic assay using a commercial diagnostic kit (Arkray, India) on the biospectrometer at 340 nm, following Murray (1994).

G. Statistical analysis

Data are presented as mean $\pm$ standard error (SE) of six observations per group. Data were analysed by Student's t-test for between-group comparisons and by one-way analysis of variance (ANOVA) for within-group comparisons across weeks, following Snedecor and Cochran (1994). Analyses were performed using SPSS software version 20 (IBM Corp., Armonk, NY, USA). Significance was set at $p < 0.05$.

Table 3: Effect of *Asparagus racemosus* root powder on plasma GGT, MDA and AST in Jersey crossbred cows during summer (Mean $\pm$ SE; n = 6 per group).

Week	GGT (U/L)		MDA (nmol/mL)		AST (IU/L)	
	Control	Experimental	Control	Experimental	Control	Experimental
0	17.26 $\pm$ 1.51	17.48 $\pm$ 1.25	8.92 $\pm$ 0.97	9.36 $\pm$ 0.60	68.20 $\pm$ 4.25	70.61 $\pm$ 2.54
1	17.43 $\pm$ 1.53	17.54 $\pm$ 1.15	9.06 $\pm$ 0.85	9.59 $\pm$ 0.61	62.38 $\pm$ 3.85	70.84 $\pm$ 3.18
2	19.73 $\pm$ 2.38	19.35 $\pm$ 1.52	8.49 $\pm$ 0.79	7.99 $\pm$ 1.10	55.90 $\pm$ 8.15	62.41 $\pm$ 7.92
3	19.77 $\pm$ 1.96	20.78 $\pm$ 1.25	10.08 $\pm$ 0.86	8.37 $\pm$ 0.95	70.50 $\pm$ 4.54	69.64 $\pm$ 4.63
4	24.29 $\pm$ 2.79	23.40 $\pm$ 1.74	10.44 $\pm$ 0.72	9.92 $\pm$ 0.49	67.33 $\pm$ 3.32	65.63 $\pm$ 3.52

No significant difference between groups or within groups ($p > 0.05$).

B. Plasma gamma-glutamyltransferase (GGT)

Mean plasma GGT concentrations in the control and experimental groups across the four weeks of the summer trial are presented in Table 3. At week one, GGT values were 17.43 $\pm$ 1.53 and 17.54 $\pm$ 1.15 U/L in the control and experimental groups, respectively, representing a non-significant numerical increase in the experimental group. At week two, the corresponding values were 19.73 $\pm$ 2.38 and 19.35 $\pm$ 1.52 U/L (a non-significant decrease in E). At week three, values were 19.77 $\pm$ 1.96 and 20.78 $\pm$ 1.25 U/L (a non-significant increase in E). At week four, values were 24.29 $\pm$ 2.79 and 23.40 $\pm$ 1.74 U/L (a non-significant decrease in E). No significant difference was detected within either group across weeks.

The plasma GGT levels observed in the current investigation remained within the normal range and are in agreement with earlier reports in dairy cattle (Antonic-Svetina *et al.*, 2011; Omur *et al.*, 2016; Watanabe *et al.*, 2014). In both groups, GGT was lowest at week one and rose progressively to a peak at week four during summer. The physiological role of GGT centres on glutathione metabolism: glutathione is critical to antioxidant defence, nutrient metabolism and the regulation of cellular events such as gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response. By modifying glutathione availability, GGT indirectly participates in these functions and serves as a biomarker of hepatic function (Hoffmann & Solter, 2008). Summer stress induces free-radical generation, and the antioxidant defence systems of the body including the glutathione system are mobilised to neutralise them. The progressive rise in GGT observed in both groups is therefore consistent with an adaptive recruitment of glutathione-dependent antioxidant capacity under sustained heat load.

C. Plasma malondialdehyde (MDA)

Mean plasma MDA values across the four-week summer trial are presented in Table 3. At week one, MDA was

RESULTS AND DISCUSSION

A. Climatic load during the trial

Weekly mean THI values ranged from 84.08 $\pm$ 0.29 to 84.63 $\pm$ 0.29 (Table 1), placing the animals continuously within the moderate-to-severe heat-stress zone defined by Du Preez *et al.* (1990).

9.06 $\pm$ 0.85 and 9.59 $\pm$ 0.61 nmol/mL in the control and experimental groups, respectively (a non-significant increase in E). At week two, values were 8.49 $\pm$ 0.79 and 7.99 $\pm$ 1.10 nmol/mL (a non-significant decrease in E). At week three, values were 10.08 $\pm$ 0.86 and 8.37 $\pm$ 0.95 nmol/mL, representing a non-significant absolute reduction of 1.71 nmol/mL in E. At week four, values were 10.44 $\pm$ 0.72 and 9.92 $\pm$ 0.49 nmol/mL (a non-significant decrease in E). No significant difference was detected within either group across weeks.

MDA is one of several low-molecular-weight end-products generated during the radical-mediated decomposition of polyunsaturated fatty acids (Janero, 1990). Lipids are among the most susceptible substrates to free-radical damage, and biomarkers of lipid peroxidation are considered the best indicators of oxidative stress (Georgieva, 2005). MDA also indexes the imbalance between pro-oxidant and antioxidant capacities in the body (Obućinski *et al.*, 2019). The values observed in the present investigation are in agreement with those of Elshahawy and Abdullaziz (2017) but are lower than those reported by Hady *et al.* (2018) in crossbred cows and buffaloes during the Egyptian summer. In both groups of the present study, MDA was lowest at week two and rose to a peak by week four, consistent with the cumulative effect of sustained heat exposure. Summer stress triggers elevated cortisol, which drives gluconeogenesis using lipids as a substrate and mobilises body fat; the concurrent generation of oxidants targets these lipids to form MDA (Hady *et al.*, 2018); a positive THI–cortisol correlation and a negative THI–total-antioxidant-capacity correlation have since been confirmed in summer-stressed Holsteins (Altyeb *et al.*, 2025). The numerically lower MDA in the experimental group from week two onwards is consistent with the well-documented antioxidant activity of *A. racemosus* (Bopana & Saxena 2007; Visavadiya & Narasimhacharya 2005; Wiboonpun *et al.*, 2004) and with broader evidence that antioxidant supplementation

across the transition period reduces oxidative risk (Abuelo *et al.*, 2013; Castillo *et al.*, 2006).

D. Plasma aspartate aminotransferase (AST)

Mean plasma AST values across the four-week summer trial are presented in Table 3. At week one, AST was 62.38 ± 3.85 and 70.84 ± 3.18 IU/L in the control and experimental groups, respectively (a non-significant increase in E). At week two, values were 55.90 ± 8.15 and 62.41 ± 7.92 IU/L (a non-significant increase in E). At week three, values were 70.50 ± 4.54 and 69.64 ± 4.63 IU/L (a non-significant decrease in E). At week four, values were 67.33 ± 3.32 and 65.63 ± 3.52 IU/L (a non-significant decrease in E). No significant difference was detected within either group across weeks.

The plasma AST levels observed in this investigation remained within the normal range and are in agreement with earlier reports (Elshahawy & Abdullaziz 2017; Hashemzadeh-Cigari *et al.*, 2015; Watanabe *et al.*, 2014). In both groups, AST was lowest at week one and reached a peak at week three, paralleling the increasing thermal load. The non-significant reduction in AST observed in the experimental group at weeks three and four is consistent with the hepatoprotective effects of *A. racemosus* reported in rodent models, where its extracts significantly reduced elevated levels of AST, ALT, and alkaline phosphatase in chemically induced hepatic damage (Agrawal *et al.*, 2008; Kamat *et al.*, 2000) and ameliorated oxidative stress and inflammatory biomarkers, including IL-6, that are frequently elevated in heat-stressed ruminants (El-senosi *et al.*, 2021). Obućinski *et al.* (2019) further noted that high-yielding cows are particularly susceptible to oxidative stress and liver injury during peak lactation, which often coincides with the metabolic demands of summer—a context in which the antioxidant action of *A. racemosus* (Bopana & Saxena 2007) is biologically plausible.

Considered jointly, the directional reductions in MDA and AST, alongside an unchanged GGT profile, suggest that *A. racemosus* at 200 mg/kg body weight/day supports lipid-peroxidation defence and hepatocyte integrity without disrupting the glutathione-recycling axis represented by GGT. The absence of statistical significance across markers should be interpreted in the light of the limited group size, which constrains statistical power, rather than as evidence against a biological effect. Recent work confirms that summer heat stress simultaneously elevates plasma MDA and modulates antioxidant-enzyme activity in dairy cows (Akköse & Vural 2026). The consistent direction of change across three independent markers—MDA falling, AST falling and GGT undisturbed—under documented heat-stress conditions (THI 82–86) lends biological plausibility to the antioxidant and hepatomodulatory action of *A. racemosus* in lactating Jersey crossbreds. Important limitations should be acknowledged: the trial was confined to a single summer season; concurrent measurement of glutathione, total antioxidant capacity and inflammatory markers such as IL-6 would have permitted more comprehensive redox–hepatic phenotyping.

CONCLUSIONS

Under sustained summer heat load, oral supplementation with *Asparagus racemosus* root powder at 200 mg/kg body weight/day directionally lowered plasma malondialdehyde and aspartate aminotransferase concentrations without disturbing gamma-glutamyltransferase homeostasis in lactating Jersey crossbred cows. All three markers remained within species-typical reference ranges throughout the trial. Although the between-group differences did not reach statistical significance, the consistent direction of effect across markers supports *A. racemosus* as a promising herbal antioxidant and hepatomodulatory adjunct for heat-stressed crossbred dairy cattle.

FUTURE SCOPE

The findings of this preliminary trial warrant confirmation in larger, multi-season studies designed with adequate statistical power. Future work may expand the redox panel to include reduced glutathione, total antioxidant capacity and inflammatory markers such as IL-6 and couple biochemical endpoints with reproductive performance.

Author Contributions. Conceived and designed the study: Guru D. V. Pandiyan, V. Leela. Conducted the experiment and collected the data: Guru D. V. Pandiyan, M. Ramachandran, V. Ranganathan. Performed the laboratory analyses: Guru D. V. Pandiyan, P. Visha. Performed the statistical analysis and data interpretation: Guru D. V. Pandiyan, V. Leela, S. Eswari. Drafted the manuscript: Guru D. V. Pandiyan. Critically revised the manuscript: V. Leela, S. Eswari, M. Ramachandran, V. Ranganathan, P. Visha.

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Conflict of Interest. Authors have declared that no competing interests exist.

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