

# *Moringa oleifera* as a concentrate replacer for enhancing immune status and ameliorating stress of Barbari goats during thermal stress

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## Abstract

To evaluate the effect of replacing 50% of concentrate feed with *Moringa oleifera* on immune status and reducing levels of stress markers, ten healthy Barbari goats were selected and divided equally into two groups (control and treatment) based on average body weight ( $\sim 20 \pm 0.2$  kg). Various environmental parameters were recorded to calculate the Temperature-Humidity Index (THI) during different seasons. Blood samples were collected to estimate stress biomarkers and immune parameters. Findings revealed that plasma cortisol levels were significantly lower ( $p < 0.05$ ) in the treatment group compared to the control group during all the season. Furthermore, plasma levels of triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ) were significantly higher ( $p < 0.05$ ) in the treatment group during winter than in the control group and during the hot dry season. Plasma catalase and leptin levels were also significantly higher ( $p < 0.05$ ) in the treatment group during the hot dry and winter season compared to the control group respectively. In terms of immune parameters, plasma interleukin-2 (IL 2) levels were significantly lower ( $p < 0.05$ ) in the treatment group than in the control group, while interleukin-10 (IL 10) levels showed an opposite trend. Overall, the results of the study indicate that replacing 50% of concentrate feed with *Moringa oleifera* is beneficial for alleviating the adverse effects of thermal stress and enhancing the immune status of Barbari goats.

**Key words:** Goats, *Moringa oleifera*, Seasons, Stress Biomarker, Temperature Humidity Index

## Introduction

Goats play an important role in rural economy of underdeveloped and developing countries of Africa, Asia and Eurasia region (Ogbuewu *et al.*, 2016; Salvan *et al.*, 2021, Koloskova *et al.*, 2021). According to the 20<sup>th</sup> Livestock Census of India, the goat population in the country stands at 148.88 million, which represents an increase of 10.1% compared to the previous census (DAHD 2019). India boasts a rich diversity of goat breeds, which are distributed across various agro-ecosystems throughout the country. Goats, often referred to as "the poor man's cow," are well-suited to achieve the interdependent goals of reducing poverty, increasing food availability, generating employment, and boosting rural incomes for many small-scale farmers in developing countries (Waiz *et al.*, 2018). Goats are more resilient to disease and have a greater range of adaptation (Khan *et al.*, 2013). The worldwide rise in temperature due to global warming and climate change has significant implications for plant and animal health, as well as production systems (Wilson *et al.*, 2014). In recent years, researchers have investigated alternative feed resources that offer potential nutritional and medicinal benefits to enhance the resilience and immune response of goats under varying seasonal conditions (Silva *et al.*, 2022). Healthy and cost-effective sources of protein include plant leaf meals, forage trees, saltbush, and shrubs. One notable example is *Moringa oleifera*, commonly known as the drumstick tree. *Moringa* is a versatile plant renowned for its high nutritional value and rich content of bioactive compounds, including vitamins, minerals, antioxidants, and phytochemicals (Misra and Sinha 2023; Leitanthem *et al.*, 2022). The inclusion of *Moringa oleifera* leaves as a non-traditional fodder in animal diets presents a promising avenue to improve both animal and human health. These leaves are rich in bioactive compounds, including antioxidants and vitamin C, which can bolster the oxidative status and immune function of livestock. It has been recognized as a valuable feed resource for livestock due to its positive effects on growth, feed efficiency, and immune function (Leone *et al.*, 2015).

The adaptability of animals to hot or cold climatic conditions relies on the integration of various physiological systems, including the respiratory, circulatory, nervous, excretory, endocrine, and enzymatic systems. However, differences in adaptability are observed not only between species but also among breeds and even among individuals within the same breed. These differences are crucial for maintaining productive capacity under thermal stress (Marai and Haebe 2010). Thermal stress encompasses both heat stress, experienced during extreme summer conditions, and cold stress, encountered during extreme winter conditions (Gupta *et al.*, 2013a; Jyotiranjana *et al.*, 2017). Although goats are generally more resistant to thermal stress, they still experience heat and cold stress when temperatures fall outside their comfort zone. Thermal stress triggers a complex response that is fundamental for the preservation of cell survival (Gupta *et al.*, 2013b). The hypothalamic-pituitary-adrenal (HPA) axis is primarily responsible for controlling the stress response in animals, with corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and glucocorticoids being its key products (Sejian *et al.*, 2010). The activation of the HPA axis may result in enhanced production of glucocorticoids like cortisol, which is indicated as the major stress-relieving hormone and also identified as a reliable biomarker for assessing the severity of stress (Shaji *et al.*, 2017; Afsal *et al.*, 2018). The HPA axis is managed by neurons located in the paraventricular nucleus of the hypothalamus, which synthesizes and releases CRH into the pituitary gland. Thyrotropin-Releasing Hormone (TRH) stimulates the anterior pituitary gland to release Thyroid-Stimulating Hormone (TSH). TSH, in turn, stimulates the synthesis and release of thyroid hormones from the thyroid gland. The two main forms of thyroid hormones produced are T3 and T4. The thyroid hormones play a crucial role in maintaining homeostasis of energy and protein metabolism, regulating thermoregulation, supporting growth, and enhancing productivity (Farooq *et al.*, 2010). Considering the significance of *Moringa oleifera* as a "wonder tree," the present study was undertaken to assess its potential in replacing traditional concentrates in the diet of Barbari goats. The aim was to evaluate the effectiveness of *Moringa oleifera* in alleviating thermal stress and enhancing the immune status of these animals.

## Materials and methods

### Study Location

The study was conducted at the Goat Section of the Livestock Research Centre (LRC) of the ICAR - National Dairy Research Institute (NDRI) in Karnal, Haryana, India, situated at 29°41' N latitude and 76°59' E longitude, with an elevation of 245 meters above sea level. The environmental temperature varies from near freezing point in winter to over 45°C in summer. The location receives approximately 700 mm of annual rainfall.

### Selection and management of experimental animals

Ten healthy Barbari goats (*Capra hircus*) (body weight ~20±0.2 kg) were selected and equally divided into two groups, i.e., control and treatment, based on their average body weight (Fig. 1). Study was conducted during winter (December -January), spring (February-March) and hot dry season (April-May). Based on the previous studies (Damor *et al.*, 2017; Choudhary *et al.*, 2018 and Meel *et al.*, 2018) a 50% replacement of concentrate with *Moringa oleifera* was decided. All the animals were fed green fodder and concentrate mixture according to the feeding standard (ICAR, 2013), except that the concentrate mixture was replaced with 50% *Moringa oleifera* (dried

leaf powder with small twigs).in the diet of the treatment group. The animals were kept in a well-ventilated shedwith *Ad libitum*supply of fresh and clean drinking water.The nutrient composition of the experimental diet (green fodder, concentrate and *Moringa oleifera*) are shown in Table 1, and the diet was analyzed according to AOAC (2005) standards.

#### Recording of climatic variables

Microclimatic data, viz., dry and wet bulb temperature, minimum and maximum temperature, and relative humidity, were recorded in the morning (9.00 AM) and afternoon (3.00 PM) using a Zeal (UK) Masons Pattern Hygrometer P2506daily throughout the experimental period. The THI was calculated using the formula of the NRC(1971):

$$\text{THI} = 0.72 \times (\text{Tdb} + \text{Twb}) + 40.6$$

Where: Tdb = dry bulb temperature (°C),

Twb = wet bulb temperature (°C)

#### Blood sampling

Blood samples were collected aseptically from the jugular vein at 20-day intervals using sterile heparin vacutainers (BD Vacutainer™, UK) throughout the experiment. Plasma was separated by centrifugation at 3000 rpm for 15 minutes then transferred to clean, dry plastic Eppendorf vials and stored at -20°C until analysis of hormones and biochemical parameters. The ELISA was conducted at the Animal Physiology Division, ICAR-NDRI, using the Infinite M200PRO NanoQuant (Tecan Group Ltd., Männedorf, Switzerland). Estimation of plasma cortisol, T<sub>3</sub>, T<sub>4</sub>, and catalase was performed using Goat Cortisol ELISA kits (Cat No. E0021Go), Goat Tri-iodothyronine ELISA kits (Cat No. E0009Go), Goat Thyroxine ELISA kits (Cat No. E0679Go) and Goat Catalase ELISA Kit: (Cat No. E0127Go) respectively supplied by Bioassay Technology, 1008 Junjiang International Building, 228 Ningguo Rd., Yangpu District, Shanghai, China. Estimation of plasma leptin, IL-2, and IL-10 was analyzed using Goat Leptin ELISA Kit: (Cat No. KLG0062), Goat IL-2 ELISA Kit: (Cat No. KLG0034) and Goat IL-10 ELISA Kit (Cat No. KLG0090) respectively supplied by KRISHGEN Bio Systems, Unit Nos. #318/319, Shah & Nahar, Off Dr. E. Moses Road, Worli, Mumbai 400018, India.

#### Statistical analysis

Data analysis was performed using SPSS software version 20 (2011). Two-way ANOVA was used to determine significant ( $p < 0.05$ ) differences between groups and seasons, as well as their interactions. Pairwise comparisons of means were carried out using the post-hoc Tukey B test.

## Results

#### Climatic Conditions

The mean monthly THI was calculated and presented in Table 2. THI served as an index for measuring seasonal stress on the experimental animals. A high THI was observed during the hot dry season, while a low THI was noted during the winter season. Two-tailed t-test correlation analysis revealed a significant positive correlation ( $p < 0.01$ ) of THI with IL-2 and catalase in both groups of goats (Tables 4 and 5). Additionally, THI exhibited a significant negative correlation ( $p < 0.05$ ) with T<sub>4</sub>, IL-10, and T<sub>3</sub> in both the control and treatment groups.

#### Stress biomarkers

During the study, significant seasonal effects were observed on the stress biomarkers of Barbari goats when concentrate was replaced with *Moringa oleifera* (Table 3). Plasma cortisol levels were lower ( $p < 0.05$ ) in the treatment group compared to the control group. Additionally, plasma cortisol levels were lower ( $p < 0.05$ ) during spring than in winter and the hot dry season. Conversely higher T<sub>3</sub> values ( $p < 0.05$ ) were observed in the treatment group compared to the control group across all seasons. Furthermore, mean plasma T<sub>3</sub> values were lower ( $p < 0.05$ ) during the hot dry season compared to those in the spring and winter seasons in both groups. The mean plasma T<sub>4</sub> levels were higher ( $p < 0.05$ ) in the treatment group compared to the control group across all seasons. Specifically, T<sub>4</sub> levels were elevated ( $p < 0.05$ ) during winter when compared to both spring and the hot dry season in both groups. Plasma leptin levels were also higher ( $p < 0.05$ ) in the treatment group than in the control group during all seasons. Notably, plasma leptin levels were elevated ( $p < 0.05$ ) during the hot dry season, followed by spring and winter. Additionally, plasma catalase levels were significantly higher ( $p < 0.05$ ) in the treatment group in comparison to the control group across all seasons. Similar to leptin, significantly higher plasma catalase levels were recorded during the hot dry season, followed by spring and winter in both groups.

#### Immune parameters

Significant differences were observed in the immune parameters of Barbari goats due to the replacement of concentrate with *Moringa oleifera*, (Table 3). Plasma IL-2 levels were lower ( $p < 0.05$ ) in the treatment group compared to the control group across all seasons. Additionally, plasma IL-2 was higher ( $p < 0.05$ ) during the hot dry season compared to the spring and winter seasons in both groups. Conversely higher plasma IL-10 values were observed in the treatment group compared to the control group during all seasons ( $p < 0.05$ ). However, plasma IL-10 values were lower ( $p < 0.05$ ) during the hot dry season compared to the spring and winter seasons in both groups.

**Table 1:** Nutrient composition (%DM) of feed and fodder fed to the experimental animals

| Parameter                 | Green fodder | Concentrate | <i>Moringa oleifera</i> |
|---------------------------|--------------|-------------|-------------------------|
| Dry Matter %              | 21.34±0.07   | 91.08±0.12  | 89.60±0.07              |
| Organic matter %          | 89.5±0.12    | 90.20±0.41  | 88.90±0.07              |
| Crude protein %           | 7.10±0.09    | 19.28±0.28  | 21.90±0.07              |
| Ether extract %           | 4.10±0.05    | 3.43±0.09   | 4.08±0.07               |
| Total ash %               | 10.50±0.12   | 9.80±0.43   | 11.10±0.07              |
| Nitrogen free extract %   | 53.70±0.27   | 39.71±0.17  | 55.14±0.07              |
| Crude fibre %             | 24.00±0.61   | 28.72±0.09  | 11.85±0.26              |
| Neutral detergent fibre % | 66.20±0.21   | 25.05±0.25  | 23.70±0.041             |
| Acid detergent fibre %    | 27.61±0.67   | 13.80±0.67  | 16.49±0.54              |

**Table 2:** Average temperature humidity index during different seasons

| Season  | THI        |            | THI (Avg)  |
|---------|------------|------------|------------|
|         | morning    | afternoon  |            |
| Winter  | 52.57±0.64 | 64.60±0.66 | 58.58±0.55 |
| Spring  | 58.90±0.67 | 74.72±0.66 | 66.81±0.61 |
| Hot Dry | 70.85±0.64 | 82.33±0.49 | 76.59±0.48 |

**Table 3:** Effect of replacement of concentrate with *Moringa oleifera* on Stress biomarkers and Immune parameters of Barbari goats during different seasons

| Stress biomarkers        | Season  | Control                   | Treatment                 | Overall                   | P-value |        |                 |
|--------------------------|---------|---------------------------|---------------------------|---------------------------|---------|--------|-----------------|
|                          |         |                           |                           |                           | Group   | Season | Group * Seasons |
| Cortisol (ng/mL)         | Winter  | 5.68 <sup>a</sup> ±0.23   | 5.35 <sup>a</sup> ±0.19   | 5.52 <sup>y</sup> ±0.17   | 0.003   | 0.000  | 0.003           |
|                          | Spring  | 4.34 <sup>p</sup> ±0.14   | 4.42 <sup>p</sup> ±0.19   | 4.38 <sup>z</sup> ±0.17   |         |        |                 |
|                          | Hot Dry | 8.30 <sup>s</sup> ±0.29   | 6.78 <sup>t</sup> ±0.33   | 7.54 <sup>x</sup> ±0.17   |         |        |                 |
|                          | Overall | 6.11 <sup>a</sup> ±0.14   | 5.52 <sup>b</sup> ±0.14   |                           |         |        |                 |
| Triiodothyronine (ng/mL) | Winter  | 3.07±0.04                 | 3.17±0.08                 | 3.12 <sup>x</sup> ±0.06   | 0.019   | 0.000  | 0.813           |
|                          | Spring  | 2.27±0.10                 | 2.45±0.11                 | 2.36 <sup>y</sup> ±0.06   |         |        |                 |
|                          | Hot Dry | 1.98±0.04                 | 2.18±0.12                 | 2.08 <sup>z</sup> ±0.06   |         |        |                 |
|                          | Overall | 2.44 <sup>b</sup> ±0.05   | 2.60 <sup>a</sup> ±0.05   |                           |         |        |                 |
| Thyroxine (ng/mL)        | Winter  | 103.55±0.34               | 106.94±0.23               | 105.24 <sup>x</sup> ±0.18 | 0.000   | 0.000  | 0.831           |
|                          | Spring  | 93.38±0.17                | 96.47±0.22                | 94.93 <sup>y</sup> ±0.18  |         |        |                 |
|                          | Hot Dry | 81.82±0.21                | 85.00±0.33                | 83.41 <sup>z</sup> ±0.18  |         |        |                 |
|                          | Overall | 92.92 <sup>b</sup> ±0.15  | 96.14 <sup>a</sup> ±0.15  |                           |         |        |                 |
| Leptin (ng/mL)           | Winter  | 1.35±0.08                 | 1.50±0.07                 | 1.43 <sup>y</sup> ±0.04   | 0.016   | 0.000  | 0.655           |
|                          | Spring  | 1.63±0.05                 | 1.68±0.03                 | 1.66 <sup>x</sup> ±0.04   |         |        |                 |
|                          | Hot Dry | 1.59±0.04                 | 1.72±0.06                 | 1.65 <sup>x</sup> ±0.04   |         |        |                 |
|                          | Overall | 1.52 <sup>b</sup> ±0.04   | 1.63 <sup>a</sup> ±0.04   |                           |         |        |                 |
| Catalase (ng/mL)         | Winter  | 55.72 <sup>p</sup> ±0.39  | 57.50 <sup>q</sup> ±0.24  | 56.61 <sup>y</sup> ±0.23  | 0.000   | 0.000  | 0.028           |
|                          | Spring  | 55.59 <sup>p</sup> ±0.26  | 58.29 <sup>q</sup> ±0.32  | 56.94 <sup>y</sup> ±0.23  |         |        |                 |
|                          | Hot Dry | 59.18 <sup>t</sup> ±0.38  | 62.66 <sup>s</sup> ±0.25  | 60.92 <sup>x</sup> ±0.23  |         |        |                 |
|                          | Overall | 56.83 <sup>b</sup> ±0.18  | 59.48 <sup>a</sup> ±0.18  |                           |         |        |                 |
| Interleukin 2 (pg/mL)    | Winter  | 73.80±0.17                | 72.67±0.26                | 73.23 <sup>z</sup> ±0.16  | 0.000   | 0.000  | 0.094           |
|                          | Spring  | 77.36±0.25                | 76.09±0.13                | 76.72 <sup>y</sup> ±0.16  |         |        |                 |
|                          | Hot Dry | 85.76±0.27                | 83.74±0.22                | 84.75 <sup>x</sup> ±0.16  |         |        |                 |
|                          | Overall | 78.97 <sup>a</sup> ±0.13  | 77.50 <sup>b</sup> ±0.13  |                           |         |        |                 |
| Interleukin 10 (pg/mL)   | Winter  | 146.46 <sup>s</sup> ±0.24 | 148.32 <sup>w</sup> ±0.41 | 147.39 <sup>x</sup> ±0.23 | 0.000   | 0.000  | 0.004           |
|                          | Spring  | 140.12 <sup>q</sup> ±0.29 | 143.66 <sup>r</sup> ±0.41 | 141.89 <sup>y</sup> ±0.23 |         |        |                 |
|                          | Hot Dry | 136.82 <sup>p</sup> ±0.26 | 140.77 <sup>q</sup> ±0.29 | 138.80 <sup>z</sup> ±0.23 |         |        |                 |
|                          | Overall | 141.13 <sup>b</sup> ±0.19 | 144.25 <sup>a</sup> ±0.19 |                           |         |        |                 |

Different superscript (x, y and z) in column and (a and b) in rows depicts significant ( $p < 0.05$ ) difference during season and group respectively. Different superscript (p,q,r and s) depicts significant ( $p < 0.05$ ) interaction between group and season.

**Table 4:** Correlation coefficient of control group among different parameters of Barbari goats during different season.

|          | THI      | Cortisol | T3       | T4       | Leptin   | Catalase | IL2      | IL10 |
|----------|----------|----------|----------|----------|----------|----------|----------|------|
| THI      | 1        |          |          |          |          |          |          |      |
| Cortisol | 0.557**  | 1        |          |          |          |          |          |      |
| T3       | -0.776** | -0.352*  | 1        |          |          |          |          |      |
| T4       | -0.922** | -0.618** | 0.836**  | 1        |          |          |          |      |
| Leptin   | 0.384**  | 0.177    | -0.313*  | -0.417** | 1        |          |          |      |
| Catalase | 0.610**  | 0.638**  | -0.450** | -0.695** | -0.073   | 1        |          |      |
| IL2      | 0.880**  | 0.725**  | -0.785** | -0.961** | 0.338*   | 0.732*   | 1        |      |
| IL10     | -0.890** | -0.424** | 0.858**  | 0.940**  | -0.410** | -0.608** | -0.873** | 1    |

\*\* . Correlation is significant at the 0.01 level (2-tailed). \* . Correlation is significant at the 0.05 level (2-tailed).

**Table 5:** Correlation coefficient of treatment group among different parameters of Barbari goats during different season.

|          | THI      | Cortisol | T3       | T4       | Leptin   | Catalase | IL2      | IL10 |
|----------|----------|----------|----------|----------|----------|----------|----------|------|
| THI      | 1        |          |          |          |          |          |          |      |
| Cortisol | 0.410**  | 1        |          |          |          |          |          |      |
| T3       | -0.636** | -0.150   | 1        |          |          |          |          |      |
| T4       | -0.908** | -0.469** | .735**   | 1        |          |          |          |      |
| Leptin   | 0.381**  | 0.229    | -0.263   | -0.387** | 1        |          |          |      |
| Catalase | 0.845**  | 0.644**  | -0.566** | -0.856** | 0.340*   | 1        |          |      |
| IL2      | 0.899**  | 0.504**  | -0.668** | -0.958** | 0.385**  | 0.385**  | 1        |      |
| IL10     | -0.866** | -0.364*  | 0.652**  | 0.894**  | -0.424** | -0.731** | -0.823** | 1    |

\*\* . Correlation is significant at the 0.01 level (2-tailed). \* . Correlation is significant at the 0.05 level (2-tailed).

## Discussion

### Climatic Conditions

The THI model is most appropriate for measuring environmental stress levels on goats (Armstrong 1994; Srivastava *et al.*, 2021). Animals are considered to be in the thermoneutral zone when THI values of the surrounding air are around 72. Heat stress is classified as moderate when THI values range from 72 to 76, and as severe when THI values fall between 76 and 80 (NRC, 1971). Seasonal stress on experimental animals can be effectively assessed using the THI (Deshpande *et al.*, 2020).

### Stress biomarkers

Plasma cortisol serves as a critical stress indicator and is one of the major endocrine regulators of stress responses mediated by the HPA axis. Cortisol is key component of this axis that modulate stress responses in animals. (Boucher and Plusquellec 2019). Elevated levels of cortisol indicate heat stress and contribute to thermoregulation; however, if these elevated levels persist, they may potentially suppress immune function. In situations of cold stress, cortisol levels also increase, facilitating energy mobilization and thermogenesis to maintain body temperature. Like heat stress, prolonged exposure to cold stress can likewise impact immune function. Therefore, cortisol is a key marker of environmental stress, influencing immune, metabolic, and behavioral responses in goats across different seasons to maintain homeostasis (de Vasconcelos *et al.*, 2022). The significantly lower plasma cortisol levels ( $p < 0.05$ ) observed in the treatment group compared to the control group suggest a potential modulatory effect of *Moringa oleifera* on stress response mechanisms in Barbari goats. These findings are consistent with previous studies (Sivakumar *et al.*, 2010; Sejian *et al.*, 2010). The lower levels of cortisol in the treatment group compared to the control group, particularly during the hot dry season, indicated lower physiological stress response. Hooda and Upadhyay (2014) and Banerjee *et al.*, (2015) also reported lower cortisol levels in *Moringa oleifera* supplemented goats during heat stress period. The plant contains high levels of flavonoids, polyphenols, vitamin C, and vitamin E, which help in reducing oxidative stress and modulating the HPA axis. The potent antioxidant and anti-inflammatory properties of *Moringa* bioactives help neutralize reactive oxygen species and reduce oxidative stress, thereby preventing overstimulation of the adrenal cortex and suppressing excess cortisol release. As a result, goats showed improved thermotolerance and enhanced immune function. These finding indicates that the dietary intervention may help attenuate the negative impact of environmental stressors on adrenal function, thus contributing to improved stress resilience in goats supplemented with *Moringa oleifera*.

Thyroid hormones, specifically T<sub>4</sub> and T<sub>3</sub>, play a key role in an animal's ability to adapt to changes in its environment. Thyroid hormone levels were found to be within the reference ranges (Neeru *et al.*, 2010) in the present experiment. In a study on goats, the author (Hooda and Upadhyay 2014) observed that when goats were exposed to high temperature, T<sub>3</sub> and T<sub>4</sub> concentration declined significantly. Thyroid hormones play a crucial role

in increasing the basal metabolic rate by enhancing oxygen demand and generating cellular heat (Farooq *et al.*, 2010). During heat stress, decreased thyroid hormone levels serve as an adaptive response, as they directly influence the HPA axis. This leads to reduced production of TRH, which results in a lowered metabolic rate in goats (Aleena *et al.*, 2016; Pragn *et al.*, 2018). Higher levels of T<sub>3</sub> were found during the winter season compared to the summer in both cold and heat adapted goats (Banerjee *et al.*, 2015). A significantly higher concentration of T<sub>3</sub> and T<sub>4</sub> ( $p < 0.01$ ) was observed in the moringa-supplemented group compared to the control group of goats (Wankhede *et al.*, 2022). In small ruminants, a decrease in T<sub>3</sub> and T<sub>4</sub> concentration can occur as a response to negative energy balance. Additionally, heat stress may prolong the duration of negative energy balance, leading to sustained low levels of thyroid hormones (Indu *et al.*, 2014).

Seasonal variations in plasma T<sub>3</sub> and T<sub>4</sub> levels indicate that *Moringa oleifera* may modulate thyroid function in Barbari goats. Elevated hormone levels in the treatment group, especially during the hot dry season, suggest enhanced thyroid activity, potentially improving metabolic homeostasis and physiological resilience under thermal stress. The observed increase in T<sub>4</sub> levels during the winter season aligns with the natural physiological response of animals to colder temperatures, where T<sub>4</sub> production is often upregulated to support thermoregulation and metabolic demands (Wankhede *et al.*, 2022).

Leptin is produced in white adipose tissues that notifies the central nervous system about the body's total fat store and as plasma leptin concentration have been correlated with adiposity; the minor seasonal changes in leptin can be explained by small drop in body mass (Bicici and Karakurum, 2024). The overall mean values of plasma leptin recorded during this study are within the reported ranges in goats (Nguyen *et al.*, 2020). The results are also in accordance with other authors who reported higher levels of leptin during hot dry period and these changes in plasma leptin can be assumed as the goats metabolic adaptive responses to heat stress, a mean to enhance heat loss while lowering heat production in order to be euthermic (Al-Dawood 2017).

The results of the present study align with those of the other authors (Gupta *et al.*, 2013a), who reported substantially higher levels of catalase during peak summer compared to peak winter and moderately cold seasons. In a study on goats, Babiker *et al.*, (2021) observed a progressive increase in plasma catalase levels corresponding with the feeding period of *Moringa oleifera*, which mirrors the trend found in the present study. The amino acid composition of *Moringa oleifera* leaves meets the protein requirements of the animals while also enhancing their immune systems. These factors contribute to the increased production and activity of endogenous enzymes, such as catalase (Leitanthem *et al.*, 2022).

#### Immune parameters

It is important to note that IL-2 is a crucial cytokine involved in regulating immune responses, particularly in the activation and proliferation of T lymphocytes (Abioja *et al.*, 2023). IL-2 levels were significantly higher in the control group than in the treatment group, suggesting that *Moringa oleifera* may have a beneficial effect on enhancing immunity in the fed group, thereby reducing cell death compared to the control group. Throughout different seasons, IL-2 levels remained higher in the control group than in the treatment group, with the highest levels observed during the hot dry season for both groups. This suggests that the summer season is the most stressful period compared to winter and spring seasons. The observed variations in IL-2 levels during different seasons may be attributed to the dynamic nature of immune responses in goats, which can be influenced by seasonal changes and environmental factors (Kumar and Singh 2019). Isothiocyanates found in *Moringa oleifera* leaves have been shown to suppress the assembly of pro-inflammatory mediators by macrophages (Waterman *et al.*, 2014). It is suggested that IL-2 acts as a heat-trapping factor, contributing to elevated body temperature and inflammation during heat stress (Kumar and Singh 2019). IL-10 is an important anti-inflammatory cytokine involved in regulating immune responses and suppressing excessive inflammation. The observed variation in IL-10 levels across different seasons may be attributed to the dynamic nature of immune responses in goats, which can be influenced by seasonal changes and environmental factors (Abioja *et al.*, 2023). The values of plasma IL-10 were significantly higher in the *Moringa oleifera* treated group compared to the control group. *Moringa oleifera* is known for its anti-inflammatory properties, which is reflected in the results observed in the treated group in contrast to the control. During the summer season, the levels of IL-10 in both groups were lower, followed by spring and winter, where higher levels were recorded.

Further research is warranted to investigate the underlying mechanisms responsible for the seasonal variations in IL-2 and IL-10 levels, as well as the potential impact of Moringa feeding on other immune markers and overall immune function in goats. Understanding the dynamics of IL-2 and IL-10 levels, along with other immune parameters throughout different seasons, will contribute to the development of effective strategies for enhancing immune responses and promoting the overall health of goats in various environmental conditions.

The results of the study clearly indicated that replacement of concentrate with *Moringa oleifera* is beneficial for ameliorating the adverse effect of thermal stress on Barbari goats by altering the levels of stress biomarkers and immunity parameters. Therefore, concentrate fed to goats can be replaced by 50% *Moringa oleifera* successfully for improving the health status of Barbari goats during thermal stress conditions.

### Author's contribution

All the authors contributed to the experimental design. Animal trial and sample collection was conducted by Dr. Apeksha Ukey and Dr. Smaranika Biswal under the guidance of Dr. Sohan Vir Singh and Dr. Arun Misra. Data analysis was performed by Dr. Apeksha Ukey with the help of Dr. Sohan Vir Singh, Dr. Gaurav Kumar and Dr. Nikita Bhalakiya. Estimation of stress marker and immunity parameters, ELISA was used with the help of Dr. Prashant Gujjalkar. Dr. Mangesh Vaidya contributed to the writing of this manuscript.

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The fund for conducting the research work was received from the ICAR-National Dairy Research Institute, Karnal (Haryana).

**Data availability** The data that support this study will be made available on reasonable request.

### Ethical approval

The experiment was conducted at the Integrated Farming System Unit of Livestock Research Centre (LRC), ICAR - National Dairy Research Institute, Karnal, Haryana (India). The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of ICAR-NDRI, constituted under Article 13 of the CPCSEA and established by the Government of India (Reg. No. 46-IAEC-20-17, dated 22-07-2020). All ethical guidelines were followed during the course of the experiment.

**Conflicts of interest** No potential conflicts of interest were reported by the authors.

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