# Effects of Combined Heat and Humidity Stress on Growth Performance, Antioxidant Capacity, and Immune Function in Tibetan Sheep and Goats (Postprint)

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## Abstract

This experiment aimed to investigate the variation patterns of serum hormones, antioxidant and immune indices in Tibetan sheep and goats under identical nutritional and heat-humidity stress conditions, and to examine the differences in the effects of heat-humidity stress on growth performance, antioxidant capacity, and immune function between Tibetan sheep and goats. Six Tibetan sheep and six goats (Boer goat × local yellow goat) with similar age and body weight [(45.83±3.54) kg] were selected. The experiment lasted for 135 d, including a 15-d preliminary period and a 120-d formal experimental period. The temperature-humidity index (THI) was measured daily, and serum-related indices of Tibetan sheep and goats were measured monthly. The results showed: 1) From May to July, the THI in the sheep house increased significantly with month ($P<0.05$), and the THI values from June to August were all greater than 72; therefore, June to August was defined as the heat-humidity stress period. 2) In July and August, the rectal temperature and respiratory frequency of both Tibetan sheep and goats were significantly higher than those in May ($P<0.05$), and during the heat-humidity stress period, the rectal temperature and respiratory frequency of Tibetan sheep were significantly higher than those of goats ($P<0.05$). 3) During the formal experimental period, heat-humidity stress caused maximum reductions in dry matter intake of 10.70% and 10.44% for Tibetan sheep and goats, respectively, and maximum reductions in average daily gain (ADG) of 50.00% and 47.82%, respectively. 4) As the THI in the sheep house increased from 71.17 (May) to 76.82 (July), serum cortisol and insulin concentrations in both Tibetan sheep and goats increased significantly ($P<0.05$), while serum glucose and triiodothyronine concentrations decreased significantly ($P<0.05$). Under heat-humidity stress, the maximum reductions in serum growth hormone and thyroxine concentrations in Tibetan sheep were greater than those in goats. 5) Except for the serum total antioxidant capacity of Tibetan sheep in July and goats in August, the serum superoxide dismutase and glutathione peroxidase activities and total antioxidant capacity of both Tibetan sheep and goats in July and August were significantly lower than those in May ($P<0.05$), while serum malondialdehyde concentrations were significantly higher than those in May ($P<0.05$). Under heat-humidity stress, the magnitudes of change in serum superoxide dismutase activity, total antioxidant capacity, and malondialdehyde concentration in goats were greater than those in Tibetan sheep. 6) Compared with May, the serum immunoglobulin A, immunoglobulin M, immunoglobulin G, and interleukin-2 concentrations of Tibetan sheep and goats in August decreased significantly ($P<0.05$), while serum tumor necrosis factor- concentration increased significantly ($P<0.05$). Under heat-humidity stress, the magnitudes of change in serum immunoglobulin, interleukin-2, and tumor necrosis factor- concentrations in Tibetan sheep were greater than those in goats. In summary, under heat-humidity stress, the respiratory frequency and rectal temperature of Tibetan sheep and goats increased, while antioxidant capacity and immune function decreased, resulting in reduced growth performance. Tibetan sheep were more affected in growth performance and immune function by heat-humidity stress, whereas goats were more affected in antioxidant capacity.

## Full Text

## Effects of Moist-Heat Stress on Growth Performance, Antioxidant Capacity, and Immune Function in Tibetan Sheep and Goats

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### Abstract

This study investigated the variation patterns of serum hormones, antioxidant markers, and immune indices in Tibetan sheep and goats under identical nutritional and moist-heat stress conditions, and examined the differential impacts of moist-heat stress on growth performance, antioxidant capacity, and immune function between the two species. Six Tibetan sheep and six goats (Boer goat × local yellow goat) of similar age and body weight [(45.83±3.54) kg] were selected. The experiment lasted 135 days, comprising a 15-day adaptation period and a 120-day formal trial period. Temperature-humidity index (THI) was measured daily, and serum-related indices were monitored monthly. The results showed that: 1) THI in the sheep barn increased significantly from May to July ($P<0.05$), with values exceeding 72 from June to August, which was therefore designated as the moist-heat stress period. 2) Rectal temperature and respiratory rate in both Tibetan sheep and goats were significantly higher in July and August compared to May (P<0.05), and Tibetan sheep exhibited significantly higher values than goats during the moist-heat stress period (P<0.05). 3) During the formal trial period, moist-heat stress caused maximum reductions in dry matter intake (DMI) of 10.70% and 10.44% for Tibetan sheep and goats, respectively, while maximum reductions in average daily gain (ADG) were 50.00% and 47.82%, respectively.

## Introduction

The sheep industry constitutes an important component of China' s animal husbandry sector, with rapid development in meat sheep production. Between 2005 and 2014, the national sheep inventory increased by 1.75% while mutton production grew by 22.31% [1]. Tibetan sheep (*Ovis aries*), evolved and selected over long periods, possess remarkable adaptability to high altitude, hypoxia, and low temperatures. As the most numerous livestock species on the Qinghai-Tibet Plateau with a population of approximately 30 million, Tibetan sheep represent a vital part of China' s sheep industry.

## Materials and Methods

**1.3 Sample Collection** Feed offered was weighed before each feeding, and residual feed in troughs was collected before morning feeding on the following day to calculate daily dry matter intake (DMI) per experimental group. All experimental animals were weighed monthly on the 1st day of each month from May to August 2015 before morning feeding to determine average daily gain (ADG). Simultaneously, 10 mL of blood was collected from the jugular vein of each animal, allowed to clot for 30 minutes, then centrifuged at 4,000 r/min for 15 minutes to harvest serum. Serum samples were aliquoted into 1.5 mL sterile centrifuge tubes and stored at -20°C for subsequent analysis.

**1.4.1 Environmental Temperature and Humidity** Three dry-wet bulb thermometers were suspended approximately 1.5 m above ground level at the middle and both ends of the sheep barn, out of animals' reach. Dry bulb temperature (Td) and wet bulb temperature (Tw) were recorded daily at 08:00, 11:00, 14:00, and 17:00. Daily average temperature-humidity index (THI) was calculated using the formula: $THI = 0.72 \times (Td + Tw) + 40.6$ [7].

**1.4.2 Rectal Temperature and Respiratory Rate** Rectal temperature and respiratory rate were measured following the methods of Johnson et al. [8]. During the formal trial period, respiratory rate was measured every 10 days using a stopwatch and counter. Measurements were taken twice daily at 08:00 and 14:00, counting breaths for 1 minute per session, with three consecutive measurements averaged. Immediately after respiratory rate measurement, rectal temperature was determined using a veterinary thermometer at the same time points (08:00 and 14:00), with the two daily values averaged. The procedure involved shaking the mercury column below 35°C, disinfecting with medical alcohol, applying lubricant (Vaseline), restraining the animal, inserting the thermometer approximately 5 cm into the rectum, removing after 5 minutes, and recording the reading.

**1.4.3 Serum Indices** Serum glucose (GLU) concentration was determined by the biochemical laboratory of Sichuan Agricultural University' s Veterinary Hospital using an AUTOLAB PM-4000 batch automatic biochemical analyzer (AMS Company, Italy). Serum concentrations of cortisol (COR), insulin (INS), growth hormone (GH), triiodothyronine (T3), thyroxine (T4), interleukin-2 (IL-2), and tumor necrosis factor- (TNF- ) were measured by enzyme-linked immunosorbent assay (ELISA). Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) concentration were determined by colorimetric methods. Immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin G (IgG) concentrations were measured by immunoturbidimetry. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

**1.5 Statistical Analysis** Basic data were organized using Excel 2016 software. Comparisons between breeds were performed using independent samples t-test with SPSS 19.0 software, while comparisons within breeds were conducted using one-way ANOVA followed by Duncan' s multiple comparison test. Significance level was set at $P<0.05$, and results were expressed as "mean ± standard deviation."

**Results**

**2.1 THI Variation in Sheep Barn** Changes in barn THI during the formal trial period are presented in and [Figure 1: see original paper]. THI increased gradually throughout the trial period, with values in June, July, and August being significantly higher than in May ($P<0.05$) and numerically exceeding 72. Accordingly, the experimental period was divided into a non-stress period (May) and a moist-heat stress period (June–August).

**2.2 Effects of THI Changes on Rectal Temperature and Respiratory Rate** As shown in , rectal temperature and respiratory rate in both Tibetan sheep and goats increased with rising THI. In Tibetan sheep, rectal temperature and respiratory rate in July and August were significantly higher than in May and June ($P<0.05$), and June values were significantly higher than May ($P<0.05$). In goats, rectal temperature in July was significantly higher than in May, June, and August ($P<0.05$), while August values were significantly higher than May ($P<0.05$). Respiratory rate in goats was significantly higher in June, July, and August compared to May ($P<0.05$). During the moist-heat stress period, Tibetan sheep exhibited significantly higher rectal temperature and respiratory rate than goats ($P<0.05$). The maximum increases were 2.19% and

0.97% for rectal temperature, and 98.49% and 33.70% for respiratory rate in Tibetan sheep and goats, respectively.

**2.3 Effects of THI Changes on Growth Performance** shows that THI variation affected DMI and ADG in both species. Tibetan sheep DMI was significantly lower in July and August compared to May and June ($P<0.05$), while goat DMI was significantly lower only in August versus May ($P<0.05$). Tibetan sheep ADG was significantly lower in August compared to May ($P<0.05$), whereas goat ADG was significantly lower in July and August versus May ($P<0.05$). No significant differences in DMI or ADG were observed between breeds during the formal trial period ($P>0.05$). Maximum reductions were 10.70% and 10.44% for DMI, and 50.00% and 47.82% for ADG in Tibetan sheep and goats, respectively.

**2.4 Effects of THI Changes on Serum Indices** demonstrates that serum COR concentration increased with THI in both species. In Tibetan sheep, COR concentrations differed significantly between all months except May and June ($P>0.05$), while in goats, COR was significantly higher in July and August versus May and June ($P<0.05$), and May values were significantly higher than June ($P<0.05$). Elevating THI from 71.17 (May) to 76.82 (July) significantly decreased serum GLU concentrations by 36.25% and 26.81% in Tibetan sheep and goats, respectively ($P<0.05$). Compared to May, serum INS concentrations increased significantly in Tibetan sheep during June and July ($P<0.05$), and in goats during June, July, and August ($P<0.05$), with maximum increases of 27.52% and 30.04%, respectively. Serum T3 concentrations decreased significantly in Tibetan sheep during June, July, and August, while T4 and GH concentrations decreased significantly only in August ($P<0.05$). In goats, serum T3 and T4 concentrations decreased significantly in July and August, and GH decreased significantly in August ($P<0.05$). Maximum reductions were 48.49% and 36.59% for T4, and 45.50% and 36.32% for GH in Tibetan sheep and goats, respectively. Tibetan sheep had significantly lower serum COR concentration than goats in July ($P<0.05$), lower INS concentration in August ($P<0.05$), and lower T3 concentration in June, July, and August ($P<0.05$).

**2.5 Effects of THI Changes on Serum Antioxidant Indices** reveals that, except for serum T-AOC in Tibetan sheep (July) and goats (August), SOD and GSH-Px activities and T-AOC in July and August were all significantly lower than in May ($P<0.05$), while serum MDA concentration was significantly higher ($P<0.05$). Maximum changes included reductions of 23.46% and 34.84% in SOD activity, 25.80% and 28.46% in T-AOC, and increases of 29.23% and 42.07% in MDA concentration for Tibetan sheep and goats, respectively. Tibetan sheep exhibited significantly higher serum SOD activity and T-AOC than goats in July ($P<0.05$).

**2.6 Effects of THI Changes on Serum Immune Indices** shows that serum IgA, IgM, and IgG concentrations decreased to varying degrees in July and August compared to May. Maximum reductions were 48.90% and 34.14% for IgA, 49.21% and 36.02% for IgM, and 40.33% and 25.39% for IgG in Tibetan sheep and goats, respectively. Serum IL-2 concentration decreased with increasing THI, being significantly lower in July and August versus May and June ($P<0.05$), with maximum reductions of 48.84% and 39.59%. Serum TNF- concentration increased with THI, being significantly higher in July and August compared to May and June ($P<0.05$), with maximum increases of 63.64% and 21.43%. Tibetan sheep had significantly lower serum IgA and IgM concentrations than goats in June, and lower IgA and IgG concentrations in August ($P<0.05$). Serum TNF- concentration was significantly lower in Tibetan sheep than goats in May ($P<0.05$), while IL-2 concentration was significantly lower in Tibetan sheep throughout the trial period ($P<0.05$).

## Discussion

**3.1 Effects of THI Changes on Rectal Temperature and Respiratory Rate** Rectal temperature and respiratory rate are important indicators of physiological status. Heat stress elevates these parameters and alters physiological condition in livestock [9]. Srikandakumar et al. [10] reported that Omani sheep exhibited lower rectal temperature and smaller increases compared to Merino sheep under heat stress, indicating stronger heat storage capacity and thermotolerance that reduces additional water and energy expenditure. Lower respiratory rate and smaller increases suggested lower maintenance energy requirements and reduced heat stress impact. Our findings showed that moist-heat stress increased respiratory rate and rectal temperature in both species, with greater increases in Tibetan sheep, indicating poorer heat storage capacity and lower thermotolerance compared to goats. The higher respiratory rate and greater increment in Tibetan sheep imply higher maintenance energy requirements and greater susceptibility to moist-heat stress.

**3.2 Effects of THI Changes on Growth Performance and Serum Indices** Previous studies demonstrated that heat stress significantly reduces DMI [11] and ADG [11-12] in animals. Our results showed that moist-heat stress decreased DMI and ADG in both species, with Tibetan sheep exhibiting greater DMI reduction in July and larger ADG decline than goats, indicating more pronounced effects on growth performance. Cortisol serves as a biomarker of stress response, with serum concentrations increasing significantly under heat stress [13]. Our findings of elevated serum COR with increasing THI are consistent with previous research. Impaired growth under heat stress is associated with reduced GH secretion [14]. Renaville et al. [14] suggested synergistic growth-promoting effects between T4 and GH, while heat stress induces hypothyroidism and decreases T3 and T4 secretion [15]. McGuire et al. [16] also reported reduced blood GH concentration in heat-stressed dairy cows. Therefore, heat stress affects growth performance by altering blood T3, T4, and GH concentrations. Our observation of decreased serum T3, T4, and GH under moist-heat stress aligns with these findings, indicating reduced thermogenesis and anabolic metabolism, which corresponds to decreased ADG. Moreover, Tibetan sheep showed greater reductions in T4 and GH than goats, consistent with their larger ADG decline, suggesting greater impact on growth performance.

Research indicates that heat stress significantly increases blood INS concentration in beef cattle [17] and lactating dairy cows [18], while effects on blood GLU are inconsistent. Mahjoubi et al. [12] attributed this discrepancy to different glucose metabolism rates between meat-producing and lactating animals. Our study found increased serum INS and decreased GLU under moist-heat stress, possibly due to elevated maintenance energy requirements and reduced energy utilization efficiency. The higher respiratory rate in Tibetan sheep indicates greater energy expenditure for maintenance, resulting in larger ADG reduction compared to goats.

**3.3 Effects of THI Changes on Antioxidant Capacity** Serum SOD and GSH-Px scavenge free radicals and represent important antioxidant enzymes [19]. Antioxidant system impairment leads to reduced enzyme activities [20]. Heat stress decreases antioxidant enzyme activity in dairy cows [21], enhancing oxidative stress and damaging the antioxidant system [22]. Megahed et al. [23] reported significantly reduced SOD activity and T-AOC in heat-stressed buffalo. Yang et al. [24] demonstrated that heat stress accelerates oxidation, causing lipid peroxide MDA accumulation and membrane damage. Our findings of decreased SOD and GSH-Px activities and T-AOC, along with increased MDA, are consistent with these reports. However, goats exhibited greater changes in SOD activity, T-AOC, and MDA concentration than Tibetan sheep, indicating more severe antioxidant capacity impairment under moist-heat stress.

**3.4 Effects of THI Changes on Immune Function** Immunoglobulins are essential immune components, with IgG comprising approximately 75% of total serum immunoglobulin concentration. Heat stress suppresses immune function in dairy cows [25]. Tao et al. [26] reported significantly reduced serum IgG concentration in heat-stressed cows. Starkie et al. [27] suggested that heat stress elevates pro-inflammatory cytokines like TNF- , exacerbating inflammation and compromising immunity. However, comparative studies on heat stress effects across breeds are limited. Our study demonstrated that moist-heat stress significantly decreased serum immunoglobulins (IgA, IgM, IgG) and IL-2 while increasing TNF- , consistent with Starkie et al. [27] and Cheng et al. [28]. Furthermore, Tibetan sheep showed greater changes in immunoglobulins, IL-2, and TNF-  than goats, indicating more substantial immune function impairment.

## Conclusion

Moist-heat stress elevated respiratory rate and rectal temperature while decreasing growth performance, antioxidant capacity, and immune function in both Tibetan sheep and goats. Tibetan sheep experienced more severe effects on growth performance and immune function, whereas goats suffered greater impairment of antioxidant capacity under moist-heat stress conditions.

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*Note: Figure translations are in progress. See original paper for figures.*