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Amelioration of Heat Stress-Induced Alterations in Immune indices, Serum Enzyme Activity, Antioxidant Ability and Gene Expression in Wistar Rats through Nutritional Strategies

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ABSTRACT

High ambient temperature and humidity have a negative impact on livestock health and can lead to heat stress in animals. The objective of this study was to assess the impacts of SeP, ZnP, P and SeZnP on the growth performance, serum enzyme activity, antioxidant status, immune function, and mRNA expression of Wistar rats exposed to high ambient temperature. Seventy-two male Wistar rats were randomly allocated to six groups: Control (CON), high temperature (HT), high temperature plus probiotics (HT+P, CFU, L. acidophilus 10^{11}/mL and S. cerevisiae 10^{9}/mL), high temperature plus zinc-fortified probiotics (HT+ZnP, 100mg/L), high temperature plus selenium-fortified probiotics (HT+SeP, 0.3mg/L), and high temperature plus selenium/zinc-fortified probiotics (HT+SeZnP, 0.3mg/L + 100mg/L). The feeding period lasted forty days, and blood and tissue samples were collected on the final day. The results demonstrated that all supplemented groups (P, SeP, ZnP, and SeZnP) significantly enhanced growth performance compared to the control group ($p < 0.05$). Notably, SeZnP supplementation significantly increased GSH content, SOD activity, GSH-Px activity, while reduced MDA content, creatinine, LDH, CK, ALP, AST, ALT, and blood urea nitrogen levels in the serum of Wistar rats. The concentrations of IFN-γ, IL-6, and IL-2 ($p < 0.05$) increased with all supplemented treatments, while IL-10 decreased. Moreover, SeP, ZnP, and SeZnP significantly upregulated the expression of GPx1 and SOD1 genes ($p < 0.05$), while downregulated Hsp90 and Hsp70 heat shock genes ($p < 0.05$). In conclusion, this product shows potential as a nutritive supplement for animals exposed to high ambient temperatures. Implementing this strategy can help producers maintain the health, comfort, and productivity of their animals during the summer season.

Keywords: Antioxidant status, Genes expression, Heat stress, Immune function, Nutritional Strategies, Wistar rats

INTRODUCTION

Heat stress poses a significant challenge in dairy production, particularly during the hot summer months when temperatures can soar up to 45°C during the day, while remaining around 30°C at night. Furthermore, the duration of daylight in this season typically ranges from 12 to 14 hours. Dairy animals are capable of maintaining a constant body temperature, a characteristic known as being homoeothermic. However, when exposed to high environmental temperatures, humidity, solar radiation, and heat waves, heat stress can occur, disrupting the animals' homoeothermic responses. If dairy animals are unable to dissipate the excess metabolic heat through processes such as conduction, convection, radiation, and evaporative cooling, they may suffer from heat stress. This condition can have adverse effects on the health, productivity, and profitability of dairy animals. Therefore, it is crucial to implement managemental and nutritional strategies to mitigate heat stress in order to alleviate its negative impacts (Frigeri et al., 2023; Wankar et al., 2021).

Heat stress in dairy animals has diverse and extensive impacts on their physiological and reproductive functions. It can lead to reduced dry matter intake, rumen acidosis, laminitis (inflammation in the hoof), decreased milk quality, leaky gut (increased gut permeability), inflammation throughout the body, decreased milk production, compromised reproductive performance, impaired fetal growth, reduced fertility rate, early embryonic death, and impaired immune function. These effects of heat stress can significantly impact the overall health and productivity of dairy animals. Observable signs of heat stress include behavioral changes such

as seeking shaded areas, increased water intake, decreased feed intake, standing instead of lying down, elevated respiration rate and body temperature, increased saliva production, open-mouth breathing and panting, decreased activity levels, sweating, and excessive drooling. These indicators serve as valuable cues for identifying and addressing heat stress in dairy animals (Das et al., 2016; Polsky & von Keyserlingk, 2017).

Nutritional strategies play a vital role in mitigating the adverse effects of heat stress on animals. Elevated temperatures can have detrimental impacts on animal health and performance, but appropriate nutrition can help alleviate these effects. Ensuring access to clean water and supplementing with electrolytes aids in maintaining hydration and mineral balance. Incorporating easily digestible, energy-dense feeds, helps reduce the production of metabolic heat. Antioxidants such as selenium, zinc, copper, manganese, and vitamins E and C can combat oxidative stress caused by heat stress. Optimizing the balance of macronutrients supports energy metabolism and muscle function during periods of heat stress. By implementing these nutritional interventions, the harmful consequences of high ambient temperature on animals can be mitigated effectively (Nzeyimana, Fan, Zhuo, Butore, & Cheng, 2023; Petrocchi Jasinski, Evangelista, Basiricò, & Bernabucci, 2023). In this study, we investigated the effects of SeP, ZnP, and P on the growth performance, antioxidant status, serum enzyme activity, immune function, and gene expression of Wistar rats exposed to high ambient temperature.

MATERIALS AND METHODS

Research design and Feeding

The average weight of Wistar rats used in the study was 165 ± 5g. These rats were obtained from the Animals Laboratory of Yangzhou University in China. The Animal Care and Use Committee of Nanjing Agricultural University granted approval and certification for the study (Certification No. SYXK (Su) 2011-0036).

The basal diet given to the rats contained 0.3 mg/L of inorganic selenium and 100 mg/L of zinc oxide. The measurement of these elements in the diet was conducted using methods outlined in a previous research article (Malyar et al., 2019). Every morning, the rats were administered 1mL of diet with different formulations through a stomach tube. The formulations included probiotics (P), selenium-fortified probiotics (SeP), zinc-fortified probiotics (ZnP), or selenium/zinc-fortified probiotics (SeZnP). Seventy-two rats were divided into six groups for the study: Control (CON), high temperature (HT), high temperature plus probiotics (HT+P, CFU, L. acidophilus 10^{11}/mL and S. cerevisiae 10^{9}/mL), high temperature plus zinc-fortified probiotics (HT+ZnP, 100mg/L), high temperature plus selenium-fortified probiotics (HT+SeP, 0.3 mg/L), and high temperature plus selenium/zinc-fortified probiotics (HT+SeZnP, 0.3 mg/L+100mg/L). Each group consisted of 12 male Wistar rats. This feeding regimen was maintained for a duration of 40 consecutive days, after which blood and tissue samples were collected at the end of the experiment.

Serum Biochemical, Inflammatory Cytokines and Antioxidant Status Analysis

The levels of IFN-γ, IL-10, IL-6, IL-2, creatinine, blood urea nitrogen, CK, LDH, ALP, AST, ALT, GSH, SOD, GSH-Px, and MDA were determined using commercially available kits obtained from Wuhan Service Biotechnology Co., Ltd., located in Wuhan, China. The selection of these kits was based on their compliance with manufacturer specifications. Established protocols were followed to ensure accurate and reliable measurements of the specific biochemical markers.

qPCR Analysis

We used real-time quantitative PCR to analyze the expression levels of β-actin, GPx1, SOD1, Hsp70, and Hsp90 genes. Gene-specific primers were designed based on the sequences of Rattus norvegicus from the NCBI database, using primer software.

Statistical Analysis

The data was represented as means ± standard errors, while statistical analysis was performed using SPSS version 23. ANOVA was utilized to evaluate significant differences among the groups, followed by the Duncan post hoc test. A significance level of $P < 0.05$ was deemed statistically significant.

RESULTS

The results of the study showed that the administration of P, ZnP, SeP, and SeZnP significantly improved the growth performance of rats compared to both the control and HT groups ($p < 0.05$). However, no significant differences were observed among the P, ZnP, and SeP groups. Notably, the SeZnP supplementation group demonstrated the most substantial improvements in growth, while the HT group experienced a significant

decrease in growth performance compared to the control and other treated groups (Figure 1). The findings of the study revealed that HT ($p < 0.05$) led to a decrease in GSH content, SOD and GSH-Px activities compared to both the control and other treated groups. Moreover, the HT group exhibited a significant increase in MDA content ($p < 0.05$). However, supplementation with P, ZnP, SeP, and SeZnP ($p < 0.05$) improved the antioxidant status of Wistar rats exposed to HT, as indicated in Table 1. In this study, we examined the effects of HT, P, ZnP, SeP, and SeZnP on serum biochemical parameters (creatinine, blood urea nitrogen, CK, LDH, ALP, AST, ALT) and cytokine levels (IFN-γ, IL-10, IL-6, IL-2) in Wistar rats exposed to HT. The HT group exhibited a significant decreases ($p < 0.05$) in IFN-γ, IL-6, and IL-2 levels, along with a increases in IL-10, creatinine, blood urea nitrogen, CK, LDH, ALP, AST, and ALT levels compared to the control and other treated groups. However, supplementation with P, ZnP, SeP, and SeZnP ($p < 0.05$) improved cytokine levels and biochemical parameters, with the SeZnP group showing the most significant improvement (Figure 2). Additionally, the supplementation of SeZnP, SeP, and ZnP resulted in a significant increase in the expression of Gpx1 and SOD1 mRNA compared to the control, HT, and P groups ($p < 0.05$). Conversely, the treatment groups showed a substantial decline in the expression of Hsp70 and Hsp90 mRNA paralleled to the HT and control groups ($p < 0.05$). Notably, the SeZnP supplemented group exhibited the most notable improvement in the expression levels of antioxidant and heat shock-related genes ($p < 0.05$) (Figure 3).

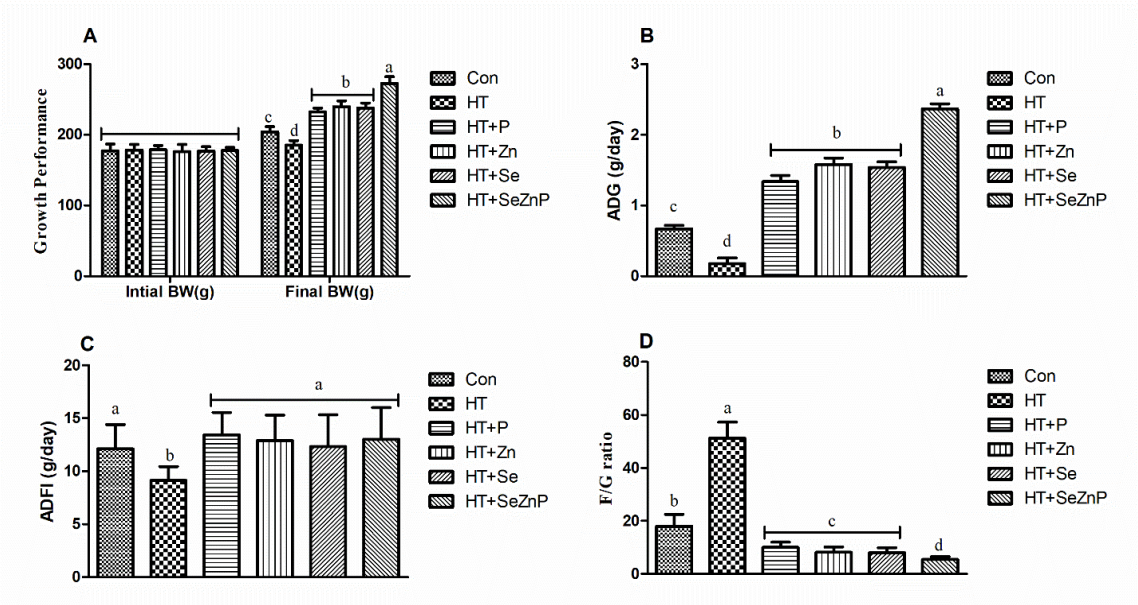

Figure 1. Illustrates the impact of HT and treatments on the growing performance of Wistar rats exposed to elevated ambient temperature. The figure includes the following parameters:
A) Growth Performance: This represents the overall assessment of the rats' growth under different conditions. **B)** Average Daily Gain (g/day): This quantifies the average weight gained by the rats per day, indicating their rate of growth. **C)** Average Daily Feed Intake (g/day): This measures the average amount of food consumed by the rats per day, reflecting their feeding behavior. **D)** Feed/Growth Ratio: This ratio evaluates the efficiency of food utilization by the rats, calculated by dividing the daily feed intake by the daily weight gain. It provides insights into the rats' ability to convert food into growth. This figure provides valuable information on how HT, P, ZnP, SeP, and SeZnP influence the growth performance of Wistar rats in a high ambient temperature environment.

Table 1. The Antioxidant contents in blood Serum

	Groups					
Antioxidant status	**Con**	**HT**	**HT+P**	**HT+ZnP**	**HT+SeP**	**HT+SeZnP**
GSH-Px Activity (U/L)	22.49±4.3d	16.23±2.2e	23.68±4.6c	53.91±5.99b	56.12±7.8b	62.56±4.5a
GSH content (µmol/g protein)	26.23±1.7d	19.78±3.9e	46.34±1.82c	59.63±2.63b	61.56±3.2b	71.98±5.6a
SOD Activity (U/mL)	51.46±4.9d	40.12±5.8e	82.05±3.79c	122.26±5.4b	125.56±7.5b	130.45±9.7a
MDA content (nmol/ML)	54.17±2.9b	75.56±8.2a	32.81±2.54c	19.27±3.23d	18.23±6.5d	14.34±4.2e

The data presented as mean±SE. (a-e) unlike letters in each row specify differences between mean ($p < 0.05$).

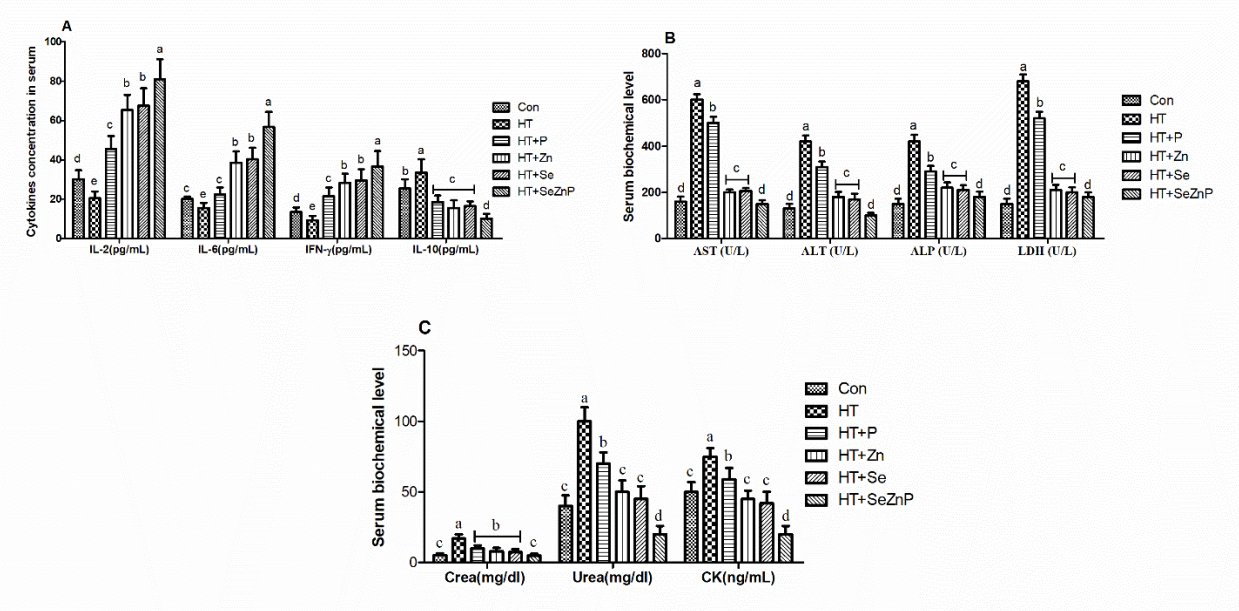

Figure 2. Depicts the effects of HT and different treatments on serum cytokine levels **(A)** and serum biochemical levels **(B and C)** in Wistar rats exposed to thermal stress.

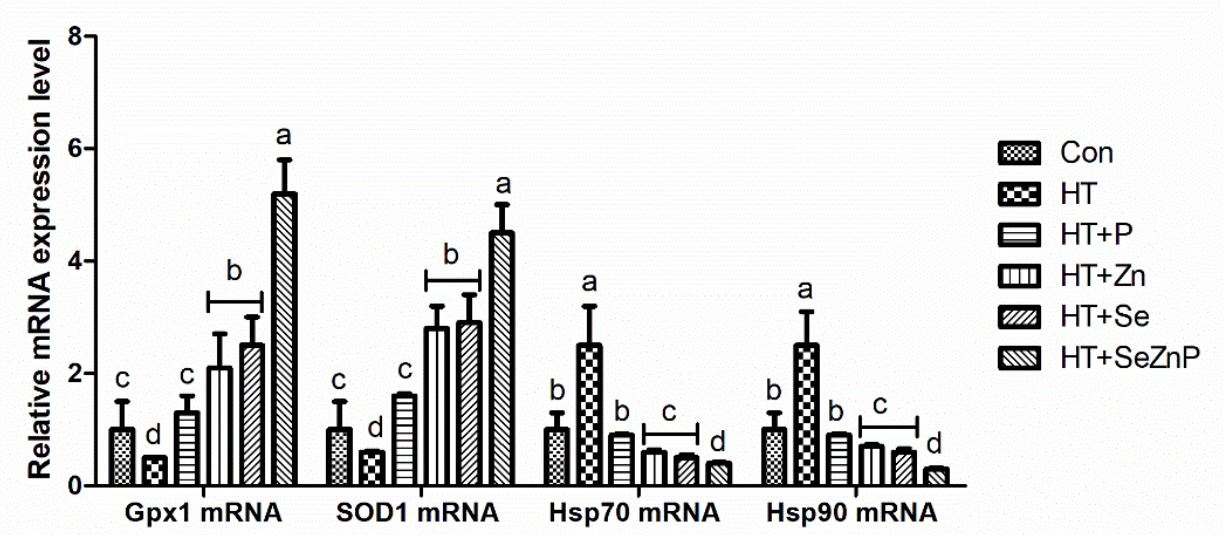

Figure 3. Illustrates the impact of HT and other treatment groups on the expression of Gpx1, SOD1, Hsp70, and Hsp90 genes in the liver of Wistar rats exposed to thermal stress conditions.

DISCUSSION

During the summer season, animals frequently experience ecological thermal stress (Bhusari, Hearne, Spiers, Lamberson, & Antoniou, 2008), which has been consistently shown to negatively impact the growth performance, food intake, and weight gain of pigs and laying hens raised in high ambient temperatures (Song, Liu, Sheikhahmadi, Jiao, & Lin, 2012; Spencer, Boyd, Cabrera, & Allee, 2003). Previous research has also indicated that heat exposure leads to a significant decrease in AKP concentrations, whereas AST and ALT concentrations increase compared to the control (Wang, Li, Cao, & Li, 2015). LDH, AST and ALT activities in the plasma serve as markers associated with heat stress and are released into the bloodstream when the body undergoes any form of injury (Khan et al., 2015). Moreover, studies on animals have revealed that serum enzymes activities increase under heat stress conditions (Ismail, Al Busadah, & El-Bahr, 2013; Mokondjimobe et al., 2012). In terms of immune status, previous studies have reported that addition with SeP increases serum IL-2 level of piglets reared under high ambient temperatures (Gan et al., 2014). Similarly, probiotics like *L.*

acidophilus have been shown to elevate IL-2 level of pigs reared under normal conditions (Babinska, Rotkiewicz, & Otrocka-Domagala, 2005; Tortuero, Rioperez, Fernandez, & Rodriguez, 1995). Moreover, previous studies have reported that zinc supplementation in Japanese quails can enhance the total antioxidant capacity and reduce malondialdehyde levels compared to the control group (Atakisi, Atakisi, & Kart, 2009). Additionally, zinc picolinate has shown superior effects on the antioxidant enzymes of thermally-stressed quails compared to zinc sulfate monohydrate (Sahin et al., 2005). In our current study, significant improvements were observed in serum biochemical parameters, antioxidant status, immune indices, and the expression of antioxidant, heat shock, and immune-related mRNA following supplementation with P, ZnP, SeP, and the SeZnP combination. These findings highlight the potential benefits of these interventions in mitigating the adverse effects of heat stress on animals.

CONCLUSION

The findings suggest that the product used in the study has potential as a nutritive supplement for animals, particularly in high ambient temperatures. Implementing this strategy of supplementation could help producers maintain the health, comfort, and productivity of their animals, especially during the summer season.

Conflict of Interest: All authors express no conflict of interest in any part of the research.

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