Effects of Heat Stress on Intestinal Health, Immune System, and Meat Quality in Pigs and the Underlying Mechanisms: Postprint

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Abstract

In commercial swine production, heat stress induces reductions in growth performance, compromises immune function, and deteriorates meat quality, thereby inflicting substantial economic losses on the industry. Therefore, investigating the influence patterns of heat stress on porcine intestinal health, immune system, and meat quality is of paramount importance for guiding commercial production. This review summarizes the effects and molecular mechanisms of heat stress on porcine intestinal health, immune function, and meat quality, and outlines feasible mitigation strategies, thereby providing a theoretical foundation and guidance for alleviating the declines in growth performance and meat quality caused by heat stress in the swine industry.

Full Text

Effects of Heat Stress on Intestinal Health, Immune System, and Meat Quality in Pigs and Its Molecular Mechanisms

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Abstract: In practical production, heat stress leads to reduced growth performance, weakened immunity, and deteriorated meat quality in pigs, causing

severe economic losses to the swine industry. Therefore, investigating the effects of heat stress on porcine intestinal health, immune system, and meat quality is of great significance for guiding production practices. This review summarizes the impacts of heat stress on pig intestinal health, immune function, and meat quality along with their underlying molecular mechanisms, and outlines feasible mitigation strategies. The objective is to provide a theoretical foundation and practical guidance for alleviating the decline in growth performance and meat quality caused by heat stress in pig production.

Keywords: heat stress; pigs; intestinal health; immune system; meat quality; molecular mechanism

Pigs are homeothermic animals with underdeveloped sweat glands. When ambient temperature exceeds the upper limit of their thermoneutral zone, pigs exhibit stress responses characterized by tail-biting, rapid breathing, and reduced feed intake, accompanied by abnormal metabolic and physiological functions [1-3]. These manifestations result in decreased growth performance, including reduced average daily feed intake (ADFI) and average daily gain (ADG), and increased feed-to-gain ratio (F/G). Heat stress also compromises immunity, elevates serum inflammatory cytokine levels, reduces disease resistance, and increases morbidity and mortality. Furthermore, it deteriorates meat quality by affecting both muscle and adipose tissue metabolism, decreasing intramuscular fat content while increasing fat deposition in adipose tissues. Notably, pigs at different growth stages exhibit varying sensitivities to ambient temperature. In southern China, the hot and humid summer conditions, which persist for extended periods, inflict substantial economic losses on the pig industry. This review examines the effects of heat stress on porcine intestinal health, immune system, and meat quality and their molecular mechanisms, and summarizes practical mitigation measures to provide theoretical guidance for alleviating performance and quality declines.

1. Effects of Heat Stress on Porcine Intestinal Health

The impact of heat stress on pig growth performance initially manifests as reduced feed intake and feed conversion efficiency. When ambient temperature ranges from 20–30°C, each 1°C increase reduces ADFI by 40–80 g, consequently decreasing ADG and increasing F/G [4]. These effects stem from heat stress-induced damage to intestinal structural integrity and barrier function, reduced digestive enzyme activity, and disrupted microbial balance in the digestive tract, which severely impairs nutrient digestion and absorption and subsequently affects growth and development. The reduction in feed intake also decreases heat increment from metabolism, alleviating the burden of heat dissipation.

1.1. Effects of Heat Stress on Porcine Intestinal Mucosa

Under heat stress conditions, the expression of heat shock proteins (HSPs) in porcine intestine is upregulated. To enhance heat dissipation, blood flow is

redirected to peripheral tissues, causing intestinal hypoxia. Intestinal cells are particularly sensitive to oxygen and nutrient deprivation, leading to ATP depletion, oxidative stress, and nitrosative stress, which ultimately compromise intestinal barrier function [5]. Heat stress induces apoptosis and shedding of epithelial cells at villus tips, significantly upregulating pro-apoptotic factors including caspase-3, caspase-8, caspase-9, and B-cell lymphoma 2-associated X protein (Bax) while downregulating the anti-apoptotic factor B-cell lymphoma 2 (Bcl-2) [6]. The villus height-to-crypt depth ratio decreases across intestinal segments, goblet cells increase within the intestinal epithelium, and mucosal structure becomes damaged [7], findings that have been corroborated in studies on heat stress effects on goose intestinal morphology [8]. These structural alterations are reflected in changes to intestinal permeability and integrity. Physiologically relevant heat stress significantly increases epithelial permeability, reduces transepithelial electrical resistance (TEER) in the ileum and colon, and compromises intestinal integrity. This is accompanied by compensatory upregulation of occludin expression mediated by heat shock factor-1 (HSF-1), concurrent with increased expression of myosin light chain kinase (MLCK) and casein kinase II-α (CK II-α), as well as upregulated tight junction (TJ) proteins including claudin-3 and occludin [9-13]. In vitro studies using intestinal epithelial T84 cells have demonstrated that heat stress disrupts barrier function via protein kinase C (PKC) and MLCK signaling pathways [14], validating findings from animal experiments. Beyond morphological and permeability changes, heat stress significantly reduces digestive enzyme activities, such as amylase and trypsin in jejunal chyme [15], thereby impairing digestive function. Additionally, heat stress alters the amino acid composition of endogenous intestinal proteins, increasing their loss [16].

Transcriptomic and proteomic studies have advanced our understanding of heat stress effects at the cellular level. Yu et al. [17] investigated heat stress impacts on porcine small intestine morphology and gene expression through microarray analysis of jejunal samples, revealing that differentially expressed genes were primarily involved in protein folding, translation regulation, cell proliferation, cell migration, and antioxidant responses. Cui et al. [18] examined the effects of chronic moderate heat stress on finishing pigs, identifying 53 differentially expressed proteins in jejunal mucosa, 18 of which were associated with cell structure and motility—potentially contributing to reduced intestinal integrity and function. Downregulated proteins were mainly involved in the tricarboxylic acid (TCA) cycle, electron transport chain, and oxidative phosphorylation, indicating that chronic moderate heat stress primarily causes energy metabolism disorders that subsequently induce oxidative stress. Altered expression of 10 proteins related to heat stress response and defense suggested adaptation and feedback regulation mechanisms in pigs under long-term heat stress.

Feed restriction alone can alter intestinal function, nutrient transport, and morphology, and the effects of heat stress may be confounded and exacerbated by markedly reduced feed intake. Pearce et al. [13,19] found that both indirect heat stress (pair-feeding at thermoneutral temperature to match intake of

heat-stressed pigs) and direct heat stress similarly reduced intestinal integrity and increased endotoxin permeability. However, comparative analysis of jejunal protein and gene expression profiles between acutely heat-stressed pigs and their pair-fed counterparts revealed that many alterations induced by direct heat stress were independent of feed restriction, indicating that reduced intake only partially accounts for heat stress effects on intestinal integrity. Direct heat stress impacts metabolism and growth performance through mechanisms beyond reduced feed intake [20].

1.2. Effects of Heat Stress on Porcine Intestinal Microbiota

Heat stress affects porcine intestinal health not only by compromising barrier function and digestive enzyme activity but also by altering intestinal microbial metabolism. The gut harbors a microbial barrier—a micro-ecosystem formed by commensal bacteria and the host. Disruption of this ecosystem facilitates invasion by potential pathogens, including opportunistic bacteria. Studies on heat stress effects in chickens have demonstrated significant damage to intestinal structure and alterations in jejunal and ileal microbiota, characterized by suppressed *Lactobacillus* proliferation and promoted *Bacteroides ovatus* growth, thereby disrupting microbial balance [21-23]. These findings align with Peng et al. [24] regarding the impact of chronic heat exposure on cecal microbiota diversity in broilers.

Supplementation with single or mixed probiotics helps restore microbial balance and enhance barrier function compromised by heat stress. Dietary selenium-enriched probiotics increased microbial diversity and improved composition in piglet intestine under high temperature conditions. Zhang [25] reported that a composite probiotic preparation of *Enterococcus faecium* HDRsEf1 and *Bacillus subtilis* HDRaBS1 significantly reduced *E. coli* populations in cecum while increasing *Lactobacillus* in ileum of heat-stressed laying hens, restoring microbial balance, repairing intestinal mucosa, and upregulating TJ protein expression to enhance barrier function at multiple levels. Song et al. [26] found that a mixed probiotic containing *Bacillus licheniformis*, *B. subtilis*, and *Lactobacillus plantarum* ameliorated heat stress-induced reductions in beneficial bacteria and damage to intestinal morphology and function in chickens. Mixed probiotic applications have been implemented in pig production to mitigate heat stress damage in a cost-effective manner.

2. Effects of Heat Stress on Porcine Immune System

Heat stress triggers immune responses that weaken disease resistance and increase morbidity and mortality in pigs. First, heat stress compromises intestinal integrity and increases endotoxin permeability, reducing jejunal TEER by 30% while increasing endotoxin levels by 45% and lipopolysaccharide (LPS) permeability coefficient by 2-fold, accompanied by increased alkaline phosphatase activity. Toxin penetration triggers immune cell infiltration, inflammatory responses, and activates detoxification mechanisms in intestine and liver [13,19].

Second, heat stress affects immune function through neuroendocrine pathways. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, causing hypersecretion of corticotropin-releasing hormone (CRH), glucocorticoids (GC), adrenocorticotropic hormone (ACTH), and proopiomelanocortin (POMC), which subsequently act on various cytokines and immune cells to modulate immune function [27]. Heat stress stimulates production of inflammatory cytokines including interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) in porcine serum, leading to increased leukocyte counts, reduced neutrophil percentages, and elevated percentages of eosinophils, basophils, and monocytes [28-29]. Chen et al. [30] demonstrated that heat stress upregulates Toll-like receptor 9 (TLR9) expression in human B lymphocytes via extracellular signal-regulated kinases (ERK) and nuclear factor-kappa B (NF- B) pathways, resulting in immune suppression and disease susceptibility. Heat stress upregulates TLR2, TLR4, plasma cortisol, and IL-2 transcription in peripheral blood mononuclear cells (PBMCs) of Bama miniature pigs, while showing no significant effect on IL-8 transcription. In vitro studies using cultured PBMCs from Bama pigs confirmed that heat stress stimulates TLR2, TLR4, and IL-12 expression but inhibits IFN-γ expression [31]. Ju et al. [32-34] further reported that heat stress significantly increases serum cortisol and TLR2/TLR4 expression, elevates inflammatory cytokine levels, reduces blood cell and granulocyte counts, and affects CD8+ T cell numbers and CD4+/CD8+ T cell ratios.

Additionally, heat stress suppresses immune organ development and induces apoptosis. In broilers, heat stress impairs development and differentiation of immune organs including thymus, spleen, and bursa of Fabricius, and adversely affects their histological structure [35-36].

3. Effects of Heat Stress on Porcine Meat Quality

Economic losses from heat stress in pig production arise not only from reduced growth performance and immune activation but also from altered organ [37] and muscle metabolism and fat deposition. Heat stress disrupts energy balance among fat, carbohydrate, and protein metabolism, reducing activities of several metabolic enzymes involved in glycolysis and the TCA pathway, thereby affecting meat quality [4,20,38-39].

3.1. Effects of Heat Stress on Porcine Muscle Metabolism

Chronic high temperature exposure suppresses muscle structural and functional development, reduces metabolic capacity, promotes apoptosis, and triggers stress responses, consequently affecting meat quality. Zhang et al. [40] found that chronic heat stress significantly increased lactate content and altered glycolytic pathways in broiler muscle, reducing muscle pH and meat quality while increasing lightness and predisposing to pale, soft, exudative (PSE) meat. This may result from delayed upregulation of calcium regulation genes including α-ryanodine receptors (αRYR), β-ryanodine receptors (βRYR), and

Ca2+-storage protein calsequestrin (CASQ) [41]. Hao et al. [42] used Illumina sequencing to investigate microRNA expression profiles in porcine longissimus dorsi muscle under heat stress, revealing impacts on glucose metabolism, cytoskeletal structure and function, and stress responses. Heat stress significantly upregulated pyruvate dehydrogenase kinase 4 (PDK4), heat shock protein 90 (HSP90), and desmin (DES) while downregulating stearoyl-CoA desaturase (SCD) and lactate dehydrogenase A (LDHA). Protein expression of calmodulin 1 (CALM1), DES, and hypoxia inducible factor 1 alpha subunit (HIF1α) was significantly increased [42]. Subsequent bisulfite sequencing analysis of DNA methylation in porcine longissimus dorsi muscle revealed no significant differences in GC islands, with changes concentrated in non-GC island regions primarily involving energy and lipid metabolism, cellular defense and stress responses, and calcium signaling pathways [43].

Muscle fiber composition influences various meat quality attributes including color, drip loss, tenderness, juiciness, and flavor. Factors affecting fiber type transition include hormones, nutrition, and environment. Heat stress-induced hormonal and nutritional changes can affect meat quality by modulating muscle fiber type transition. Based on morphological, functional, and physiological characteristics, muscle fibers are classified as type I (slow-twitch, oxidative, red fibers) and type II (fast-twitch, white fibers). Type I fiber proportion positively correlates with meat flavor. Chronic moderate heat stress significantly increases white fiber number and proportion while decreasing red fiber number and proportion in pigs. However, contradictory findings suggest that chronic moderate heat stress may induce fiber type transition from fast-twitch (type II) to slow-twitch (type I) via peroxisome proliferative activated receptor gamma coactivator 1 alpha (PGC-1α)-mediated calcineurin/nuclear factor of activated T-cells (calcineurin/NFAT) pathway [44-45]. The discrepancy may arise from differences between in vitro satellite cell models and whole-animal stress responses, with the former resembling local hyperthermia rather than systemic stress. Additionally, Ohno et al. [46] reported no change in type I fiber proportion in rat soleus muscle following heat stress, suggesting potential species-specific effects.

3.2. Effects of Heat Stress on Porcine Fat Metabolism

Intramuscular fat content, which correlates with muscle tenderness and flavor, represents a crucial meat quality indicator and precursor for muscle flavor compounds, and is associated with traits including muscle pH, water-holding capacity, and tenderness. Chronic high temperature significantly reduces intramuscular fat content in porcine longissimus dorsi muscle, thereby compromising meat quality [47].

Heat stress alters adipose tissue metabolism by downregulating lipolytic gene expression while upregulating lipid uptake and synthesis genes, leading to fat deposition and altered fatty acid composition. Kouba et al. [48] reported that heat stress enhanced hepatic and adipose tissue lipid metabolism in finishing pigs. Qu et al. [49] demonstrated that heat stress significantly increased lipopro-

tein lipase (LPL) activity in porcine mesenteric cells and upregulated genes related to fatty acid uptake and triglyceride (TAG) synthesis, including fatty acid synthase (FAS), adipocyte protein 2 (aP2), fatty acid translocase 36 (CD36), fatty acid transport proteins 4 and 6 (FATP4, FATP6), LPL, glucose transporter type 4 (GLUT4), phosphoenolpyruvate carboxykinase 1 (PCK1), and glycerol kinase (GK). Heat stress increased abdominal, subcutaneous, and extramuscular fat deposition in broilers while reducing the unsaturated-to-saturated fatty acid ratio in abdominal and subcutaneous fat [50]. In growing pigs, heat stress decreased circulating non-esterified fatty acids by 20% and reduced non-esterified fatty acid stress by 71% [51]. Elevated PCK1 expression in porcine subcutaneous and mesenteric adipose tissues under heat stress indicated that glyceroneogenesis contributed to fat deposition, with significantly upregulated CCAT/enhancer-binding homologous protein (CHOP) content in mesenteric fat [52].

4. Feasible Measures for Alleviating Heat Stress in Pigs

In summary, heat stress causes intestinal dysfunction, immune suppression, and reduced meat quality, inflicting substantial economic losses on the pig industry. Therefore, implementing practical, economical, and convenient mitigation strategies is essential.

Current approaches include selecting heat-tolerant breeds, improving housing conditions (ventilation, floor cooling, water spraying when relative humidity is low), enhancing management practices, adjusting feeding schedules, providing adequate drinking water, and modifying dietary nutrient levels and formulations. Nutritional strategies are particularly advantageous as they require less time, labor, and financial investment compared to genetic or environmental modifications, making them more practical for widespread implementation.

The theoretical basis for dietary modifications lies in compensating for reduced feed intake-induced energy deficiency that impairs growth performance. Increasing net energy while reducing heat increment can alleviate this issue. Since protein digestion generates more heat than starch or fat, reducing heat increment from nutrient metabolism improves the efficiency of converting metabolizable energy to net energy. High-fat diets are commonly used in practice to increase energy density while reducing crude protein content, thereby decreasing heat increment, improving net energy availability, and enhancing palatability and feed intake to promote growth and improve carcass meat quality [53]. Adjusting dietary formulations (e.g., adding lysine to balance amino acids while reducing crude protein) also effectively mitigates heat stress [54]. Additionally, heat stress disrupts electrolyte balance, and maintaining dietary electrolyte equilibrium prevents energy expenditure for acid-base regulation. Therefore, increasing energy concentration, reducing crude protein while maintaining amino acid levels, improving the net energy-to-metabolizable energy ratio, and preserving electrolyte balance collectively alleviate heat stress [55].

Beyond macronutrient modifications, supplementation with anti-heat stress nutrients—including trace elements (zinc, selenium, chromium) [56-57], antioxidants (vitamin C, vitamin E, daidzein) [58], anti-stress agents (herbal additives, zinc methionine, chromium picolinate, acidifiers) [59], and probiotics [60-61]—can ameliorate heat stress-induced declines in growth performance, immunity, and meat quality.

Due to the complex mechanisms of heat stress and regional climate variations, research findings remain inconsistent, largely attributable to substantial differences in experimental conditions, temperatures, and durations across studies. For example, Pearce et al. [20] used 35°C for heat stress versus (20$±$1)°C for control, with durations of 1, 3, and 7 days; Hu et al. [7] simulated summer conditions with cyclic temperature variation between 26–39°C (4 hours at 39°C) versus 24°C control for 10 days; Yu et al. [17] applied 40°C for 5 hours daily (04:00–09:00) with 26°C for remaining time versus 23°C control for 10 days; Cui et al. [18] gradually increased temperature from 27°C to 30°C over 3 weeks versus 22°C control. Our laboratory [62] employed different conditions (35°C vs. 22°C for 30 days). The temperature range spans 26–40°C with inconsistent durations, complicating cross-study comparisons and potentially causing resource waste from duplicated efforts. Therefore, experimental conditions should simulate local production realities: short-term acute heat stress can model transport conditions, while chronic heat stress should mimic natural climate variations to better understand heat stress impacts and guide production accurately.

Current research has progressed from animal experiments to molecular mechanisms, with substantial advances in transcriptomic and proteomic studies establishing a foundation for understanding heat stress effects on intestinal health, immunity, and meat quality, and mitigation measures have achieved limited application. However, numerous knowledge gaps remain requiring further validation, and comprehensive molecular mechanisms underlying whole-animal responses warrant continued investigation.

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