REVIEW

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Heat stress on microbiota composition, barrier integrity, and nutrient transport in gut, production performance, and its amelioration in farm animals

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Abstract

Livestock species experience several stresses, particularly weaning, transportation, overproduction, crowding, temperature, and diseases in their life. Heat stress (HS) is one of the most stressors, which is encountered in livestock production systems throughout the world, especially in the tropical regions and is likely to be intensified due to global rise in environmental temperature. The gut has emerged as one of the major target organs affected by HS. The alpha- and beta-diversity of gut microbiota composition are altered due to heat exposure to animals with greater colonization of pathogenic microbiota groups. HS also induces several changes in the gut including damages of microstructures of the mucosal epithelia, increased oxidative insults, reduced immunity, and increased permeability of the gut to toxins and pathogens. Vulnerability of the intestinal barrier integrity leads to invasion of pathogenic microbes and translocation of antigens to the blood circulations, which ultimately may cause systematic inflammations and immune responses. Moreover, digestion of nutrients in the guts may be impaired due to reduced enzymatic activity in the digesta, reduced surface areas for absorption and injury to the mucosal structure and altered expressions of the nutrient transport proteins and genes. The systematic hormonal changes due to HS along with alterations in immune and inflammatory responses often cause reduced feed intake and production performance in livestock and poultry. The altered microbiome likely orchestrates to the hosts for various relevant biological phenomena occurring in the body, but the exact mechanisms how functional communications occur between the microbiota and HS responses are yet to be elucidated. This review aims to discuss the effects of HS on microbiota composition, mucosal structure, oxidant-antioxidant balance mechanism, immunity, and barrier integrity in the gut, and production performance of farm animals along with the dietary ameliorations of HS. Also, this review attempts to explain the mechanisms how these biological responses are affected by HS.

Keywords: Heat stress, Gut microbiota, Oxidative status, Barrier integrity, Mucosal structure, Amelioration

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Availability of data and material
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INTRODUCTION

Livestock commonly encounters a number of biotic and abiotic stressors in their life time such as heat and cold, overcrowding, transportation, weaning, infection, overproduction, diseases, and fear. Heat stress (HS) is considered as one of the most significant stressors in all types of animal production systems, especially in the tropical parts of the world [1,2]. Moreover, a threat in global rise of environmental temperature has compelled research for better understanding of HS response mechanisms of animals and its mitigation. High environmental temperatures adversely affect the well-being, production and welfare of animals [3,4]. Overall, production performances of all types of livestock species including poultry are adversely affected by HS due to decreased feed intake with reduced nutrient utilization and feed efficiency resulting in considerable economic losses [5,6]. A wide array of patho-physiological changes, such as immune dysregulation and increases in production of oxidants causing lipid peroxidation of cell membranes and cellular oxidative stress occur due to HS, resulting in greater susceptibility to infectious diseases and increased mortality rate in farm animals [1,2].

The gastrointestinal tract is particularly vulnerable to HS-induced alterations, including impairment of intestinal development, gut barrier dysfunction, improper immune responses, and imbalance of the oxidative-anti-oxidative mechanisms [1,7]. These alterations permit the translocation of antigens, toxins, and pathogens present in the lumen of intestine through the tight junction (TJ) barrier and further stimulate the immune system via toll-like receptor (TLR) signaling, cytokines, and heat shock proteins (HSP), eventually causing alterations in intestinal mucosal microstructures, initiation of inflammation and injury [1,8,9].

A growing number of evidence suggests a strong relationship between the complex and diverse gut microbial communities and health/disease including normal physiological and behavioral changes, immune functions and stress responses of animals and humans [10]. Different types of stress responses including HS can change the composition of gastrointestinal microbiota [10,11]. The gut microbiota has recently been considered as a key factor of adaption and immune response to stressors including HS in the body [10]. HS in animal production systems can cause alterations in the normal microbial communities and epithelial structures in the intestine, which may lead to greater colononization of pathogenic and undesirable microbiota [12]. There are many reviews focusing biochemical, cellular and metabolic changes that occur during thermal stress [13], production performance and welfare [2,14,15] along with HS mitigation strategies [3,4]. This review mainly focuses on the effect of HS on gut microbiota composition and intestinal health including mucosal morphology, barrier and nutrient transport functions, immunity and antioxidant status in the intestine with some discussion on the production performance and dietary HS mitigation strategies in livestock and poultry.

MICROBIOTA CHANGES IN THE GUT BY HEAT STRESS

The gastrointestinal tracts of animals harbors a complex microbiota comprising of commensal microbiota (bacteria, fungi, protozoa, archaea, and viruses) that is dominated by bacteria [16]. An optimal gut microbiome composition is essential for proper nutrient digestion and absorption, feed utilization efficiency, animal performances, immunity development, pathogen exclusions, gut barrier function, health, behavior, and welfare of animals [16,17]. Gut microbiota can also influence the growth and production by producing extra nutrients through the fermentation of plant fibers that the animals cannot digest themselves [18]. The gut microbiota is influenced by several interacting factors, particularly feed composition, water quality, stressors including hyperthermia, farm

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management, feed additive use, and bio-security [16]. Also, the diversity of the gut microbiota is largely influenced by the age of the animals, breed, and location in the digestive tract [16]. In the following sections, effects of HS on the alpha- and beta-diversity of gut microbiota in different animals are discussed along with the mechanisms of their alterations.

Effect of heat stress on alpha-diversity

The effect of HS on the alpha-diversity is variable depending upon the species, duration, intensity of exposure, and segments of the gut (Table 1). High temperature (21℃ vs. 31℃ from 28 to 42 days) significantly affected the alpha-diversity in ileum with greater number of observed species, Chao 1 and whole-tree phylogenetic diversity, but the Shannon, Simpson, and Pielou indices were not altered, indicating that HS increased the microbiota species richness in the ileum of broiler chickens [19]. In feces of pigs, HS (25℃ vs. 29℃ for 21 days) also increased species richness, but the Shannon index was similar [20]. Relatedly, species richness was greater in hot climatic conditions than in the temperate climatic conditions in feces of pigs [20]. In other studies, HS did not show alterations of alpha-diversity such as Shannon index of the gut microbiota in cecum of laying hens [21], cecum of broiler chickens except phylogenetic diversity [22], ileum and jejunum of ducks [23], feces of pigs [24,25] to a large extent. There is limited information on alpha-diversity in ruminants as affected by HS. In Holstein heifers, different environmental temperatures (20℃, 28℃, and 33℃) and humidity (60% and 80%) did not alter the operational taxonomic unit (OTU) numbers and Shannon diversity in the ruminal fluid [26]. In Hanwoo steers, HS (15℃ vs. 35℃) did not alter alpha-diversity indices such as OTU richness, Shannon index, Simpson index, Chao1, and phylogenetic diversity of ruminal bacteria or archaea [27]. It seems that HS has a smaller effect on the alpha-diversity of microbiota except species richness in the gut of livestock species.

Effect of heat stress on microbiota communities based on principal coordinate analysis

Although alpha-diversity of gut microbiota is minimally affected by HS, gut microbiota at the community level is usually influenced by HS, especially due to changes of the microbiota at the lower taxonomic levels such as family and genus levels. The principal coordinate analysis based on beta-diversity of the overall microbial community composition suggests that microbiota in ileum [19] and cecum [22] of broiler chickens of HS groups formed a distinct cluster separated from the control group. In pigs, heat challenge at 25℃ vs. 29℃ for 21 days from 23 to 26 weeks of age [20] and 25℃ vs. 35℃ for one day [25] also resulted in two distinct microbiota communities. But, HS effect has differential impacts on the microbiota communities in the different segments of guts. For example, microbial communities in the jejunum formed separate clusters of HS vs. thermoneutral conditions, but not in the ileum and cecum of ducks [23]. Moreover, the microbial community changes with the duration of heat exposure. No difference in the gut microbiota community was evident between HS and thermoneutral condition on day 1 and 3 of heat exposure as the communities did not form separate clusters, while a tendency toward change in the communities occurred on day 7 and a distinct cluster was formed on day 28 in the cecum of broiler chickens [28]. In Hanwoo steers, HS (15℃ vs. 35℃) caused a change in microbial community composition in the rumen on day 6 of heat exposure [27]. Although microbial communities may be associated with decreased feed intake, but HS-induced changes in bacterial communities was independent of reduced feed intake because there was no difference in microbial communities in pair-feeding (when feed intake level in the thermoneutral condition was controlled to the intake level of HS group) and thermoneutral animals [25]. From the above discussion, it is clear that heat challenge usually changes overall microbial community composition independent of feed intake, but impacts

Table 1. Effect of heat stress on microbial diversity and microbiota composition at the predominant phylum level in the gut of livestock

Reference	Animal	Thermoneutral vs. HS	Site	Alpha-diversity		Beta-diversity[1)]			
				Chao1	Shannon	*Firmicutes*	*Bacteroidetes*	*Proteobacteria*	Other phyla
[22]	Broiler chickens	22℃ vs. 32℃ for 14 days	Cecum	428 vs. 440	5.48 vs. 5.46	↑ by 14%	↓	=	=
[28]	Broiler chickens	24℃–26℃ vs. 34℃–38℃ for 28 days	Cecum (day 3)	-	-	57.7 vs. 55.9	37.0 vs. 39.4	4.97 vs. 3.62	=
			Cecum (day 7)	-	-	58.5 vs. 68.5↑	33.2 vs. 22.5↓	7.29 vs. 6.76	*Tenericutes:* 1.0 vs. 0.74↓
			Cecum (day 14)	-	-	62.5 vs. 71.7↑	32.8 vs. 23.6↓	3.01 vs. 4.03↑	*Cyanobacteria*: 0.52 vs. 0.13↓
			Cecum (day 28)	-	-	68.6 vs. 69.3	26.2 vs. 26.7	3.43 vs. 2.22	=
[36]	Cows (high HS sensitivity)	THI of 65.5 vs. 80.2	Feces	-	-.	↓	=	=	=
	Cows (low HS sensitivity)	THI of 65.5 vs. 80.2	Feces	-	-	=	↑	=	=
[19]	Broiler chickens	21℃ vs. 31℃ from 28 to 42 days	Ileum	95.5 vs. 176↑	1.90 vs. 2.27	85.6 vs. 92.2↑	5.07 vs. 2.58↓	7.39 vs. 1.93↓	=
[21]	Hens	21℃ vs. 29℃–35℃ for 28 days	Cecum	=	-	18.7 vs. 17.4	49.9 vs. 54.8	=	=
[31]	Laying hens	25℃ vs. 35℃ for 24 h	Feces	-	-	54.7% vs. 45.9% ↓	26.1% vs. 43.1%↑	6.57% vs. 3.70% ↓	*Fusobacteria*↓
[23]	Cherry-valley ducks	25℃ vs. 32℃ for 8 h/day for 21 days	Jejunum	-	=	45.4 vs. 12.0 ↓	=	39.0 vs.72.7↑	=
			Ileum	-	=	=	=	=	=
			Cecum		=	=	=	=	=
[24]	Sows	18℃–22℃ vs. 28℃–32℃ for 30 days	Feces	754 vs. 797	4.85 vs. 4.95	=	=	=	*Spirochaeta*↓ (tended)
[20]	Pigs	25℃ vs. 29℃ for 21 days	Feces	-	=	66.9 vs. 70.8↑	24.9 vs. 17.6↓	0.79 vs. 0.77	*Spirochaetes*: 1.4 vs. 2.11↑
									Fibrobacteres: 0.25 vs. 0.40↑
									Tenericutes: 0.01 vs. 0.03↑
[25]	Pigs	25℃ vs. 35℃ for 24 h	Feces	-	=	=	↓	↑	=
[27]		15℃ vs. 35℃ for 6 days	Rumen	=	=	=	=	↓	Planctomycetes↓, Chloroflexi↓

[1)]Relative abundances (%) are presented depending upon the availability of the information in the literature.

The symbols '↓' or '↑' indicate that abundances of microbiota at phylum level decreased ($p < 0.05$) or increased ($p < 0.05$) in the heat stress conditions compared with the control thermoneutral conditions.

The symbol '=' indicates that abundances of microbiota at phylum level in the heat stress conditions was similar ($p > 0.10$) to control thermoneutral conditions.

HS, heat stress; THI, temperature humidity index.

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may differ in different gut segments depending upon duration of exposure.

Effect of heat stress on beta-diversity

Poultry

The modern poultry genotypes are more susceptible to HS due to marked growth rate along with greater metabolic activity [1]. An earlier study with the use of a culture technique showed that the number of *Enterobacteriaceae* was decreased, whereas *Streptococcus* and *Staphylococcus* numbers were increased by HS in the small intestine of chickens [29]. Microbial community composition in the ileum and cecum of chickens was also altered by heat exposure to 30℃ for 24 h [12]. Moreover, ileal tissues from chickens subject to 30℃ for 24 h increased ex-vivo attachment of *Salmonella* compared with chickens kept at 23℃ [12]. Similarly, HS (33℃ for 10 h vs. 22℃) lowered the viable numbers of beneficial *Lactobacillus* and *Bifidobacterium*, but increased viable numbers of coliforms and *Clostridium* in the small intestine of broiler chickens [7]. In the ileum and ceca of laying hens, the pathogenic *E. coli* number decreased, whereas lactobacillus decreased due to HS (26℃ vs. 33℃ for 20 days) on both day 10 and 20 in the ileum and ceca of laying hens [30]. The culture dependent study clearly showed that HS increased pathogenic bacterial populations and decreased beneficial microbiota in the gut.

More recently, culture-independent study has been used to assess the impacts of HS on the gut microbiome of farm animals. Exposure of heat has shown variable effects on the abundances of gut microbiota at the phylum level. In laying hens, the abundances of *Firmicutes* (54.7%), *Bacteroidetes* (26.1%), *Fusobacteria* (7.05%) and *Proteobacteria* (6.57%) in feces dominated the phylum level in the thermoneutral conditions (21℃ to 28℃ and 36% to 45% relative humidity), which were altered in HS conditions [31]. In HS conditions (25℃ to 34℃ and 56% to 79% humidity), the abundances of phylum *Firmicutes* (45.9%) also dominated, followed by *Bacteroidetes* (43.1%), *Proteobacteria* (3.70%), and *Euryarchaeota* (2.03%) in feces [31]. But, this study suggested that HS increased the relative abundance of *Bacteroidetes*, whereas the abundance of *Firmicutes, Fusobacteria*, and *Proteobacteria* reduced in the feces of layer chickens [31]. Similarly, the relative abundances of *Firmicutes* (45.4% vs. 12.0%) reduced, whereas phylum *Proteobacteria* (39.0% vs. 72.7%) markedly increased in the jejunum of heat stressed-ducks (32℃ for 8 h/day for 21 days) compared with the ducks in the thermoneutral (25℃) condition [23]. In contrast, HS (21℃ vs. 31℃ for 2 weeks) increased relative abundance of *Firmicutes* (92.2% vs. 85.6%), and decreased relative abundance of *Proteobacteria* (1.93% vs. 7.39%) and *Bacteroidetes* (2.58% vs. 5.07%) in the ileum of broiler chickens [19]. In another study with laying chickens, HS increased *Bacteroidetes*, but had no effect on other predominant phyla in cecum [32]. At the phylum level, no changes in the abundances have also been reported. For instance, *Bacteroidetes* (49.9%, 54.8%, and 46.0%) and *Firmicutes* (18.7%, 17.4%, and 16.0%) were dominant in the cecal samples of thermoneutral and HS and pair-feeding laying hens, which were not significantly different among the groups [21].

The contrasting reports on the abundances of microbiota at the phylum level are not clear, but the variations may arise due to duration and intensity of exposure and feed intake changes caused by HS. For instance, in a study with broiler chickens, the relative abundances of *Firmicutes* (58.5% vs. 68.5%) and *Tenericutes* (0.1% vs. 0.74%) increased, whereas the abundances of *Bacteroidetes* (33.2% vs. 22.5%) reduced in the cecal content of HS group (34℃ to 38℃) compared with the control group (24℃ to 26℃) on day 7 [28]. The relative abundance of *Firmicutes* (71.7% vs. 62.5%) and *Proteobacteria* (4.03% vs. 3.01%) increased, whereas *Bacteroidetes* (23.6% vs. 32.8%) and *Cyanobacteria* (0.1% vs. 0.52%) reduced in the ceca of HS group on day 14 [28]. However, HS did not cause any changes in the gut microbiota communities at the phylum level on day 1, 3, and

28 [28]. The changes in abundances of bacteria at the phyla levels, particularly for *Firmicutes* and *Bacteroidetes* may not be considered to have its practical inferences for health benefits and diseases unless their compositional changes are studied at the lower taxa levels such as genus and species [33].

Compared with the phylum level, as expected, gut microbiota communities are markedly influenced by HS at the lower taxa levels, particularly at the lower family and genus levels and a number of orders, classes, families and genera are changed. At the order level, relative abundance of the *Lactobacillales* (25.0% vs. 12.9%) was greater and relative abundance of *Clostridiales* (66.9% vs. 72.7%), *Enterobacteriales* (1.56% vs. 7.30%), and *Bacterioidales* (2.55% vs.5.07%) was lower in the ileum of the HS group. At the genus level, the relative abundances of *Faecalibacterium, Rothia, Alistipes, Clostridium* XIVb, *Streptophyta*, *Azospirillum*, and *Oscillibacter* were greater, whereas that of *Coprococcus* and *Streptococcus* were lower in heat-stressed broiler chickens [19]. Also at the genus level, the abundance of *Bacteroides* on day 7 (26% vs. 18%), 14 (5% vs. 14%), and 28 (10% vs. 5%), *Faecalibacterium* on day 1 (8% vs. 5%) and 7 (12% vs. 9%), and *Oscillospira* on day 1 (12% vs. 8%) and 3 (11% vs. 9%) decreased after HS in broiler chickens [28]. In laying hens, more than 40 genera were altered due to HS. *Bacteroides* (13.6% vs. 19.8%) and *Alistipes* (5.99% vs. 9.91%) abundance under *Bacteroidetes* phylum increased in heat-stressed layers [31]. Conversely, *Fusobacterium* (6.73% vs. 0.49%) belonging to phylum *Fusobacteria* (13.6% vs. 19.8%), *Clostridium* (9.22% vs. 7.82%), *Ruminococcaceae* (4.29% vs. 1.99%), *Lactobacillus* (3.44% vs. 1.98%), and *Turicibacter* (2.46% vs. 0.30%) under *Firmicutes* were less abundant in HS conditions [31]. Among the less abundant genera (< 3%), *Bifidobacterium, Cloacibacillus,* and *Synergistes* in the heat-stressed layers (0.20%, 0.21%, and 0.10%, respectively) increased compared with those in the control layers (0.12%, 0.072%, and 0.038%, respectively) and *Dorea* abundance (0.35% vs. 0.30%) was decreased by HS [31].

In ducks, the relative abundances of class *Bacilli*, *Lactobacillales* order, *Lactobacillaceae* family, and *Lactobacillus* genus reduced, whereas phylum *Proteobacteria* (39.0% vs. 72.7%), order *Pseudomonadales*, family *Moraxellaceae* and genus *Acinetobacter* markedly increased in the jejunum of heat stressed-ducks (32℃ for 8 h/day for 21 days) compared with the ducks in the control temperature (25℃) condition [23]. In the ceca, the relative abundance of *Rickettsiales* was more prevalent, while the relative abundances of class *Negativicutes* and order *Selenomonadales* markedly reduced in heat stressed-ducks compared with the controlled-ducks [23]. In the ileum, there were no significant changes of microbiota abundances in ducks due to HS [23]. This study suggested that the most impacts of HS may occur in the jejunum section of gut of ducks [23]. In another study with laying hens, *Bacteroidales* (49.9%, 54.8%, and 46.0%) and *Clostridiales* (12.7%, 12.2%, and 10.0%) were dominant in the cecal samples of thermoneutral, HS and pair-feeding laying hens, which were not significantly different among the groups [21]. At the genus level, abundances of 15 genera were different between thermoneutral and HS groups, notably *Bacteroides, Lactobacillus, Ruminococcaceae* UCG_005, and *Prevotellaceae* Ga6A1 group were greater in the thermoneutral group, whereas *Gallibacterium, Escherichia, Shigella, Barnesiella, Anaerosporobacter, Sphaerochaeta, Odoribacter, Clostridium sensu stricto* 1, and *Rikenellaceae* RC9 gut group increased in the HS group [21]. Among these 15 genera, feed intake was significantly related with the relative abundances of 10 genera, which indicates that reduction in feed intake in the HS group may be one of the factors of HS-induced microbiome changes [21]. The reasons for the differences in abundances of microbiota at taxonomic levels among the studies are not clearly known, but may be attributed to the differences in feed composition, species, location in the gut, and intensity and duration of HS [31].

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Swine

In Landrace × Large White crossbred sows, HS (18℃–22℃) vs. (28℃–32℃) conditions applied from 85 days of gestation to farrowing altered beta-diversity [24]. Particularly, HS increased the relative abundances of genera and OTUs related to *Clostridiales* and *Halomonas*, but reduced the relative abundances of genera and OTUs related to *Bacteroidales* and *Streptococcus* [24]. Similarly, Large White × Créole pigs subject to HS (25℃ vs. 29℃ for 21 days) from 23 to 26 weeks of age had more OTU abundances in the families related to *Prevotellaceae* (41% of the OTU) and the *Lachnospiraceae* (17%), whereas *Clostridiaceae* 1 (24% of OTU), *Erysipelotrichaecae* (5% of the OTU) and *Peptostreptococcaceae* (2.3% of the OTU) were less abundant in feces of HS conditions [20]. Also, the total number of OTU was more abundant in hot environment (under tropical climate or after a heat exposure) in the feces of pigs [20]. The metabolic pathway associated with steroid hormone biosynthesis was less-represented and the G protein-coupled receptor was over-represented under HS conditions [20]. Even, acute HS (25℃ vs. 35℃ for one day) changed the composition and diversity of fecal microbial community by decreasing the abundances of *Bacteroidetes* phylum and it lower taxa of class *Bacteroidia*, order *Bacteroidales*, and family *Prevotellaceae*, and increasing the abundances of phylum *Proteobacteria* and its class *Proteobacteria*, order *Pseudomonadales*, family *Moraxellaceae* and genus *Acinetobacter* in feces of pigs [25]. These changes were independent of feed intake reduction due to HS as pair-feeding group only decreased the abundances of *Bacteroidales* and *Prevotellaceae* compared with the thermoneutral group [25]. Like poultry [28], duration (7 days vs. 14 days) of HS redistributed the microbiota phylum composition in pigs [34].

Ruminants

There is relatively limited research investigating HS on gut microbiota composition in ruminants. In an earlier study with Holstein heifers exposed to different environmental temperatures (20℃, 28℃ and 33℃) and humidity (60% and 80%),16S rDNA sequence library composition was different between 20℃ and 33℃ at 80% humidity, but not at 60% humidity [26] suggesting that environmental temperature along with humidity has more impact in this study. In the phylum level, *Firmicutes, Bacteroidetes, Proteobacteria,* and *Spirochetes* in ruminal fluid were not changed at different temperatures and humidity levels [26]. In the same study, the quantification of the bacterial composition using oligonucleotide probes, the relative abundances of the *Fibrobacter* genus decreased, whereas that of *Clostridium coccoides–Eubacterium rectale* group, and *Streptococcus* genus increased due to increasing temperature [35]. In lactating cows, the relative abundance of fecal *Firmicutes* was lower and that of fecal *Bacteroidetes* was higher in the HS (temperature humidity index of 80.5 vs. 66.0) conditions [36]. A recent study applying Ilumina MiSeq platform reported that HS (15℃ vs. 35℃ for 3 or 6 days) lowered the relative abundances of *Proteobacteria*, *Chloroflexi* (on day 6), and *Planctomycetes* (on day 3) among 15 phyla [27]. The relative abundances of the family *Prevotellaceae*, genera *Prevotella* and YRC22 (increased) under phylum *Bacteroidetes; Ruminococcaceae* family (decreased), *Lactobacillaceae* family, *Lactobacillus* genus (increased) under phylum *Bacteroidetes*: *Succinivibrionaceae*, *Moraxellaceae* families, *Ruminobacter* genus (increased), *Desulfovibrionaceae* (decreased) under *Proteobacteria* phylum, *Anaerolineaceae* and *Pirellulaceae* families (decreased) were impacted by HS [27]. Ruminal archaeal populations at the phylum *Euryarchaeota*, families *Methanobacteriaceae*, *Methanomassiliicoccaceae*, genera *Methanobrevibacter*, *Methanosphaera*, and vadinCA11 were not altered by HS [27]. Overall, these studies indicated that HS can change a few bacterial taxa in the rumen.

Implications of gut microbiota changes due to heat stress

The alteration of abundances some of the above discussed bacteria due to HS might have

implications on the development of diseases and nutrient harvest from feeds. For example, *Escherichia, Clostridium,* and *Shigella* can produce α-toxin and are associated with the development of enteric necrotic colitis [37]. Increased abundances of *Fusobacteria* spp. could be detrimental as it may perform as a proinflammatory factor promoting tumorigenesis in the intestine [38]. *Lactobacillus* can lower lumen pH in the intestine, creating an environment that is unfavorable for potential pathogens [39]. The members of *Bifidobacterium* provide health benefits to the host, including prevention of gut disorders, immunomodulation [40] and protection from pathogenic bacteria [41]. The higher relative prevalence of *Proteobacteria* is considered as an indication of intestinal microbial dysfunction [42]. *Cloacibacillus* spp. are potential pathogens related to intestinal infections and bacteremia [43]. The members of *Clostridium* XIVb are frequently observed in ulcerative enteritis of poultry [44]. On the other hand, lower abundance of *Coprococcus* spp. has been associated with irritable bowel syndrome, including bloating, gut discomfort, and colonic hypersensitivity in humans [45]. *Dorea* population reduced in alcohol-related diseases [46]. The abundances of *Faecalibacterium* decreases in gut inflammation, and this bacterium is important in maintaining gut health because of its anti-inflammatory activity within the intestine [47,48]. *Oscillospira* and *Faecalibacterium* genera that are butyrate producers and are reduced in gut disorders, were also decreased by HS [49,50]. *Lachnospiraceae* members can produce butyric acid, which may promote the development of epithelial cells and gut health [51,52]. The effects of HS on these taxa may be associated with poor gut health and production performance of animals, but this assumption needs additional direct evidence [21].

Metabolites and metabolic profile changes in gut by heat stress

The changes in microbiota composition by HS may also change the metabolites in the digest or feces and Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways at the lower levels like bacterial taxa. Usually, HS does not cause major changes of major KEGG pathways at first and second levels in the fecal microbiota of control and heat-stressed layer hens [31]. A few KEGG pathways at the third level are changed by HS. For example, cysteine and methionine metabolism and benzoate degradation pathways increased, but pathways related to retinol metabolism, phenylpropanoid biosynthesis, type II diabetes mellitus, and mitogen-activated protein kinase signaling tended to decrease by HS [31]. In HS conditions, the concentrations of total short-chain fatty acids, propionate, butyrate, fumarate, malate, lactate, succinate, β-alanine and niacin, aspartate, and ethanolamine were lower, whereas concentrations of fructose and azelaic acid were greater [24]. The changes in HS-induced microbiota were correlated with metabolites, suggesting that the shift in HS-induced microbiota likely changed intestinal metabolism [24].

In lactating cows, fecal *Firmicute* abundance reduced fecal *Bacteroidetes* abundance increased in the HS (temperature humidity index of 80.5 vs. 66.0) conditions [36]. The KEGG pathway analysis of microbial function showed that heat sensitive cows in HS environment up-regulated expression of the pathways associated with diseases, including infectious and immune system disease, cancer, genetic information processing (degradation, folding, and sorting), and environmental adaptation [36]. High microbial diversity (Simpson or Shannon diversity) had negative correlation with plasma cortisol, interleukin 1 beta (IL1B), and tumor necrosis factor alpha (TNFA) levels [36]. In growing-finishing pigs, HS altered about 35 most enrichment pathways at the third level of KEGG hierarchy, including carbon fixation pathways in prokaryotes, activation of secretion system, other ion coupled transporters, and pyruvate metabolism pathways [25]. Nutrient metabolism pathways such as amino acid, starch and sucrose metabolism, peptidases, and pathways involved in DNA replication and repair were depressed by HS [25]. These changes in metabolic pathways by HS also reflected in the metabolites in feces such as decreased concentrations of total short-chain fatty

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acids, acetic acid, propionic acid, butyric acid, valeric acid, and isovaleric acid. These alterations were independent of feed intake reduction except for acetic acid concentration that was also reduced by reduced feed intake in the pair-feeding group compared with the thermoneutral group [25]. In dairy goats, hippurate and other phenylalanine derivative compounds in urine increased in HS (15℃ to 20℃ vs. 30℃ to 37℃) condition, which has been suggested due to overgrowth of harmful bacteria in the gut [53]. The above discussion suggests that HS has marked influences on the KEGG pathways, mainly at the lower levels, due to changes in bacterial composition that resulted in metabolite profile in digesta.

Mechanisms of microbiota changes

The drivers of taxonomic perturbation in the gut microbial community composition due to HS are not clearly recognized. Feed intake is reduced during high temperature exposure, which might cause the alterations of microbiota in the gut. But studies have shown that acute HS-induced microbiota community changes may occur independent of feed intake in animals, for example, in the feces of pigs [25]. In a long term study with laying hens, HS (21℃ vs. 29℃ to 35℃ for 28 days), however, did not separate the microbial community structure in the cecum of pair-feeding group from that of HS group, but there was a distinct separation between the pair-feeding and thermoneutral groups, suggesting that a shift in microbial community of cecum in HS condition may be related to reduced feed intake [21]. Nonetheless, some of the beta-diversity in this study did not relate to feed intake. This means other factors besides feed intake may contribute to the microbiota changes in the HS groups.

Gastrointestinal microbiota is likely governed through bidirectional crosstalk of microbiota-gut-brain axis during homeostatic as well as stress conditions. The microbiota-gut-brain axis has been considered a dynamic matrix of tissues and organs consisting of brain, glands, gastrointestinal tract, immune cells and gut microbiota, which regulates homeostasis by communicating in a complex multidirectional manner [10]. Under homeostatic conditions, microbiota regulates the release of cytokines and chemokines from mucosal immune cells by modulating the differentiation of immune cell subsets, which in turn maintains local levels of bacteria in the gut [54]. It has been reported that polysaccharide A of *Bacteroides fragilis* can stimulate regulatory T cells, which have an anti-inflammatory effect and diminish immune responses; whereas segmented filamentous bacteria can persuade T helper 17, which are pro-inflammatory [54]. Gut microbiota, thus, influencing local immune responses can also synthesize neurotransmitters and microbial products and influence the release of hormones and neuropeptides from enteroendocrine cells [10,55,56]. The microbial by-products, cytokines, chemokines, and endocrine messengers can infiltrate the blood and lymphatic systems, or persuade neural messages through the vagal and spinal afferent neurons to influence the local and centrally-mediated responses, including regulation of hypothalamus-pituitary-adrenal (HPA) axis activity [10,55,56]. Heat exposure can impair these mechanisms of homeostasis and consequently influence gut microbiota composition through release of stress hormones and neurotransmitters that alter gut physiology [10,57]. In broiler chickens, HS increased serum corticosterone levels and systematic inflammatory cytokines (e.g., TNFA and IL2), and lowered immunity [58–60]. This indicates that HS activates HPA axis and increases corticosterone levels, which in turn may decrease the immune system activity in the intestine, leading to changes in microbiota composition. Exposure of heat increases core body temperature including rectal temperature in pigs [61,62] and poultry [63]. Bacterial populations are sensitive to temperature changes and consequently the increase temperature in the gut digesta may also contribute to the changes in the microbiota composition due to HS. Overall, the precise mechanisms how microbiota composition is modified by HS and how altered microbiome orchestrates a response

communication between the microbiota and hosts are yet to be elucidated. A better understanding of the functions of gut microbiota in fundamental physiological and pathophysiological responses would be helpful to ameliorate the HS-related adverse effects in animals.

GUT HEALTH

HS causes a range of adverse effects on intestinal morphology, mucosal immunity, integrity, digestive enzyme secretions, antioxidant status, and HSP expressions in different domestic animals, which have been summarized in Table 2 from different studies.

Heat stress on morphological changes in the gut

Intestinal villi perform a number of functions, such as secretion, absorption, and immunity. Proper villus structures are required for optimum functions. The villi are broader and tongue shaped in the duodenum and jejunum, which become finger shaped in the ileum. Usually, length and surface area of villi are greater at the beginning of small intestine, reducing gradually to reach a minimum in the ileum close to the ileo-cecal junction [64]. Shorter villus length reduces surface area for nutrient absorption. The crypts can be considered as the villus factory, which replenish damaged tissues with newer ones [64]. Exposure of heat to livestock species is detrimental to the intestinal morphology including villus architecture, crypt depth, and mucosal layer thickness.

Various histopathological changes in the gut occur due to HS in animals. The intestinal villi structures of HS-laying hens were damaged with extensive desquamation, mainly at the tip and exposed lamina propria, compared to the normal intestinal villi in the control group [30,65]. Sloughing of epithelial cells of tips and sides of villus, vacuolization, and desquamation of epithelial mucosa with denuded lamina propria were noted under HS (21℃ vs. 35℃) for short period (from 09:00 to 13:00 and 21℃ from 13:00 to 09:00) for 30 days in layer chickens [66]. Also, HS has been shown to cause mild acute multifocal lympho-plasmocytic type enteritis and moderate infiltrates with foci of heterophils in intestinal lamina propria in broiler chickens [58,59,65,67]. This mild enteritis worsen when there is pathogenic infection in gut, e.g., *Salmonella* during the HS conditions in chickens [67]. In cows, HS (15℃ vs. 28℃) has been shown to increase the number of infiltrating cells in sub-mucosa of jejunum but not in mucosa [68]. In pigs, HS (23℃ vs. 40℃) for 5 h/day for 10 days resulted in increased mitochondria numbers with shortened internal cristae and organelle debris within lysosomes in jejunal epithelium and caused desquamation at tips of intestinal villi and exposed lamina propria in jejunum within 3 days of HS; however the recovery of the intestinal mucosal damage initiated in 6 days of HS [69,70]. Moreover, microarray analysis revealed that 110 genes were down-regulated and 93 genes were up-regulated, which were associated with pathways in unfolded protein and regulation of cell migration, antioxidant mechanism, translation initiation, and cell proliferation [70].

Detrimental alterations of the microstructures of the intestinal mucosa caused by HS are common features in different livestock species depending upon duration and intensity of heat exposure. In broiler chickens, HS decreased villus surface area, villus height, epithelium cell area, and relative intestinal weight. Heat exposure (37℃ for 8 h for 15 days) damaged the jejunal and ileal villus structures with shorter intestinal villi, deeper crypts, and a reduced villus height to crypt depth (V/C) ratio along with decreased numbers of goblet cells and lymphocytes compared with the thermoneutral (24℃) condition in chickens [71]. Shorter villus, deeper crypt depth, and lower ratio of V/C ratio in jejunum were observed due to HS (33℃ for 10 h vs. 22℃) in broiler chickens [7]. The villi height and V/C of ileum and ceca were decreased by HS in laying hens compared with the control laying hens [30]. However, very short-term (24 h) HS (24℃ vs. 30℃) to birds reduced

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Table 2. Effect of heat stress on mucosal morphology, antioxidant status, integrity, immunity, and production performance in the gut of farm animals

Reference	Animal	TN vs. HS conditions with RH	Major effect in comparison with TN versus HS conditions
[130]	21-day old male broiler chickens	TN (21℃) vs. HS (32℃) with 64% RH for 14 days	• HS decreased BW, ADG and increased FCR • HS decreased villus length, villus surface area, epithelium cell area, and relative weight of intestine.
[66]	Lohmann LSL-classic layer cockerels	TN (21℃, RH 62%) vs. HS (35℃, 64% RH, from 09:00 to 13:00 and rest of the time at 21℃ for 30 days	• HS decreased duodenal, jejunal and ileal villus height, crypt depth and absorptive epithelial cell area. • Sloughing of epithelial cells of tips and sides of villus, vacuolization, and desquamation of mucosal epithelia with denuded lamina propria.
[63]	26-day-old broiler chickens	TN (20℃, 24 h/day) vs. HS (30℃, 24 h/day and 35℃ for 4 h/day and then 20℃ for 18 h) for 10 days	• HS reduced BW, ADG and increased FCR. Feed intake increased in the acute HS condition. • Increased lipopolysaccharide, corticosterone, TNFA, and IL2 in blood, and a higher prevalence of *Salmonella* spp. in livers and meat.
[12]	Male Ross 308 broiler chickens	TN (23℃) vs. HS (30℃) for 24 h	• Ileal tissue had fewer bands on HS than TN. • Crypt depth reduced but no effect on villus height and VCR after 24 h of HS.
[131]	Male Wenchang chickens	TN (25.7℃, 79 to 88% RH) vs. HS (40.5℃, 52.4% RH 2 h/day) for 15 days	• Small intestinal mucosal epithelial cells dispersed outwards, indicative of compromised structural integrity. • Mucosal epithelia were detached with ruptured small intestinal villi and exposed lamina propria. • HS reduced in villus length, mucosa thickness, intestinal wall thickness, and crypt depth in all three segments.
[97]	Male Wenchang chickens	Ambient temperature vs. HS (40.5℃, 52.4% RH for 2 h) for 15 days	• HS declined BW, ADG, and feed intake, but no effect on FCR. • HS decreased villus length, crypt depth, mucosa thickness, and intestinal wall thickness in duodenum and ileum, and goblet cells in duodenum and jejunum.
[76]	Male Arbor Acres plus broiler chickens	TN (20℃) vs. HS (32℃–33℃ 8 h/day) for 42 days	• HS decreased ADG, ADFI, and FCR. • HS increased jejunal mucosal MDA content, and lowered SOD activity in ileal mucosa at 42 day. • HS reduced villi height and VCR in jejunum and ileum, and increased jejunal crypt depth. • HS decreased mRNA abundance of *CLDN3* in jejunum, but not in ileum at 21 days. • HS reduced mRNA levels of jejunal *MUC2* and *OCLN*, and ileal *MUC2*, *ZO1*, *OCLN*, and *CLDN3* at 42 days.
[132]	Castrated crossbred male pigs	TN (22℃) vs. HS (30℃) and 55% RH for 21 days	• HS reduced villus height and crypt depth, but no effect on VCR. • HS increased plasma D-lactate concentration and lowered alkaline phosphatase activity in intestinal mucosa. • HS upregulated HSPH1, HSPB1, HSPA5, and HSPA1A.
[90]	White Leghorn hens (350-day- old)	TN (20℃–22℃, 50%–60% RH) vs. HS (30℃–33℃, 70%–80% RH for 24 h) for 28 days	• HS decreased egg weight, eggshell thickness, eggshell percentage, and eggshell density. • HS decreased calcium binding protein (calbindin) in ileum, cecum, and colon.
[61]	1-week post-weaned crossbred gilts	TN (28℃) vs. HS (38℃ for 6 h/day and rest 18 h/day at 32℃) with 40%–60% RH for 3 days	• TER reduced in ileum. • FITC-d and FITC-LPS flux increased in ileum. • HSP70 protein increased in ileum.
[89]	Male broiler chickens (28 days old)	TN (20℃, 50% RH) vs. HS (30℃, 70% RH) vs. pair-feeding like HS in TN for 14 days	• HS and pair-feeding reduced BW gain. • Only HS lowered plasma thyroid hormones and increased corticosterone than TN and pair feeding groups. • HS reduced fresh weight and length of jejunum compared to TN and pair-feeding. • No change in proximal end of jejunum, but villus length reduced on HS, followed by pair feeding and then TN. • HS increased the activity and expression of apical SGLT1 by approximately 50%, with no effects in the pair-feeding group.
[77]	21-day-old mixed Cobb broiler chickens	TN (20℃) vs. HS (27.8℃) with 53.0% RH for 14 days (21 to 35 days of age)	• HS decreased BW, ADG, ADFI, and feed efficiency. Breast meat quality was not affected by HS. • HS decreased jejunal TER values. • No effect on serum LPS concentration. • HS had no effect on jejunal gene expressions of *OCLN*, *ZO1*, *CLDN1*, and *JAM2*.
[84]	Male broiler chickens	TN (21℃) vs. HS (0, 2, 3, 5 and 10 h at 36℃ for a day	• In jejunal mucosa, HS decreased level of lactic acid dehydrogenase in 3, 5 and 10 h, but no change of GPx. • In jejunum, SOD increased after 2 h of HS, but T-AOC tended to increase with HS time and peaked at 10 h after HS. • HS increased T-AOC and MDA content after 10 h in jejunum.

Table 2. Continued

Reference	Animal	TN vs. HS conditions with RH	Major effect in comparison with TN versus HS conditions
[68]	German Holstein cows	Pair-feeding (15℃, 63% RH) vs. HS (28℃, 52% RH) for 4 days	• Villus height and crypt depth of jejunum was not affected. • HS increased number of infiltrating cells in submucosa of jejunum but not in mucosa. • HS increased *ZO1* and tended to increase *CLDN1* mRNA abundance in jejunum. • HS tended to lower ZO1 protein abundance, but CLDN1 protein was similar in jejunum. • No significant differences for *MLCK*, *ZO2*, and *OCLN* mRNA abundances. • *No effect on TNFA*, *IL6*, *IL10*, *CXCL5*, and *haptoglobin* mRNA levels, but *IL4* mRNA expression tended to be higher in jejunum of HS animals. • No effect on IL1β and IL4 protein levels in jejunum. • HS tended to increase catalase mRNA expression, but mRNA abundances of *SOD1* and *GPx1* were similar. • No effect on catalase and lysozyme activities, but HS increased alkaline phosphatase activity in jejunal mucosa.
[133]	Crossbred pigs 50% male and 50% female	TN (21℃–23℃, 30.2% RH for 6 h) vs. HS (39.3℃, 15.9% RH for 3 h followed by rapid cooling to TN or gradual cooling in 3 h to TN)	• Jejunum and ileum villus height was reduced in gradual cooling pigs compared to rapid cooling and TN pigs. • Jejunum and ileum VCR reduced in gradual cooling pigs. • On day 4, FITC-d permeability was higher than on day 0. • Jejunal *CLDN1* gene expression was higher on day 0 than on day 4, but Ileal *CLDN1* gene expression was lower for gradual cooling than for TN on day 2, and was higher for gradual cooling than for rapid cooling and TN on day 4. • Increased *ZO1* gene expression in gradual cooling pigs.
[134]	Male Arbor Acres broilers from 28 to 42 days	TN (23℃) vs. cyclic HS (28℃–35℃–28℃ for 12 h daily) for 21 days	• HS lower ADFI, ADG, and feed conversion ratio. • HS reduced villus height and VCR in duodenum and jejunum on day 28. • HS increased serum D-lactic acid concentration on day 28. • HS increased serum TNFA, IL6 levels and tended to increase pro-inflammatory cytokine IL1β, but decreased anti-inflammatory cytokine IL10 levels.
[69]	2-month-old male Chinese mini pigs	TN (23℃) vs. HS (40℃, 5 h/day for 10 days)	• HS decreased villus height in duodenum and jejunum on day 3. • Crypt depth in duodenum and jejunum was shallower, but illeal crypt depth was similar. • HS increased VCR on day 3, but no effect on day 6 in duodenum. HS increased VCR on day 1 in jejunum, and no effect in ileum. • HS increased mitochondria numbers with shortened internal cristae in jejunal epithelium. • HS decreased protein expression of EGF in jejunum. • Desquamation found at tips of intestinal villi, and exposed lamina propria in jejunum.
[71]	28 day old female Xuefeng black-boned chickens	TN (24℃ for 24 h/day) vs. HS (37℃ for 8 h/day; remaining 16 h/day at 24℃) for 15 days	• HS reduced intestinal villi and VCR, deepened crypt depth. • HS decreased numbers of goblet cells and lymphocytes in intestine. • HS increased mRNA and protein levelss of *HSP70*, *HSP90* and *NF-κB*, and decreased *EGF* in jejunal mucosa.
[94]	Female growing pigs	TN (20℃) vs. HS (35℃ during 09:00–17:00 h and 28℃ for rest of the day) at 38% RH for 2 days	• HS increased Intestinal *HSP70* mRNA abundance. • Increased FITC-d permeability and decreased TER. • Decreased GPx activity.
[22]	Arbor Acres male broiler chickens	TN (22℃, 24 h/day) and HS (32℃, 10 h/day) for 14 days	• HS decreased villus height in duodenum and ileum but not in jejunum, increased crypt depth and decreased VCR in all intestinal segments. • HS increased MDA content in small intestine.
[62]	Crossbred gilts (50 kg BW)	TN (21℃, 47% RH) vs. HS (30℃, 35% RH for 10 h/day) for 21 days	• HS decreased ADFI. ADG and final BW, but no effect on feed efficiency.
[135]	Male Cobb 500 broilers birds	32℃–27℃ vs. 37℃–33℃, decreased 2℃/week until reaching 33℃ in third week; for 5 h/day from 29 to 42 day of age	• HS decreased villus length, crypt depth and VCR. • Decreased body weight in HS birds
[78]	Crossbred pigs	TN (21℃, 35%–50% RH) vs. HS (35℃, 24%–43% RH) for 24 h	• HS decreased feed intake and body weight. • Rectal temperature increased. • HS decreased TER and increased FITC-d permeability in ileum and colon. • HS increased protein expression of casein kinase II α (trend) and MLCK in ileum. • No effect on protein expression of CLDN1, but CLDN3 and OCLN protein up-regulated. • Active glucose and glutamine (trend) transport increased in ileum and glucose level in blood. • HS increased GLUT2 protein expression (trend) and increased Na^+/K^+-ATPase activity, but no effect on SGLT1 protein abundance in ileum. • HS decreased sucrase and maltase activities, but no effect on ileal mucosal aminopeptidase N activity. • HS increased ileal HIF1A and HSP70 protein expression. • No differences in IL1B and IL8 concentrations, but myeloperoxidase activity increased in ileum. • Protein expression of ileal mast cell tryptase increased.

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Table 2. Continued

Reference	Animal	TN vs. HS conditions with RH	Major effect in comparison with TN versus HS conditions
[72]	Crossbred gilts (43 kg BW)	TN (20℃, 35%–50% RH) vs. HS (35℃, 20%–35% RH) vs. pair-feeding in TN conditions (PFTN) for 7 days	• HS decreased TER, increased FITC-LPS permeability in jejunum, and increased endotoxin in plasma. • Villus height and VCR decreased linear and quadratic pattern) on day 1, 3, and 7. Crypt depth increased for the first 3 days of HS, and afterward decreased at day 7 compared with day 0 TN. • On day 7, HS tended to increase FITC-LPS permeability compared with PFTN. • Jejunal 4-HNE level (oxidative stress marker) and Na^+/K^+-ATPase activity increased within first 24 h of HS. • In jejunum, no effect on IL8. • Myeloperoxidase activity (neutrophil infiltration marker) increased in jejunum on day 3 and 7. • Intestinal lysozyme and alkaline phosphatase activity decreased. • Gene expressions of *OCLN, CLDN3,* and *ZO1* increased on day 7 compared with day 0, 1, and 3 of HS. • Jejunal HSP27 up-regulated on day 1 and 3 compared with day 0 and 7. HIF1A level numerically peaked at day 3 of HS and down regulated by day 7. • Gene expression of *MLCK* in jejunum increased.
[98]	Crossbred gilts (63.8 kg BW)	TN (21℃, 70% RH for 6 h) vs. HS (37℃, 40% RH for 2, 4, and 6 h)	• HS reduced feed intake and increased rectal temperature. • Ileum TER decreased but no change in colon TER. • Increased ileal FITC-d permeability but not colon FITC-d permeability. • Reduced Ileum villus length and VCR, but not crypt depth. • Increased villi tip autolysis at 4 h and 6 h. • MUC2 protein expression increased at 6 h, but not at 2 and 4 h. • Increased protein expression of HSP70 in ileum and colon. HIF1A protein expression was not affected in ileum, but increased (trend) in colon. • Increased gene expressions of *HSP27, HSP70* and *HSP90*, but no effect on *HSF1* expression in ileum. • Increased (linear trend) *CLDN3* and *MUC2* gene expression in ileum. • No effect on ileal gene expressions of *OCLN, MLCK, GLUT2, sodium-glucose transporter 1,* Na^+/K^+*-ATPase, TGFB1, IL1β,* and *IL6*.
[136]	Crossbred gilts (64 kg BW)	TN (21℃, 70% RH) vs. HS (37℃, 40% RH) vs. PFTN condition for 12 h	• HS reduced feed intake and BW in pigs. • HS up-regulated HSP27, HSP90α, HSP90β, HSP70, and HSP65 in ileum. • Peroxiredoxin 1 protein (related to oxidative stress) decreased. • Increased ileal *HSP27* and *HSP70* gene expression in HS pigs compared with both TN and PFTN pigs. • No effect on ileal *HSF1* and *HIF1A* gene expressions. • Ileal *HIF2* tended to increase in HS pigs compared to both TN and PFTN pigs. • No effect on gene expressions of SGLT1, Na^+/K^+-ATPase, AMP-activated protein kinase-α, GLUT2, citrate synthase, hexokinase, or catalase.
[99]	Crossbred gilts (64 kg BW)	TN (21℃, 70% RH) vs. HS (37℃, 40% RH) vs. PFTN (pair-feeding to their HS-CON counterparts and exposed to TN conditions) for 12 h	• Reduced feed intake in HS compared with TN. • Ileum villus height and crypt depth decreased in both PFTN and HS. • PFTN and HS increased dextran flux and reduced TER in ileum compared with the TN. • Ileal MUC2 protein abundance increased in HS and PFTN condition. • Colonic TER and dextran flux were similar in HS or PFTN treatments. • HSP70 protein expression increased in ileum of HS compared with the TN and PFTN. • Increased HSP70 protein expression in colon of HS-CON group compared with TN. • Ileum and colonic HIF1A protein expression did not differ.
[59]	Broiler chickens	TN (21℃) vs. low HS (31℃) vs. high HS (36℃). HS was applied for 10 h/day from 35 days to 42 days	• HS decreased BW gain and ADFI in both HS, but feed conversion ratio increased on high HS. • No effect of HS on villus height, crypt depth, VCR, and intraepithelial lymphocyte numbers in jejunal mucosa. • Cellularity increased in jejunal lamina propria on low HS. • Mild multifocal acute enteritis on HS. • Mild acute multifocal lymphoplasmocytic enteritis found in jejunal lamina propria of low HS.
[65]	Male broiler chickens	TN (21℃ 24 h/day) vs. HS (31℃ for 10 h/day and rest 14 h/day at 21℃)	• HS decreased feed intake, BW gain, and feed conversion. • No effect on villus height, crypt depth, VCR, and intraepithelial lymphocyte numbers. • Multifocal lympho-plasmocytic enteritis in jejunum. • Moderate infiltrates with foci of heterophils.
[67]	Male broiler chickens	TN (21℃, 24 h/day) vs. positive *Salmonella* TN group (21℃, 24 h/day) vs. positive *Salmonella* HS group (31℃ from 35 to 41 days from 8:00 am to 6:00 pm 10 h/day) vs. negative *Salmonella* HS group	• HS decreased ADG and ADFI in *Salmonella*-infected and non-infected birds. HS birds infected with *Salmonella* Enteritidis exhibited an increased feed conversion. • No effect on VCR, villus height, crypt depth and intraepithelial lymphocyte numbers in any segments of small intestine. • HS without *Salmonella* infection caused mild acute multifocal lympho-plasmocytic enteritis and foci of heterophil infiltrates in all segments of small intestine, but HS with *Salmonella* infection increased these changes from mild to moderate.
[137]	Cobb 500 male chickens	TN (24℃) vs. HS (35℃) with 55 RH from 21 day to 42 days	• HS decreased body weight, feed intake, and feed efficiency on day 28, 35, and 42. • Increased serum FITC-d in HS chickens on day 35 and 42.

Table 2. Continued

Reference	Animal	TN vs. HS conditions with RH	Major effect in comparison with TN versus HS conditions
[138]	28-day old male broiler chickens	TN (25℃) vs. HS (33℃) with 40%–55% RH for 8 h/day) for 10 days	• HS reduced villous height and ileal TER.
[139]	Male Cobb 500 broiler chickens	TN (26℃) vs. HS (34℃ for 8 h daily) for 21 days	• HS decreased feed intake and BW. • HS decreased genes expressions of *CLDN3* and *OCLN* but not *CLDN1*. • HS increased genes expressions of *HSPA1A*, *HSPD1*, and *HSPB1*.
[140]	17-day-old Ross broiler chickens	TN (25℃) vs. HS (39℃) for 8 h/day for 4 days	• HS decreased villus height, epithelial and total villus areas in all small intestine segments. • Decreased villus breadth at the tip and increased crypt depth in jejunum. • VCR decreased in duodenum and ileum. • Increased GPx activity and decreased T-AOC capacity.
[141]	Ross-708 chicks of mixed sex	Control (35℃ at day 1 and decreased 3℃ per week to 26℃ and then maintained constant) vs. (35℃ from day 1 to 42)	• HS decreased BW on day 21 and 42. • HS increased FCR and decreased, feed intake. • HS reduced villus height, width, surface area, and crypt depth on day 21 and 42.
[142]	Crossbred gilts (43 kg BW)	TN (19℃, 61% RH) vs. HS (36℃, 50% RH) for 1 or 7 days	• HS reduced feed intake and BW gain. • Colonic TER decreased as HS progressed. • Colonic FITC-LPS flux tended to increase from days 1 to 7.
[143]	Crossbred gilts (39 kg BW)	TN (19℃, 46% RH) vs. HS (32℃, 26% RH) for 24 h	• Feed intake and body weight decreased.
[7]	21-day-old Ross male broiler chickens	TN (22℃, RH 70%) vs. HS (33℃ for 10 h/day, RH 70%) from 22–42 days	• Reduced TER value and increased FITC-d permeability in jejunum. • HS caused shorter villus height, deeper crypt depth, and lower VCR in jejunum. • HS downregulated protein levels of OCLN and ZO1.
[1]	15-day-old Ross broilers	TN (22℃–23℃) vs. HS (38℃–39℃ for 8 h and remaining time at 22℃–23℃) for 5 days	• In jejunum, HSF-3, HSP70, HSP90, CDH1, CLDN5, ZO1, TLR-4, IL6, and IL8 mRNA expression and HSP70 protein expression increased. • Increased all gene expressions in ileum as in jejunum along with *HSF1*, *CLDN1*, and *HIF1A* mRNA levels.
[144]	21-day-old Arbor Acres broiler chickens	36℃ for 10 h/day for 20 days	• Decreased villus height, increased crypt depth, D-lactic acid concentration and diamine oxidase activity, and soluble intercellular adhesion molecule-1, TNFA, and IL10 concentrations. • Reduced ZO1, CLDN1, and OCLN expression levels.
[70]	Chinese mini pigs	TN (23℃) vs. HS (40℃, 5 h/day for 10 days)	• HS reduced VCR. • HS increased the number of shortened internal cristae mitochondria, organelle debris within lysosomes, and altered enterocyte tight junction morphology. • Up-regulated *HSP70*, *HSP90*, and *HSP27* mRNA expressions, but down-regulated EGF and EGF receptor mRNA expression in jejunum.
[93]	Cobb male chickens	TN (26℃,) vs. HS (36℃ from 08:00 to 18:00 and 26℃ from 18:00 to 08:00)	• HS decreased feed intake, BW gain, plasma concentrations of triiodothyronine and thyroxine; increased FCR. • HS decreased intestinal VH, VCR, mucosal ATP level, activities of alkaline phosphatase and digestive enzymes. • HS increased intestinal crypt depth, mucosal AMP and MDA levels, and mRNA levels of *HSP70*, caspase 3, heme-oxigenase, xanthine oxidoreductase, and AMP-activated protein kinase.
[30]	Hy-Line Brown commercial laying hens (40 weeks old)	TN (26℃) vs. HS (33℃), with 60%–70% for 20 days	• HS decreased egg production rate, feed intake, and egg weight; increased feed to egg ratio, broken egg ratio, and mortality. • HS caused typical fractures in villi and exposed lamina propria and reduced villus height in ileum and cecum. • Down-regulated expression levels of OCLN, ZO1, and JAM-A in ileum and cecum.
[86]	21-day-old Cobb male broilers	TN (22℃) vs HS (33℃) with 70% RH for 10 h and remaining time at 22℃ for 21 days	• HS lowered final BW, ADG, and feed intake. • HS decreased villus height, VCR, and goblet cell numbers; deepened crypt depth. • Increased numbers of *Escherichia coli*, *Salmonella*, and *Clostridium*, and lowered *Lactobacillus* and *Bifidobacterium* numbers. • Reduced intestinal mucosal *CLDN1*, *OCLN*, *ZO1*, *CDH1*, and *MUC2* mRNA levels.

TN, thermoneutral; HS, heat stress; RH, relative humidity; BW, body weight; ADG, average daily gain; FCR, feed conversion ratio; TNFA, tumour necrosis factor α; IL, interleukin; VCR, villus height to crypt depth ratio; ADFI, average daily feed intake; MDA, malondialdehyde; SOD, superoxide dismutase; CLDN, claudins; MUC, mucin; OCLN, occludin; ZO1, zonula occludens 1; HSP, heat shock protein; TER, transepithelial electrical resistance; FITC-d, fluorescein isothiocyanate–labeled dextran (4.4 kDa); LPS, lipopolysaccharide; PFTN, pair-fed thermal neutral; SGLT1, sodium-dependent glucose cotransporter 1; GPx, glutathione peroxidase; T-AOC, total antioxidant capacity; MLCK, myosin light chain kinase; EGF, epidermal growth factor; NF-κB, nuclear factor kappa B; 4-HNE, 4-hydroxynonenal; TGFB1, transforming growth factor beta 1; CDH1, E-cadherin; HIF1A, hypoxia inducible factor 1α; HSPA1A, heat shock protein family A (HSP70) member 1A; HSPD1, heat shock protein family D (HSP60) member 1, HSPB1, heat shock protein family B (small) member 1; HSF, heat shock factor; GLUT, facilitative glucose transporter; VH, villus height.

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crypt depth, but had no effect on villus height or V/C ratio in the ileum of broiler chickens, which has been suggested due to the short duration and greater resistance of ileal mucosal structural change than other parts of the small intestine [12].

There are a few mechanisms of histopathological and architectural changes in the mucosa and villi due to HS. With response to HS, blood is increasingly diverted to the skin away from the splanchnic bed as a result of peripheral vasodilatation and vasoconstriction in the gut, which consequently cause hypoxic, oxidative and nitrosative stress, and eventually, apoptosis of the epithelial cells can occur [72]. Heat challenge induces damages of the intestinal mucosa, which may be also caused by down-regulation of the epidermal growth factors (EGF) in the intestine [69]. In the mucosa, EGF, a mitogen, has been shown to improve epithelial recovery and intestinal morphology by activating the proliferation and differentiation of enterocytes [73]. Many studies have reported a decrease in mRNA and protein expression of EGF or EGF receptor in the intestine due to HS [69–71], which might be responsible for reduction of enterocyte cell growth and consequently villi length and crypt depth. The mitotic divisions in the crypts contribute to a large extent (60%) for epithelial cell proliferation, followed by the middle (32%) and apical (8%) regions of villus. Because of the high proliferative activity of the crypt, it is likely that alterations in cell proliferation would occur first in the stem cells of crypts rather than in the villus. Shorter villus length caused by HS occurs due to increased mucosal cell turnover and reduced cell mitosis or size. Deeper crypt due to HS results from greater number of proliferating stem cells to replenish the damaged villus epithelial cells and indicates rapid tissue turnover and increased protein and energy requirement in the gut tissues [64]. Reduced V/C ratio is a crucial indicator of gut morphology alterations as it reflects in reduced surface area and/or increased stem cell proliferation together. Broiler chickens spend approximately 12% of their synthesized protein on gastrointestinal tissue turnover [64]. Therefore, the restructuring of the gut morphology due to HS has remarkable impacts on the gut absorptive and catabolic function. The changes in gut morphology may be attributed to the direct effect of HS on the gut epithelia such as hypoxia, reduced antioxidant status and hormone secretion or indirectly through changes in gut microbiota that regulate mucosal cell differentiations.

Heat stress on tight junction function in the gut

The TJ proteins are considered as gate guards and border protectors formed by zonula occludens (ZO), claudins (CLDN), occludin (OCLN), and junctional adhesion molecule (JAM) proteins, which regulate the passage of molecules through selective paracellular pores, especially preventing entry of pathogenic bacteria, endotoxins and antigenic compounds [68,74,75]. TJ function has usually been shown to alter due to HS as evidenced from reduced mRNA expressions of TJ genes, their protein amount along with changes in regulatory proteins in several studies, but the changes depend upon the duration, intensity and period of heat exposure. The expressions of *OCLN*, *ZO1*, and *JAM-A* in the ileum and ceca reduced in heat stressed-hens compared with control hens (26℃ vs. 33℃ for 20 days), particularly, at 20 days [30]. In broiler chickens, HS (20℃ vs. 30℃–33℃ for 8 h/day) decreased mRNA abundance of *CLDN3*, but not *ZO1*, *CLDN2, OCLN,* and mucin 2 (*MUC2*) in jejunum; however, any of these genes was not affected in ileum at 21 days [76]. In the same study, HS reduced mRNA levels of jejunal *MUC2* and *OCLN*, and ileal *MUC2, ZO1, OCLN,* and *CLDN3* on day 42 [76]. In contrast, HS (20℃ vs. 27.8℃ for 14 days) in broiler chickens had no effect on jejunal gene expressions of *OCLN, ZO1, CLDN1,* and *JAM2*, although it decreased jejunal transepithelial resistance (TER) values at 35 days of age [77], which might be attributed to the less intensity of the HS for 14 days, resulting in restoration of the gene expressions. Serum lipopolysaccharide (LPS) concentrations were also not affected by HS [77].

In cows, HS (pair-feeding 15℃ vs. 28℃) for 4 days increased *ZO1* and tended to increase

CLDN1 mRNA abundance in jejunum, but tended to lower ZO1 protein abundance in jejunum; whereas no significant differences were noted for myosin light chain kinase (*MLCK)*, *ZO2*, and *OCLN* mRNA abundances [68]. In pigs, *ZO1* and *CLDN3* mRNA abundance increased after 7 days of heat exposure, but *ZO1* mRNA abundance was not affected at 3 days [72]. In chickens, HS (38℃–39℃, 8 h/day for 5 days vs. 22℃–23℃) up-regulated mRNA levels of *CLDN5, ZO1,* and E-cadherin (*CDH1*) in the jejunum, and the gene expressions were more pronounced in ileum where mRNA expressions of *CLDN1* was also up-regulated [1]. These studies may suggest that when heat exposure lasts for short time, some TJ gene expressions may be up-regulated as a result of protective response related to intestinal mucosal restitution.

Epithelial cells also consist of a peri-junctional actin cytoskeleton, which mediates TJ permeability and is regulated by MLCK [75]. A cyclical effect of HS on TJ and MLCK gene expressions has been reported, which were rapidly up-regulated in acute HS, but then decreased drastically by day 3 [72]. Similarly, ZO1 protein level also decreased, suggesting TJ disruption in HS conditions [72]. Heat challenge has also been shown to upregulate *OCLN* gene expression, which is an important TJ protein for regulating barrier function, and an increased expression may be an indicative of protective response related to intestinal epithelial restoration.

The TJ gene and protein expressions though sometimes vary depending upon the duration and intensity of HS and type of genes and proteins, the permeability (increased) and TER (decreased) values are usually altered. HS decreased jejunal TER and increased paracellular permeability of fluorescein isothiocyanate dextran 4 kDa (FITC-d) and down-regulated protein levels of OCLN and ZO1 in jejunum [7]. Pigs exposed to HS conditions (21℃ vs. 35℃) for 24 h decreased TER in ileum and colon, increased protein expressions of MLCK and casein kinase II α, CLDN3 and OCLN in ileum, while there were no differences in ileal CLDN1 expression [78]. Plasma endotoxin levels increased 45% in HS crossbred gilts (35℃, 20%–35% humidity) compared with thermoneutral crossbred gilts (20℃, 35%–50% relative humidity), while jejunal TER decreased by 30% and intestinal FITC-labeled lipopolysaccharide permeability increased by 2-fold [72]. Furthermore, day 7 HS pigs tended to have increased (41%) lipopolysaccharide permeability compared with the pair-feeding control [72]. Sows in their gestation period subject to HS (18℃–22℃ vs. and HS 28℃–32℃) from 85 days of gestation to furrowing had higher serum HSP70, lipopolysaccharide and lipopolysaccharide-binding protein levels [24]. HS (30℃ vs. 20℃) in broiler chickens impaired barrier integrity in the intestine, which resulted in greater intestinal permeability to endotoxin and lipopolysaccharide in serum, translocation of intestinal pathogens (*Salmonella* spp.) to liver, spleen and meat, and serum inflammatory cytokine (TNFA and IL2) concentrations [63,67].

The gastrointestinal tract is highly vulnerable to HS-induced alterations, including changes in the microbiota composition, dysfunctions of immune response, impairment of intestinal barrier integrity, imbalance of the oxidative-anti-oxidative mechanism, and alterations of the mucosal structures and injury [1,7]. These alterations cause to allow the translocation of antigens and pathogens through the TJ of intestine epithelium and stimulate the innate immune system via TLR signaling, ultimately leading to intestinal inflammation and injury [1]. Besides, HSP that are recognized by TLR in many cell types can directly facilitate inflammatory responses [1,9]. The barrier integrity in the intestine can be modulated by different cytokines [1,8], and increased amounts of pro-inflammatory cytokines, i.e., IL6 and IL8, are found in intestinal epithelial cells after barrier disruption [1]. It has also been recognized that the upregulation of HSP, particularly HSP70, confers a protective mechanism by inhibiting the expressions of pro-inflammatory cytokines [1,9].

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Heat stress on heat shock protein in the gut

HSPs are well-recognized as stress proteins and molecular chaperones that protect the internal cell environment by participating in protein folding, repair, localization and degradation influencing essential processes such as cell signaling, transcription, protein synthesis, metabolism, and regulation of cellular redox conditions and thus regulating cell growth and survival [1,79,80]. The synthesis and expressions of HSP are stimulated under biotic and abiotic stress stimuli, particularly hyperthermia, oxidative stress, infections, diseases, and hypoxia, to protect cell proteins from oxidative stress and other harmful environmental conditions [79,80]. The multifaceted response to stress is mediated by heat shock factors (HSF) that regulate HSP expressions [1,81]. Stress initiates phosphorylation and trimerisation of HSF and these HSF trimers bind to the heat shock elements in the promoter region of HSP genes, mediating HSP gene transcription [1]. Heat exposure has thus been shown to upregulate mRNA and protein expressions of HSP and HSF in different tissues of animals including in the intestinal mucosa [24].

In chickens, HS (38℃–39℃, 8 h/day for 5 days vs. 22℃–23℃) up-regulated HSF3, HSP70, HSP90 mRNA expressions and HSP70 protein expression in the jejunum, and the gene expressions were more pronounced in ileum where mRNA expressions of HSF1, and hypoxia inducible factory 1 alpha (HIF1A) were additionally upregulated [1]. In HS-chickens, expression of HSF3 gene, an avian-specific HSF family member, upregulated in jejunum and ileum, whereas the HSF1 mRNA level increased in the ileum [1]. A species- and tissue-specific differences of heat-induced HSF1, HSF3, and their protein expressions have been reported, which may be associated with the extent of oxidative damage [81,82].

Up-regulated HSF and activate the major heat inducible proteins such as HSP70 and HSP90. In HS-chickens, HSP70 and HSP90 mRNA levels up-regulated in both jejunum and ileum, but the corresponding protein expression significantly increased only for HSP70 in jejunum and ileum [1]. However, HSP70 and HSP90 mRNA levels were not altered in duodenum and colon, indicating the differences in the susceptibility of the individual intestinal segment [1]. Even short-term (2 to 3 days) HS increased gene expression of *HSP70, HSP90,* and *HSF1*, as well as induced TJ proteins, which was independent of adenosine monophosphate-activated protein kinase in chickens [83]. Pigs exposed to HS conditions (35℃ vs. 21℃) for 24 h increased ileum HSP70 expression [78]. HS also upregulated gene and protein expressions of HSP70 and HSP90 in the jejunal mucosa of chickens [71]. HSPs, including HSP70, play a major role in the protection of intestinal mucosal integrity from heat-stress injury by correctly fixing the non-native proteins together and keeping them functional, inhibiting lipid peroxidation, and improving the antioxidant capacity, thereby contributing to the cell functions under stress conditions [84,85].

Heat stress on transport function and digestive enzymes in the gut

Heat exposure has been shown to decrease intestinal barrier integrity, and also reduces nutrient absorption in different studies in growing pigs [78], broilers [1,63,83,86], and laying hens [30], which may be attributed to the imbalance of the gut microbiome coupled with systemic effects from dysregulation of neuroendocrine responses, subsequently affecting the intestinal mucosa. There are conflicting reports of HS on the nutrient transport in livestock. Glucose transport in the intestine and blood glucose increased due to HS (21℃ vs. 35℃ for 24 h) along with increased Na^+/K^+-ATPase activity in the ileum of HS pigs [78]. Protein expression of sodium-dependent glucose cotransporter 1 (SGLT1) was not altered; but, HS increased ileal facilitative glucose transporter 2 (GLUT2) protein expression [78]. In broiler chickens, chronic HS (22℃ vs. 33℃ for 14 days) increased the gene expressions of *GLU2* and peptide transporter 1 (*PEPT1*) in jejunum [87]. Acute

HS (24℃ vs. 32℃ for 8 h) did not alter the mRNA levels of *SGLT1* and amino acid transporters *CAT1, r-BAT, y + LAT1,* and *PEPT1*, but decreased the expressions of *GLUT2*, *FABP1*, and *CD36* in the jejunum, suggesting that periodic HS may affect glucose and lipid transport, but not amino acid transport in the jejunum [88]. In chickens, HS (20℃ vs. 30℃) increased the activity and expression of apical SGLT1 in intestine by approximately 50%, but no effects on this transporter was noted in the pair-feeding, indicating that increased transported activity was not resulted from decreased feed intake [89]. In leghorn hens, HS (20℃–22℃, 50℃–60% relative humidity vs. 30℃–33℃, 70%–80% relative humidity) for 28 days decreased calcium binding protein (calbindin) in ileum, cecum, and colon [90]. In the above discussion, it is apparent that gene or protein expressions of some nutrient transporters are elevated in the intestine under HS, which seems due to adaptive mechanisms to allow proper nutrient absorption by over expressing the transporters compensating HS-induced reduction in absorptive surface area caused by reduced villi height and mucosal damage.

During hyperthermia, redistribution of blood away from the splanchnic area to the periphery occurs in order to maximize radiant heat dissipation from the body [91]. Therefore, thermal stress may decrease blood flow to the intestine, motility of the digestive system, and secretion of digestive enzymes [12] which could affect digestion, absorption and metabolism of nutrients including minerals. High temperature (32℃ vs. 20℃) reduced digesta passage and the activity of amylase, trypsin and chymotrysin in the intestinal juice of broiler chickens [92]. Additionally, HS, independent of reduction of feed intake, elevated Na^+/K^+-ATPase activity in the intestine, which was likely due to maintain osmotic homeostasis in the intestine [72]. Ion pumps are involved in the active transport of ions across the plasma membrane with the hydrolysis of ATP resulting in depletion of ATP and increased amount of AMP [72,93]. Overall, above discussions may imply that nutrient transport is a function of changes in the expressions of the nutrient transport system, digestive enzyme secretion, and microvilli ultrastuctures in the intestine, which are affected by HS.

Heat stress on oxidative stress in gut mucosa

Heat challenge usually causes oxidative stress such as increased malondialdehyde (MDA) content and decreased antioxidant enzyme activities, i.e., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, in the intestine resulting the damages and structural changes of the mucosa, and reduced barrier integrity. In chickens, chronic cyclic HS (20℃ vs. 32℃–33℃ 8 h/day) for 42 days increased jejunal mucosal MDA content, and lowered SOD activity in ileal mucosa at 42 day [76]. Cyclic HS (22℃, 24 h/day vs. 32℃, 10 h/day) for 2 weeks in chickens increased MDA content in small intestine [22]. In growing pigs, GPx activity was decreased by HS (28℃ to 35℃ vs. 20℃) for 2 days in ileum and jejunum [94] and 4-hydroxynonenal, an oxidative stress marker, increased within first 24 h of HS [72]. In cattle, HS (28℃, 52% relative humidity vs. 15℃, 63% relative humidity with pair-feeding) for 4 days tended to increase catalase mRNA expression, but mRNA abundances of *SOD1* and *GPx1* and activities of catalase and lysozyme were similar [68]. Even, HS for a short period results in oxidative stress in the mucosa. For example, HS at 36℃ for 0, 2, 3, 5 and 10 h vs. 21℃ for one day increased SOD activity after 2 h, but total antioxidant capacity and MDA content increased after 10 h in jejunum [84]. Gene expression of hypoxia inducible factor 1, subunit alpha (HIF1A), an oxidative stress marker, upregulated in the ileum of heat-exposed chickens [1] and pigs [78]. It has also been reported that HIF1A expression tended to increase at day 3 of HS, but then reached to normal expression level by day 7, probably due to restoration of this function by protective response of the intestine [72].

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Heat stress on mucosal immunity in gut

HS induces the release of pro-inflammatory cytokines and mediators (e.g., nuclear factor kappa B (NF-KB) p50 and IL4) in the broiler chickens. In chickens, HS (38℃–39℃, 8 h/day for 5 days vs. 22℃–23℃) up-regulated mRNA expressions of *TLR4, IL6,* and *IL8* in the jejunum [1]. In Holstein cattle, HS (28℃, 52% humidity vs. 15℃, 63% humidity in pair-feeding condition) for 4 days had no effect on jejunal mRNA levels of *TNFA*, *IL6*, *IL10*, *CXC-5*, and haptoglobin, and protein expressions of IL1B and IL4, but *IL4* mRNA level tended to be higher in jejunum [68]. Transcription factor NF-KB p50 has a major role in gene regulations induced by inflammatory cytokines, pathogens and oxidative stress [95]. Generally, IL4 is considered as a crucial cytokine that control naive T-helper cell differentiation into T-helper 2 effector cells that promote immunity and inflammation [96].

In poultry, HS (31℃ from 35 to 41 days of age) increased serum corticosterone levels and *Salmonella* colonization and invasion to crop, cecum, livers and spleen, and lowered plasma IgA and interferon gamma (IFNG) levels, mRNA expression of *IL6, IL12,* and *TLR2* in spleen and *IL1B, IL10,* transforming growth factor beta 1 (*TGFB1*), *TLR2,* avian beta defensin (*AvBD4*) and *AvBD6* in cecal tonsils of chickens challenged with *Salmonella* Enteritidis [60]. This indicates that HS activates HPA axis and increases corticosterone levels, which may decrease the immune system activity, resulting in an impairment of the mucosal barrier and inflammation in the intestine, subsequently increasing susceptibility to the invasion of pathogenic microorganisms [60,65].

HS crossbred gilts (35℃ vs. 20℃, 35%–50% humidity) had lower alkaline phosphatase and lysozyme activity (59% and 46%, respectively) over time in HS pigs, while myeloperoxidase activity (a immune cell marker) was increased in the jejunum on day 3 and 7 [72]. Decreased lysozyme and alkaline phosphatase activities may due to inflammatory and LPS responses observed during HS [72]. Compromised lysozyme and alkaline phosphatase activity in mucosa may increase transmucosal passage of enteric pathogens and toxins, and decrease the protection against LPS-induced inflammation [72].

Heat stress on the mucus barrier

Mucin layer, composed of gel like mucin substances, covers over the epithelial mucosa. Mucins are synthesized mainly by gastric foveolar mucous cells and intestinal glandular goblet cells [8,75]. Mucin 2 polypeptide is the major contributor of mucin layer in the GI tract [8]. This mucus layer in the gut provides physical barrier between luminal content and epithelial cells in the intestine by restricting large particles from directly contacting the intestinal epithelium, including bacterial attachment [74,75]. Small molecules such as nutrients can easily diffuse through the mucus layer. Colonization of gut microbiota can only occur at the outer loose mucus layer, but they are mostly restricted at the inner adherent mucus layer [74,75]. A defective or thin mucus layer may lead to susceptibility to pathological changes due to increased adhesion of antigenic bacteria on the mucosa. HS has been shown to reduce goblet cell numbers in the intestinal mucosa of chickens [86,94,97] and *MUC2* mRNA levels [86]. But a few studies with short duration of heat challenge caused a linear trend in increased *MUC2* gene expression and increased MUC2 protein expression in HS pigs (21℃ vs. 37℃ for 2, 4, and 6 h) at 6 h [98] and at 12 h [99] compared with the thermoneutral pigs. The above studies suggest that HS can affect the physical mucin barrier in the gut leading to increased invasion of pathogens and vulnerability to mucosa.

PRODUCTION PERFORMANCE

Feed intake

The adverse effects of HS on feed intake have been well-recognized in different studies on various livestock species [100]. Pigs exposed to HS (35℃ and 24%–43% relative humidity) for 24 h showed elevated respiration rates by 2-fold and rectal temperatures by 1.6℃ and reduced voluntary feed intake by 53% relative to the pigs in thermoneutral (21℃ and 35%–50% humidity) condition [78]. Acute HS (35℃) in crossbred pigs for 24 h decreased feed intake compared with the thermoneutral (25℃) conditions [25]. In laying hens, HS also decreases feed intake, egg production and weight, eggshell thickness, and increases mortality rate [21,101–104]. HS effects differ due to genotypes age and group size for the production performance, of which genotype selection may be useful for selection of breeds for hot temperature conditions [102,103].

The negative effects of HS on feed intake is one of the reasons responsible for reduced growth performance in heat-stressed livestock and poultry [105]. During the recovery period, feed intake and body weight gain in pigs exposed to thermal stress returned to thermoneutral levels; but, pigs in the pair-feeding group had increased daily feed intake (21%) and weight gain (32%) above thermoneutral levels [62]. Reasons why appetite during recovery was blunted in HS pigs compared with the PF pigs are not clearly known. Also, pigs exposed to a 3-h HS period had a progressively greater feeding behavior relative to the thermoneutral pigs during a 3-h recovery period [106]. Mechanisms of compensatory growth and feed intake are difficult to understand because ability of animals to recover may depend upon the nature, severity, and duration of nutrient restriction during HS [107,108].

Production performance and feed efficiency

Broiler birds were exposed to a thermoneutral condition (20℃), chronic HS (30℃; 24 h/day) and acute HS (35℃ from 09:00 to 13:00 and 20℃ from 13:00 to 09:00) for 10 days and it was noted that both HS conditions decreased body weight gain and lowered feed conversion efficiency, whereas feed intake and mortality rate were greater in the acute HS condition [58]. It indicates that short-term HS in the day may be detrimental to growth performance. Heat-stressed birds (30℃ vs. 20℃) increased serum concentrations of corticosterone [58] Heat reduced feed intake (115 vs. 84 g/day), egg production (87.7% vs. 72.4%), and egg weight (63.3 vs. 60.0 g) in laying hens and egg weight was significantly lower in the HS group than that in the pair-feeding group (feed intake is similar to the heat-stress group, but in the thermo-neutral condition), suggesting that reduction in egg weight occurs due to HS independent of feed intake [21]. Feed efficiency (feed:egg ratio) was not significantly better in the HS group (1.95) compared to thermoneutral group (2.08), whereas it was non-significantly better in the pair feeding group (1.79) compared to the HS group [21]. The meta-analysis study demonstrated that feed intake, hen-day egg production, shell strength, and egg mass were more sensitive to HS than the other variables as these traits reduced by 9.0% to 22.6% in HS (30℃ to 35℃) compared with thermo-neutrality (15℃ to 20℃), whereas yolk and albumen proportions or Haugh units are less affected by temperature [103]. In broiler chickens, HS reduced body weight gain and feed intake, but not feed utilization efficiency [67]. However, feed utilization efficiency lowered when both HS and *Salmonella* Enteritidis infection were combined [67].

Heat stress damages the integrity and morphology of the intestine, resulting in poor nutrient absorption and decreased animal performance along with increased intestinal permeability and pathogen invasion [30,61]. The loss of intestinal barrier function lead to increased translocation of luminal content (e.g., bacterial endo- and exotoxins, food-borne antigens) resulting in LPS appearance in portal and systemic circulation [72]. Endotoxin initiates an immune response

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associated with greater circulating inflammatory biomarkers [63,91]. As a result, endotoxemia and inflammation caused by HS may alter metabolism and nutrient partitioning, consequently decreased productivity [105]. HS reduces the enzymatic activities (i.e., amylase, lipase and trypsin) for nutrient digestion in the gut [109]. In heat acclimatized laying hens (42℃ for 4 h), the levels of amylase, but not maltase, decreased compared to those of the control hens both in the intestine and pancreas and it has been suggested that the pancreas may regulate intestinal amylase activity during the adaptation of chickens to heat [110]. The overexpression of HSP70 significantly increased the amylase, lipase, and trypsin activity in the intestine of chickens under HS [85]. Compared with thermoneutral group, HS changed the serum biochemical parameters and hormones related to energy metabolism, stress response and immune indicators. Most of these changes in serum profile were independent of feed intake reduction [25]. Plasma triiodothyronine concentration was reduced in heat stressed-hens [102]. Egg production performance and eggshell quality were impaired by HS probably due to the disturbed oxidant and antioxidant balance and HSP homoeostasis. In addition, HS increases serum corticosterone levels, showing a HPA axis activation [65,67]. These results suggest that HS reduces feed intake and intestinal integrity and increases permeability of endo- and exotoxin and inflammation. These events may contribute to reduced performance during HS conditions [72].

AMELIORATION OF HEAT STRESS

The common effects of HS are the disturbance of gut microbiome and the oxidative stress caused by excessive generation of reactive oxygen species and reduced antioxidant defense in cells [1]. Probiotics and prebiotics are known to maintain the gut homeostasis by enhancing the beneficial bacterial populations and reducing pathogens, and also by improving gut-associated immunity and gut barrier functions [111]. Therefore, the use of probiotics, prebiotics, and substances with antioxidant activities, such as selenium, vitamin E, herbs, and different organic acids, including α-lipoic acid, have been suggested to include the diets to alleviate HS in different studies (Table 3).

Probiotics, prebiotics and postbiotics

HS elicits alterations of composition, diversity and functionality of gut microbial community along with dominance of undesirable pathogenic microbiota. Hence, a number of studies have been employed to ameliorate imbalance of the gut microbiota using probiotic, prebiotic and postbiotic interventions. Supplementation of probiotic mixture (*Bacillus subtilis* and *Enterococcus faecium*) reduced *E. coli* number and increased beneficial *Lactobacillus* number, which were altered due to HS in the ileum and ceca of laying hens [30]. Supplementation of probiotics (mixture of *Bacillus subtilis*, *Bacillus licheniformis*, and *Lactobacillus plantarum*) increased viable counts of *Bifidobacterium* and *Lactobacillus* in the small intestine [7], jejunal villus height, and protein level of OCLN, and decreased coliforms bacteria in the small intestine [7]. The intestinal villi structure of heat stressed laying hens were damaged with extensive desquamation, mainly at the tip and exposed lamina propria, compared to the normal intestinal villi in the control group [30,61]. The supplementing the probiotic mixture (*Bacillus subtilis* and *Enterococcus faecium*) in the heat-stressed hens restored the villus structure [30].

Supplementation of galacto-oligosaccharides in diets of chickens prevented the HS-related effects such as upregulated mRNA expressions of *HSF3, HSP70, HSP90, CLDN5, ZO1, CDH1, TLR4, IL6,* and *IL8* and protein expression of HSP70 in the jejunum, but it did not alter these effects in ileum [1]. In a study with different postbiotics (produced from *Lactobacillus plantarum* strains) fed (3 g/kg diet) to broiler chickens exposed to HS (36℃ for 3 h from 22 to 42 days),

Table 3. Amelioration of heat stress in livestock using different nutritional interventions

Reference	Animal	HS condition	Amelioration	Major effects in comparison with HS versus ameliorating agents under HS
[145]	Hubbard chickens	35℃, 75% RH, 8 h/day from 21 to 42 days	MOS (0.5% in diet) and/or probiotics (0.1% in diet)	• MOS increased crypt depth in HS birds. • Villus height and surface area increased in ileum due to MOS and probiotic supplementation under HS. • Villus width and crypt depth increased in HS-probiotic group. • MOS, probiotic and MOS+probiotic enhanced activity of goblet cells under HS.
[84]	Broiler chickens at 36 days of age	36℃ for 0 to 10 h for one day	Enhancer (L-glutamine injected intraperitoneally, 0.75 mg/kg BW) vs. inhibitor (quercetin injected intraperitoneally, 5 mg/kg of BW), 1 day before HS	• Increased GPx and SOD activity, T-AOC and HSP70 in jejunal mucosa by enhancer than by inhibitor. • LDH in jejunal mucosa was lower in enhancer group than inhibitor group. • LDH activity rapidly increased in jejunum during the first 2 h of HS. • Increased serum corticosterone in inhibitor group compared with enhancer group.
[85]	Male broiler chickens	36℃ for 0 to 10 h for one day	Intraperitoneally injected with L-glutamine (enhancer, 0.75 mg/kg of BW) or quercetin (inhibitor, 5 mg/kg of BW) for one day before HS	• No effect on villus height, crypt depth (except decrease in 5 h), or VCR (except increase in 10 h) *HSP70* mRNA expression in enhancer group was higher than inhibitor group in jejunum. • Trypsin, amylase, and lipase activities in enhancer group were higher than in inhibitor group.
[141]	Ross-708 of mixed sex chickens	35℃ from 1 to 42 days	0.5% MOS or 0.1% PM	• MOS and PM increased BW gain and ADFI, and lowered FCR compared with HS-control group. • Probiotic increased villus width and surface area compared with HS-control treatment. • MOS and PM partially alleviated changes of intestinal microstructures damaged by HS.
[146]	Wenchang male chickens	40℃, 52.4% RH for 3 h	0.2 mL gamma-amino butyric acid solution (50 mg/kg of BW) daily for 35 days	• Increased GPx, SOD, catalase, T-AOC content in intestinal mucosa. • Decreased MDA content in jejunal and ileal mucosa.
[147]	21-day-old Ross male broilers	33℃ from 08:00 to 06:00 from 21 to 42 days	COS at 1.5 g/kg diet	• No effect on BW gain, feed intake, and FCR. • Increased number of Lactobacillus, decreased *E. coli*, and had no effect on *Bifidobacterium* and *Clostridium* counts. • Increased villus height and VCR, no effect on crypt depth. • Reduced jejunal FITC-d permeability.
[142]	Crossbred gilts (43 kg BW)	36℃, 50% RH for either 1 or 7 days	100 (Zn220) and 200 (Zn320) mg/kg diet of Zn as Zn-amino acid complexes	• BW and feed intake were not affected. • Improved Ileal and colonic TER in Zn220-fed pigs • Ileal and colonic FITC-d permeability increased, and colonic FITC-LPS permeability tended to increase due to HS from days 1 to 7; but Zn supplementation has no effect. • Overall, no effects on ileal glucose, lysine, methionine, and glutamine transport. • Glucose transport linearly decreased with increasing levels of Zn-amino acid on day 1. • No differences on villi height or crypt depth.
[143]	Crossbred gilts (39 kg BW)	32℃, 26% RH for 24 h	WP (80% 98% and 100%)	• BW, BW gain, and feed intake were not affected by whey protein. • Plasma L-lactate and D-lactate levels increased and tended to increase with HS. • After 24 h of HS, 100% WP-fed pigs had lower plasma D-lactate relative to control-fed pigs. • Decreased Ileal TER (37%) in 80% WP-fed pigs. • Ileal TER decreased in HS whey protein groups.
[148]	Hubbard male broiler chicks	35℃ and 64% RH from 09:00 to 14:00 and 21℃ from 14:00 to 09:00	*Bacillus subtilis* (1 g/kg diet; 2.3×10^8 CFU/g of *B. subtilis* spores)	• Increased BW, ADG and feed efficiency, but no effect on feed intake by *B. subtilis*. • *B. subtilis* increased villus height, villus surface area, and absorptive epithelial cell area in duodenum and ileum. • *B. subtilis* increased *Lactobacillus* and *Bifidobacterium* and decreased coliforms and *Clostridium* counts in intestine.
[131]	Male Wenchang chickens	40.5℃, 52.4% RH for 2 h for 15 days	GABA (50 mg/kg of BW)	• HS decreased activity of sucrase, maltase, alkaline phosphatase, contents of secretory IgA, glutathione, d-xylose, number of lymphocytes, and Na^+-K^+-ATPase activity of intestinal mucosa. • GABA ameliorated the above effects.

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Table 3. Continued

Reference	Animal	HS condition	Amelioration	Major effects in comparison with HS versus ameliorating agents under HS
[7]	21-day-old Ross male broiler chickens	33℃ for 10 h/day, from 22 to 42 days	PM (*B. licheniformis, B. subtilis,* and *Lactobacillus plantarum*) at 1.5 g/kg diet	• Probiotic decreased FCR, but no effect on ADG and ADFI. • Probiotics had no effect on TER value and FITC-d permeability in jejunum compared with control diet. • Probiotics increased protein level of OCLN. • Probiotics increased villus height than the control diet.
[97]	Male Wenchang chickens	40.5℃, 52.4% RH for 2 h for 15 days	GABA (0.5%) fed with 0.2 mL	• GABA increased BW, but no effect on ADG, FCR and fed intake on day 15. • GABA enhanced villus height, crypt depth, mucosa thickness, intestinal wall thickness, and number of goblet cells in duodenum and ileum.
[135]	Male Cobb 500 broilers birds	32℃–27℃ vs. 37℃–33℃, decreased 2℃/week until reaching 33℃ in third week; for 5 h/day from 29 to 42 day of age	10 g/kg diet of glutamic acid	• Glutamic acid increased villus height, crypt depth, and VCR. • Glutamic acid increased BW.
[149]	Cobb 500 male broiler chickens	34℃, 65%–70% RH for 5 h/day from 29 to 42 days	GSE 150, 300, 450 mg/kg of diet and vitamin C at 300 mg/kg of diet	• GSE or vitamin C did not affect lengths of duodenum, jejunum, ileum, large intestine and cecum. • GSE increased villus height, tended to increase crypt depth, but no effect on VCR and muscle layer thickness jejunum. • No effect of vitamin C on jejunum villus width, crypt depth, VCR, and muscle layer thickness. • GSE, but not vitamin C, lowered ileal coliforms and *E. coli* populations.
[99]	Crossbred gilts (64 kg BW)	37℃ and 40% RH for 12 h	ZnAA	• No differences of BW loss and feed intake between HS-control and HS-ZnAA pigs. • ZnAA decreased BW and feed intake under HS. • ZnAA had no effect on FITC-d permeability, but increased ileal TER level under HS. • Ileal IL1-β decreased and plasma LBP increased in HS-ZnAA. • Colon *MUC2* mRNA abundance elevated in HS-ZnAA AA pigs • Intestinal alkaline phosphatase gene abundance increased in HS-ZnAA. • No effect on ileal and colonic HIF1A protein expression. • No effect on ileal HSP70 protein expression but lowered HSP70 protein expression in colon of HS-ZnAA pigs.
[1]	15-day-old Ross broiler chickens	38℃–39℃ for 8 h for 5 days	GOS at 10 or 25 g/kg diet (6 days prior to HS)	• GOS prevented HS-induced upregulation of *HSF3*, *HSP70*, and *HSP90* mRNA in jejunum. • Heat-induced effects on *CDH1* mRNA expression and pan-cadherin protein expression in jejunum prevented by GOS. • HS-induced increase in mRNA expressions of *CLDN5* and *ZO1* dose-dependently alleviated in jejunum by GOS. • GOS (25 g/kg) prevented heat-induced gene *TLR-4* induction in jejunum. • Up-regulation of *IL6* and *IL8* mRNA expressions in jejunum and ileum by HS was prevented by GOS.
[71]	28 day old female Xuefeng black-boned chickens	37℃ for 8 h/day for 15 days	200, 400, or 600 mg/kg diet of resveratrol for 15 days	• Resveratrol improved villus morphology, increased goblet cell and lymphocyte numbers at 400 mg/kg. • Resveratrol reduced HSP70, HSP90, and NF-κB protein levels in jejunal villi after 15 days of HS and increased EGF level.
[130]	21 day old Hubbard male broiler chickens	32℃; 64% RH	0.5 g butyric acid/kg of feed	• Butyric acid increased BW and ADG in HS birds. • Butyric acid increased recovery of villus height, villus surface area, relative intestinal weight, and absorptive epithelial cell area in duodenum of HS-birds. • Butyric acid increased intestinal *Lactobacillus* and *Bifidobacterium* populations in HS-birds.
[94]	Female growing pigs	35℃ 8 h/day, 35% RH for 2 days	0.2–1.0 mg/kg of Se and 17–200 IU/ kg of vitamin E for 14 days	• Linearly increased TER value. • Decreased FITC-d permeability quadratically. • Increased *GPx2* mRNA abundance. • Se and vitamin E did not affect *HSP70*, *HIF1A*, *IL8*, or *TNFA* mRNA levels.
[30]	Hy-Line Brown laying hens (aged 40 weeks)	33℃ and 60%–70% RH, for 20 days	PM (*Enterococcus faecium* and *B. subtilis*)	• Probiotic increased egg production rate, feed intake, and egg weight under HS. • Probiotic improved eggshell strength, eggshell thickness, and albumen height. • Probiotics increased VCR in ileum (10 days and 20 days) and cecum (20 days) in HS-hens. • Probiotic upregulated expression levels of OCLN, ZO1, and JAM-A in ileum and cecum. • Probiotics reduced mRNA level of *HSP70*.

Table 3. Continued

Reference	Animal	HS condition	Amelioration	Major effects in comparison with HS versus ameliorating agents under HS
[93]	Cobb male chickens	36℃ from 08:00 to 18:00 and 26℃ from 18:00 to 08:00 from 8 to 35 days	NAC (1 g/kg in diet)	• NAC increased ADFI, ADG, and reduced FCR. • NAC increased VCR. • NAC increased ATP level in jejunal mucosa. • NAC inhibited the increase of jejunal AMP under HS. • NAC decreased MDA concentration and mRNA levels of *HSP70*, AMP-activated protein kinase, and heme-oxigenase in intestine. • NAC elevated catalase and trypsine activity in jejunum under HS.
[66]	Lohmann layer cockerels	TN (21℃, 62% RH) vs. HS (35℃, 64% RH from 09:00 to 13:00 and 21℃ from 13:00 to 09:00) for 30 days	Butyrate at 0.35 g/kg of diet	• Butyrate increased villus height, surface area, and absorptive epithelial cell area in all intestinal parts under HS. • No effect of butyrate on crypt depth in duodenum and jejunum under HS, but it was greater in ileum. • Butyrate alleviated villi damages and epithelial cell damage in all intestinal sections. • Butyrate increased intestinal and cecal *Lactobacillus* and *Bifidobacterium* numbers and decreased *Clostridium* and coliforms numbers.
[86]	21-day-old Cobb male broiler chickens	33℃, 70% RH for 10 h for 21 days	400 mg/kg diet of resveratrol	• Resveratrol increased final BW but no effect on ADG and ADFI. • Resveratrol increased villus height, VCR and goblet cell numbers, and lowered crypt depth. • Resveratrol increased numbers of *Lactobacillus* and *Bifidobacterium* and lowered the numbers of *Escherichia coli* colonization. • Resveratrol increased intestinal mucosal *CLDN1*, *OCLN*, *CDH1*, and *MUC2* mRNA levels. • Resveratrol decreased serum D-lactic acid and FITC-d concentrations in HS birds.
[62]	Crossbred gilts (50 kg BW)	27℃–30℃, 35% humidity for 7 days	Zn at 120 mg/kg diet (60 mg as Zn-sulfate + 60 mg as Zn-amino acid)	• No effect on ADFI, ADG, and final BW, but FCR tended to decrease. • No effect on TNFA and LPS-binding protein in serum.
[144]	21-day-old Arbor Acres broiler chickens	36℃ for 10 h/day for 20 days	5 and 10 g/kg diet of glutamine	• Glutamine increased villus height in HS birds. • Glutamine decreased D-lactate and diamine oxidase activity in HS birds. • Glutamine mediated secretion of cytokines (TNFA and IL10), increased *ZO1*, *CLDN1*, and *OCLN* mRNA levels in HS birds.
[76]	Male Arbor Acres plus broiler chickens	Control (20℃) vs. HS (32℃–33℃ 8 h/day) for 4–42 days	MOS at 250 mg/kg diet	• MOS increased ADG, ADFI, and FCR. • MOS decreased mucosal MDA content in jejunum at 42 days under HS. • MOS increased jejunal and ileal villus height and VCR. • MOS increased mRNA abundances of *OCLN*, and ileal *MUC2* and *ZO1* in jejunum and ileum, and ileal *CLDN3* genes expressions.
[138]	28-day old male Ross-308 broiler chickens	33℃ and 40%–55% humidity for 8 h/day for 10 days	1 g/kg of betaine in finisher diet	• Betaine increased ileal villus height and TER. • Betaine decreased Evans blue dye concentration in jejunum and ileum.
[134]	Male Arbor Acres broilers from 28 to 42 days	TN (23℃) or subjected to cyclic HS (28℃–35℃–28℃ for 12 h daily)	Probiotic (*Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Enterococcus faecalis*) at a dose of 1.5 × 10^8 CFU/kg	• Probiotics had no effect on growth performance except for increased ADFI on days 22–42. • Probiotic increased villus height and VCR in duodenum and jejunum of HS birds on day 42. • Probiotic decreased serum D-lactic acid concentration on day 28. • Probiotic reduced serum TNFA and IL6, but increased IL10 and TGFB1 in HS birds.
[139]	Male Cobb50 broiler chickens	TN (26℃) vs. HS (34℃ for 8 h daily for 21 days)	112.5 mg ginseng extract /kg feed	• Ginseng increased feed intake, BW and FCR. • Ginseng decreased *HSPA1A*, *HSPD* and *HSPB1* gene expression. • Ginseng upregulated *CLDN3*, *OCLN* and *CLDN1* (Caco 2).

HS, heat stress; RH, relative humidity; MOS, mannan-oligosaccharides; PM, probiotic mixture; BW, body weight; GPx, glutathione peroxidase; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; HSP, heat shock protein; LDH, lactate dehydrogenase; VCR, villus height to crypt depth ratio; ADFI, average daily feed intake; FCR, feed conversion ratio; PM, probiotic mixture; MDA, malondialdehyde; FITC-d, fluorescein isothiocyanate labeled dextran; TER, transepithelial electrical resistance; WP, whey protein; GABA, γ-aminobutyric acid; GSE, grape seed extract; ZnAA, zinc amino acid complex; IL, interleukin; LBP, lipopolysaccharide binding protein; MUC, mucin; GOS, galacto-oligosaccharides; CDH1, E-cadherin; CLDN, claudin; ZO1, zonula occludens 1; TLR, toll-like receptor; NF-κB, nuclear factor kappa B; EGF, epidermal growth factor; HIF1A, hypoxia-induced factor-1α; TNFA, tumour necrosis factor α; JAM-A, junctional adhesion molecule A; NAC, *N*-acetylcysteine; AMP, adenosine monophosphate; TN, Thermoneutral; OCLN, occludin.

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postbiotics usually increased cecal total bacteria, *Lactobacillus* and *Bifidobacterium* numbers, and lowered *Enterobacteriaceae, E. coli* and *Salmonella* numbers compared to the groups without any added postbiotics or antibiotic in heat exposed-chickens [112].

Herbs

Different plant bioactive compounds have strong antimicrobial properties [113], which may be effective against the pathogenic microorganisms that become prevalent during HS condition. Supplementation of plant bioactive compounds have been shown to improve intestinal integrity, nutrient transport and antioxidant status, especially in infection and HS conditions in livestock [75,114]. Supplementation of ginger improved feed intake, egg production and antioxidant status, which were reduced by HS in layer chickens [101]. Heat challenge elevated HSP70 expression and cortisol levels in broiler chickens, but supplementation of *Zingiber officinale* and *Zingiber zerumbet* at 20 g/kg diet enhanced HSP70 expression compared with the diet without these additives [115]. Supplementation of epigallocatechin gallate at 200 and 400 mg/kg diet [116] and genistein at 200 to 800 mg/kg diet [117] increased in feed intake and body weight, improved feed efficiency and carcass traits, and reduced MDA content in serum and liver in heat stressed quails. In Japanese Silkie fowls exposed to chronic (21 days) HS (24℃ vs. 35℃), supplementation of clove extract (0 to 600 mg/kg diet) improved daily body weight gain, feed intake and feed efficiency [118]. Soursop (*Annona muricata*) juice has been shown to ameliorate the serum oxidative stress in heat stressed rabbits [119]. Fermented herbal tea residues increased feed intake and reduced serum HSP70 level, SOD and GPx activities in Holstein heifer under HS (temperature humidity index of 79) conditions [120].

The effects of genistein on MDA content and growth performance were better in the HS (34℃ for 8 h/day) condition than the thermoneutral (22℃ for 24 h/day) condition [117]. Supplementation of ginger root powder (7.5 g/kg diet) increased body weight and body weight gain of heat-stressed broiler chickens compared to the control group at 22 day of age, but not at 42 and 49 days of age with higher total antioxidant capacity and lower MDA level in serum [121]. Dietary supplementation of resveratrol at 400 mg/kg diet improved the villus morphology, increased the goblet cell and lymphocyte numbers, attenuated the gene and protein overexpressions of HSP70, HSP90, and NF-KB, and activated the expression of EGF in the jejunal mucosa [71].

Antioxidant minerals

HS increases free radical formation causing oxidative damage to lipids, proteins, and DNA and impairs immune responses by altering the expression of cytokine profiles and causing the immune cells more vulnerable to oxidative stress [122]. Selenium, as a part of specific selenoproteins, increases antioxidant status, thus preventing damages to tissues and important biomolecules [122]. Selenium supplementation in diet improves feed intake, body weight gain, egg production and quality, feed efficiency, and antioxidant status in heat-stressed poultry [122]. Selenium also enhances immune responses by changing the production of certain cytokines by immune cells and enhancing the resistance mechanism of immune cells to oxidative stress [122]. In sheep, HS (28℃–40℃ vs. 18℃–21℃), which reduced feed intake by 13% and caused oxidative stress and supplementation of selenium (0.24 and 1.2 mg/kg diet) and vitamin E (10 and 100 IU/kg diet) ameliorated oxidative stress, but did not improve feed intake [123].

In boiler breeders, HS (21℃ vs. 32℃) decreased Mn content and SOD activity in the liver and heart, and increased in MDA level, up-regulated expressions of HSF1, HSF3, and HSP70 mRNA and protein in heart, liver and muscle [124]. Dietary supplementation of Mn (120 mg/kg diet as Mn sulfate or Mn proteinate) enhanced the antioxidant capacity and lowered HSP70 expression in

breast muscle [124]. HS decreased laying rate, egg weight, eggshell strength, thickness and weight, and increased feed:egg ratio, broken egg rate, misshapen egg rate, rectal temperature, SOD activities and HSP70 mRNA levels in liver and pancreas, as well as metallothionein level in pancreas of laying broiler breeders [125]. Organic Zn supplementation increased Zn content in the liver, as well as metallothionein levels in the liver and pancreas relative to those birds fed the control diet under HS [125]. Maternal dietary Zn and Mn supplementation as an epigenetic modifier have been shown to protect the offspring embryonic development against maternal HS via enhancing the epigenetic-activated antioxidant and anti-apoptotic ability [126,127].

Supplementation of chromium-picolinate (1.60 mg; 12.4% chromium) or chromium-histidinate (0.788 mg; 25.2% chromium) delivering 200 μg/kg of chromium were effective in alleviating production performance variables of layer chickens under the HS conditions, but did not alleviate the deteriorations in egg quality parameters caused by HS [104]. Chromium can reduce cortisol level in the blood and reverse the immuno-suppression caused by HS [128].

Antioxidant vitamins

Various antioxidant vitamins have been shown to ameliorate the HS-related adverse effects in animals. Exposure of HS (20℃ vs. 35℃ during 09:00–17:00 h and 28℃ for rest of the day) to pigs for 2 days resulted in increased intestinal *HSP70* mRNA abundance and FITC-d permeability and decreased TER and GPx activity in the intestine, which were alleviated by dietary supplementation of both vitamin E (17 to 200 IU/kg diet) and Se (0.2 to 1 mg/kg diet) for 14 days as evident from the reversal of these variables [94]. In laying quails, HS (34℃ vs. 22℃) increased serum corticosterone concentration and HSP70 expression, but vitamin C or E supplementation reduced serum corticosterone level in HS quails [129]. Feed intake and egg production were not influenced by supplementation of vitamin C and E under thermoneutral conditions (22℃), but were greater due to supplementation of vitamin C or E either singly or in combination in heat-stressed (34℃) quails [129].

Other anti-stress agents

Administration of gamma amino butyric acid (GABA) orally (0.2 mL of 0.5% GABA solution) alleviated HS-induced reductions in body weight gain, jejunal villus length, crypt depth, and mucous membrane thickness (33℃ for 14 days) in broiler chickens [87]. Supplementation with GABA decreased GLUT2, peptide transporter 1, and HSP70 mRNA expressions in the jejunal mucosa, which were overexpressed in HS conditions relative to the thermoneutral condition [87]. Moreover, GABA supplementation also elevated the triiodothyronine hormone level and antioxidant status (decreased MDA level and increased GPx activity) in liver of chickens [87].

The role of betaine in heat-stress management has been reviewed [5,6]. Betaine can alleviate HS-induced changes by protecting intestinal cell proteins and enzymes and regulating water and electrolyte balance with more stable tissue and cell metabolism due to its osmoregulatory functions. Thus, betaine supplementation has shown to improve growth performance, egg production, egg quality traits and immune indices in HS animals [5,6].

CONCLUSION

The gastrointestinal tract is one of the most vulnerable organs affected by HS. Several physiological and pathological alterations occurs in the gut including changes in the microbiome composition with greater establishment of pathogenic microbiota groups, damages of microstructures of the mucosal epithelium, increased oxidative insults, reduced immunity, and increased permeability

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of the gut. Vulnerability of the intestinal integrity leads to translocation of pathogenic microbes and antigens to the blood circulations, which ultimately may cause systematic inflammations and immune response. Moreover, transports and digestion of nutrients in the guts may be impaired due to reduced enzymatic activity in the digesta, reduced surface areas for absorption and injury to the mucosal structure and expressions of the nutrient transport proteins and genes. The systematic hormonal changes due to HS along with alterations in immunity and inflammatory responses often cause reduced feed intake and production performance in livestock and poultry. It seems that many physiological alterations in immunity, barrier function and nutrient transport in the intestines may arise due to imbalances of gut microbiome caused by HS, which may then cause systemic response. However, the precise mechanisms how microbiota communicates with the host physiological responses under HS are yet to be elucidated. A better understanding of the role of gastrointestinal microbiota in physiological and pathophysiological processes in the intestine is required to properly mitigate the HS-induced adverse effects in animal production system.

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