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

Amlan Kumar Patra1* and Indrajit Kar2

1Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal 700037, India
2Department of Avian Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal 700037, India

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 reproduction in any medium, provided the original work is properly cited.

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

Acknowledgements
Not applicable.

Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors’ contributions
Conceptualization: Patra AK.
Writing - original draft: Patra AK, Kar I.
Writing - review & editing: Patra AK.

Ethics approval and consent to participate
This article does not require IRB/IACUC approval because there are no human and animal participants.
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°C vs. 31°C 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 feaces of pigs, HS (25°C vs. 29°C 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°C, 28°C, and 33°C) 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°C vs. 35°C) 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°C vs. 29°C for 21 days from 23 to 26 weeks of age [20] and 25°C vs. 35°C 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°C vs. 35°C) 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 |
|-----------|--------|----------------------|------|-----------------|----------------|
|           |        |                      | Chao1| Shannon         | Firmicutes | Bacteroidetes | Proteobacteria | Other phyla |
| [22]      | Broiler chickens | 22°C vs. 32°C for 14 days | Cecum | 428 vs. 440 | 5.48 vs. 5.46 | ↓ by 14% | = | = | = |
| [28]      | Broiler chickens | 24°C-26°C vs. 34°C-38°C 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 | Tenenclutes: 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 | = |
| [28]      | Broiler chickens | 24°C-26°C vs. 34°C-38°C for 28 days | Cecum | - | - | 428 vs. 440 | 5.48 vs. 5.46 | = | = | = |
| [23]      | Cherry-valley ducks | 25°C vs. 32°C for 8 h/day for 21 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 | = |
|           |        |                      | Jejunum | - | - | 45.4 vs. 12.0 | = | 39.0 vs. 72.7 | = |
| [31]      | Laying hens | 25°C vs. 35°C 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°C vs. 32°C for 8 h/day for 21 days | Ileum | - | - | 66.9 vs. 70.8 | 24.9 vs. 17.8 | 0.79 vs. 0.77 | Spirochaetes: 1.4 vs. 2.11 |
|           |        |                      | Cecum | - | = | = | = | = | = |
| [24]      | Sows | 18°C-22°C vs. 28°C-32°C for 30 days | Feces | 754 vs. 797 | 4.85 vs. 4.95 | = | = | = | Spirochaetes: 0.01 vs. 0.03 |
| [20]      | Pigs | 25°C vs. 29°C for 21 days | Feces | - | = | 66.9 vs. 70.8 | 24.9 vs. 17.8 | 0.79 vs. 0.77 | Spirochaetes: 1.4 vs. 2.11 |
|           |        |                      | Rumens | - | = | = | = | = | = |
| [25]      | Pigs | 25°C vs. 35°C for 24 h | Feces | - | = | = | = | = | = |
| [27]      | Pigs | 15°C vs. 35°C for 6 days | Feces | - | = | = | = | = | = |

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.
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°C for 24 h [12]. Moreover, ileal tissues from chickens subject to 30°C for 24 h increased ex-vivo attachment of Salmonella compared with chickens kept at 23°C [12]. Similarly, HS (33°C for 10 h vs. 22°C) 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°C vs. 33°C 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°C to 28°C and 36% to 45% relative humidity), which were altered in HS conditions [31]. In HS conditions (25°C to 34°C 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°C for 8 h/day for 21 days) compared with the ducks in the thermoneutral (25°C) condition [23]. In contrast, HS (21°C vs. 31°C 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°C to 38°C) compared with the control group (24°C to 26°C) 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
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 *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 *Bacteroidales* (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, *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°C for 8 h/day for 21 days) compared with the ducks in the control temperature (25°C) 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*, *Anaeropersibacter*, *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].
Swine

In Landrace × Large White crossbred sows, HS (18°C–22°C) vs. (28°C–32°C) 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°C vs. 29°C 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 Clostridiales 1 (24% of OTU), Erysipelotrichiae (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°C vs. 35°C for one day) changed the composition and diversity of fecal microbial community by decreasing the abundances of Bacteroidetes phylum and its 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°C, 28°C and 33°C) and humidity (60% and 80%), 16S rDNA sequence library composition was different between 20°C and 33°C 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 cocoides-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 Illumina MiSeq platform reported that HS (15°C vs. 35°C for 3 or 6 days) lowered the relative abundances of Proteobacteria, Chloroflexi (on day 6), and Planctomyetes (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
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°C to 20°C vs. 30°C to 37°C) 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°C vs. 29°C to 35°C 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 °C vs. 35 °C) for short period (from 09:00 to 13:00 and 21 °C 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 °C vs. 28 °C) has been shown to increase the number of infiltrating cells in sub-mucosa of jejunum but not in mucosa [68]. In pigs, HS (23 °C vs. 40 °C) 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 °C 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 °C) condition in chickens [71]. Shorter villus, deeper crypt depth, and lower ratio of V/C ratio in jejunum were observed due to HS (33 °C for 10 h vs. 22 °C) 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 °C vs. 30 °C) to birds reduced
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°C) vs. HS (32°C) 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°C, RH 66%) vs. HS (35°C, 64% RH, from 09:00 to 13:00 and rest of the time at 21°C 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°C, 24 h/day) vs. HS (30°C, 24 h/day and 35°C for 4 h/day and then 20°C, 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°C) vs. HS (30°C) 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°C, 79 to 88% RH) vs. HS (40.5°C, 52.4% RH) for 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 decreased villus length, mucosa thickness, intestinal wall thickness, and crypt depth in all three segments. |
| [97]      | Male Wenchang chickens | Ambient temperature vs. HS (40.5°C, 52.4% RH for 2 h) for 15 days | • HS declined BW, ADG, and feed intake, but no effect on FCR. |
|           |        |                             | • HS increased plasma D-lactate concentration and lowered alkaline phosphatase activity in intestinal mucosa. |
|           |        |                             | • HS decreased mRNA abundance of CLDN3 in jejunum, but not in ileum at 21 days. |
|           |        |                             | • HS reduced villi height and VCR in jejunum and ileum, and increased jejunal crypt depth. |
|           |        |                             | • HS decreased ileum thickness. |
| [76]      | Male Arbor Acres plus broiler chickens | TN (20°C) vs. HS (32°C–33°C) for 42 days | • HS decreased ileum thickness. |
|           |        |                             | • HS increased jejunal mucosal MDA content, and lowered SOD activity in ileal mucosa at 42 day. |
|           |        |                             | • HS reduced villus height, villus surface area, epithelium cell area, and relative weight of intestine. |
|           |        |                             | • HS decreased villus length, villus surface area, epithelium cell area, and relative weight of intestine. |
|           |        |                             | • HS decreased villus length, villus surface area, epithelium cell area, and relative weight of intestine. |
|           |        |                             | • HS decreased ileum thickness. |
|           |        |                             | • HS decreased villus length, villus surface area, epithelium cell area, and relative weight of intestine. |
| [132]     | Castrated crossbred male pigs | TN (22°C) vs. HS (30°C) and 65% RH for 21 days | • HS decreased villus height and crypt depth, but no effect on VCR. |
|           |        |                             | • HS increased plasma D-lactate concentration and lowered alkaline phosphatase activity in intestinal mucosa. |
| [90]      | White Leghorn hens (350-day-old) | TN (20°C–22°C, 50–60% RH) vs. HS (30°C–33°C, 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°C) vs. HS (38°C for 6 h/day and rest 18 h/day at 32°C) with 40–60% RH for 3 days | • TER reduced in ileum. |
|           |        |                             | • FITC-d and FITC-LPS flux increased in ileum. |
|           |        |                             | • HS decreased ileum thickness. |
|           |        |                             | • HS increased the activity and expression of apical SGLT1 by approximately 50%, with no effects in the pair-feeding group. |
| [89]      | Male broiler chickens (28 days old) | TN (20°C, 50% RH) vs. HS (30°C, 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 increased activity and expression of apical SGLT1 by approximately 50%, with no effects in the pair-feeding group. |
|           |        |                             | • HS increased ileal 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. |

https://doi.org/10.5187/jast.2021.e48
Table 2. Continued

| Reference | Animal | TN vs. HS conditions with RH | Major effect in comparison with TN versus HS conditions |
|-----------|--------|-----------------------------|--------------------------------------------------------|
| [133]     | Crossbred pigs 50% male and 50% female | TN (21°C–23°C, 30.2% RH for 6 h) vs. HS (39.3°C, 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. |
|           |        |                             | No effect on catalase and lysozyme activities, but HS increased alkaline phosphatase activity in jejunal mucosa. |
| [68]      | 2-month-old male Chinese mini pigs | TN (23°C) vs. HS (40°C, 5 h/day for 10 days) | Decreased body weight in HS birds |
|           |        |                             | HS decreased villus height in duodenum and jejunum on day 3. |
|           |        |                             | Cryst depth in duodenum and jejunum was shallower, but ileal 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°C; for 24 h/day) vs. HS (37°C; for 8 h/day; remaining 16 h/day at 24°C) 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 levels of HSP70, HSP90 and NF-κB, and decreased EGF in jejunal mucosa. |
| [94]      | Female growing pigs | TN (20°C) vs. HS (35°C during 09:00-17:00 h and 28°C for rest of the day) at 38% RH for 2 days | HS decreased intestinale HSP70 mRNA abundance. |
|           |        |                             | Increased FITC-d permeability and decreased TER. |
|           |        |                             | Decreased GPx activity. |
| [22]      | Arbor Acres male broiler chickens | TN (22°C, 24 h/day) and HS (32°C, 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. |
| [62]      | Crossbred gilts (50 kg BW) | TN (21°C, 47% RH) vs. HS (30°C, 35% RH for 10 h/day) for 21 days | HS increased ADFI, ADG and final BW, but no effect on feed efficiency. |
| [135]     | Male Cobb 500 broilers birds | 32°C-27°C vs. 37°C-33°C, decreased 2°C/week until reaching 33°C 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°C, 35%-50% RH) vs. HS (35°C, 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 HIF1α and HSP70 protein expression.
- No differences in IL1β and IL8 concentrations, but myeloperoxidase activity increased in ileum.
- Protein expression of ileal mast cell tryptase increased.

<https://www.ejast.org>
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°C, 35%-50% RH) vs. HS (35°C, 20%-35% RH) | • HS decreased TER, increased FITC-LPS permeability in jejunum, and increased endotoxin in plasma. | |
|           |        | (pair-feeding in TN conditions (PFTN) for 7 days) | • 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 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°C, 70% RH for 6 h) vs. HS (37°C, 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 villip 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 IL8. | |
| [136]     | Crossbred gilts (64 kg BW) | TN (21°C, 70% RH) vs. HS (37°C, 40% RH) vs. PFTN condition for 12 h | • HS reduced feed intake and BW in pigs. | |
|           |        |                             | • HS up-regulated HSP27, HSP90a, HSP90b, 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-δ, GLUT2, citrate synthase, hexokinase, or catalase. | |
| [99]      | Crossbred gilts (64 kg BW) | TN (21°C, 70% RH) vs. HS (37°C, 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. | |
|           |        |                             | • 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°C) vs. low HS (31°C) vs. high HS (36°C). 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 lymphohistiocytic enteritis found in jejunal lamina propria of low HS. | |
| [65]      | Male broiler chickens | TN (21°C) vs. 24 h/day vs. HS (31°C) for 10 h/day and rest 14 h/day at 21°C | • 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 heterophil. | |
| [67]      | Male broiler chickens | TN (21°C, 24 h/day) vs. positive Salmonella TN group (21°C, 24 h/day) vs. positive Salmonella HS group (31°C from 31 to 41 days from 6:00 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°C) vs. HS (35°C) 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. | |
Heat stress on gut microbiota and health, and production performance

Crossbred gilts (43 kg)

- HS reduced villous height and ileal TER.
- HS decreased feed intake and BW.
- HS decreased genes expressions of CLDN2 and OCLN but not CLDN1.
- HS increased genes expressions of HSPA1A, HSPD1, and HSPB1.

17-day-old Ross broiler chickens

- 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.

Ross-708 chicks of mixed sex

- 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.

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°C) vs. HS (33°C) 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°C) vs. HS (34°C) for 8 h daily for 21 days | HS decreased feed intake and BW. |
| [140]     | 17-day-old Ross broiler chickens | TN (25°C) vs. HS (39°C) for 8 h/day for 4 days | HS decreased villus height, epithelial and total villus areas in all small intestine segments. |
| [141]     | Ross-708 chicks of mixed sex | Control (35°C at day 1 and decreased 3°C per week to 26°C and then maintained constant) vs. (35°C from day 1 to 42) | HS decreased BW on day 21 and 42. |
| [142]     | Crossbred gilts (43 kg BW) | TN (19°C, 61% RH) vs. HS (36°C, 50% RH) for 1 or 7 days | HS reduced feed intake and BW gain. Colonic TER decreased as HS progressed. |
| [143]     | Crossbred gilts (39 kg BW) | TN (19°C, 46% RH) vs. HS (32°C, 26% RH) for 48 h | HS reduced feed intake and BW gain. Feeding intake and body weight decreased. |
| [7]       | 21-day-old Ross male broiler chickens | TN (22°C, RH 70%) vs. HS (33°C) for 10 h/day, RH 70% from 22-42 days | Reduced TER value and increased FITC-d permeability in jejunum. |
| [1]       | 15-day-old Ross broilers | TN (22°C-23°C) vs. HS (38°C-39°C) for 8 h and remaining time at 22°C-23°C for 5 days | In jejunum, HSF-3, HSP70, HSP90, CDH1, CLDN5, ZO1, TLR-4, IL6, and IL8 mRNA expression and HSP70 protein expression increased. |
| [144]     | 21-day-old Arbor Acres broiler chickens | TN (36°C for 10 h/day for 20 days | Decreased villus height, increased crypt depth, D-lactic acid concentration and diaminodioxate activity, and soluble intercellular adhesion molecule-1, TNFα, and IL10 concentrations. |
| [70]      | Chinese mini pigs | TN (23°C) vs. HS (40°C), 5 h/day for 10 days | HS reduced VCR. |
| [93]      | Cobb male chickens | TN (26°C, 50%) vs. HS (36°C) from 08:00 to 18:00 and 26°C from 18:00 to 08:00 | HS decreased feed intake, BW gain, plasma concentrations of triiodothyronine and thyroxine; increased FCR. |
| [30]      | Hy-Line Brown commercial laying hens (40 weeks old) | TN (26°C) vs. HS (33°C), 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. |
| [86]      | 21-day-old Cobb male broilers | TN (22°C) vs. HS (33°C) with 70% RH for 10 h and remaining time at 22°C for 21 days | HS lowered final BW, ADG, and feed intake. |

TN, thermoneutral; HS, heat stress; RH, relative humidity; BW, body weight; ADG, average daily gain; FCR, feed conversion ratio; TNFA, tumour necrosis factor a; 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, zona 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; TGFβ1, 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.
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°C vs. 33°C for 20 days), particularly, at 20 days [30]. In broiler chickens, HS (20°C vs. 30°C–33°C 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°C vs. 27.8°C 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°C vs. 28°C) for 4 days increased ZO1 and tended to increase
Heat stress on gut microbiota and health, and production performance

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°C–39°C, 8 h/day for 5 days vs. 22°C–23°C) 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°C vs. 35°C) 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°C, 20%–35% humidity) compared with thermoneutral crossbred gilts (20°C, 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°C–22°C vs. and HS 28°C–32°C) from 85 days of gestation to farrowing had higher serum HSP70, lipopolysaccharide and lipopolysaccharide-binding protein levels [24]. HS (30°C vs. 20°C) 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].
**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°C–39°C, 8 h/day for 5 days vs. 22°C–23°C) 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°C vs. 21°C 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°C vs. 35°C for 24 h) along with increased Na+/K+ -ATPase activity in the ileum of HS pigs [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].
HS (24°C vs. 32°C 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°C vs. 30°C) 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°C–22°C, 50%–60% relative humidity vs. 30°C–33°C, 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°C vs. 20°C) 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°C vs. 32°C–33°C 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°C, 24 h/day vs. 32°C, 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°C to 35°C vs. 20°C) 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°C, 52% relative humidity vs. 15°C, 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°C for 0, 2, 3, 5 and 10 h vs. 21°C 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].
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°C–39°C, 8 h/day for 5 days vs. 22°C–23°C) up-regulated mRNA expressions of TLR4, IL6, and IL8 in the jejunum [1]. In Holstein cattle, HS (28°C, 52% humidity vs. 15°C, 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 jejenum [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 naïve T-helper cell differentiation into T-helper 2 effector cells that promote immunity and inflammation [96].

In poultry, HS (31°C 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 (TGFβ1), 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°C vs. 20°C, 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°C vs. 37°C 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°C and 24%-43% relative humidity) for 24 h showed elevated respiration rates by 2-fold and rectal temperatures by 1.6°C and reduced voluntary feed intake by 53% relative to the pigs in thermoneutral (21°C and 35%-50% humidity) condition [78]. Acute HS (35°C) in crossbred pigs for 24 h decreased feed intake compared with the thermoneutral (25°C) 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°C), chronic HS (30°C; 24 h/day) and acute HS (35°C from 09:00 to 13:00 and 20°C 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°C vs. 20°C) 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°C to 35°C) compared with thermo-neutrality (15°C to 20°C), 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
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°C 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 homeostasis. 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°C for 3 h from 22 to 42 days),
| Reference | Animal | HS condition | Amelioration | Major effects in comparison with HS versus ameliorating agents under HS |
|-----------|--------|--------------|--------------|---------------------------------------------------------------|
| [145]     | Hubbard chickens | 35°C, 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°C 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°C for 0 to 10 h for one day | In 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 in inhibitor group.  
● Trypsin, amylase, and lipase activities in enhancer group were higher than in inhibitor group. |
| [141]     | Ross-708 of mixed sex chickens | 35°C 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°C, 52.4% RH for 3 h | 0.2 mL gamma-aminobutyric 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°C 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°C, 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°C, 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°C and 64% RH from 09:00 to 14:00 and 21°C 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°C, 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. |
### 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°C 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°C, 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 feed 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°C–27°C vs. 37°C–33°C, decreased 2°C/week until reaching 33°C 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°C, 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 jejenum. |
|           |        |              |              | No effect of vitamin C on jejunal 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°C 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 IL-1β decreased and plasma LBP increased in HS-ZnAA. |
|           |        |              |              | Colon MUC2 mRNA abundance elevated in HS-ZnAA. |
|           |        |              |              | 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°C–39°C 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 HS3, HSP70, and HS900 mRNA in jejenum. |
|           |        |              |              | 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 prevented by GOS. |
| [71]      | 28-day-old female Xuefeng black-boned chickens | 37°C 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°C; 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°C, 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°C, 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. |
| Reference | Animal | HS condition | Amelioration | Major effects in comparison with HS versus ameliorating agents under HS |
|-----------|--------|--------------|--------------|-------------------------------------------------|
| [93]      | Cobb male chickens | 36°C from 08:00 to 18:00 and 26°C 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 tyrosine activity in jejunum under HS. |
| [66]      | Lohmann layer cockerels | TN (21°C, 62% RH) vs. HS (35°C, 64% RH from 09:00 to 13:00 and 21°C 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. |
| [66]      | 21-day-old Cobb male broiler chickens | 33°C, 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°C–30°C, 35% humidity for 7 days | Zn at 120 mg/kg diet (60 mg as Znsulfate + 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°C 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°C) vs. HS (32°C–33°C 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 mRNAs abundances of OCLN, ileal ZO1 and MUC2 and ZO1 in jejunum and ileum, and ileal CLDN3 genes expressions. |
| [138]     | 28-day old male Ross-308 broiler chickens | 33°C 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°C) or subjected to cyclic HS (26°C–36°C–28°C for 12 h daily) | Probiotic (Lactobacillus acidophilus, Lactobacillus plantarum, and Enterococcus faecalis) at a dose of 1.5 × 10<sup>9</sup> 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 TGFβ1 in HS birds. |
| [139]     | Male Cobb50 broiler chickens | TN (26°C) vs. HS (34°C for 8 h daily for 21 days) | 112.5 mg ginseng extract/kg feed | • Ginseng increased feed intake, BW and FCR.  
  • Ginseng decreased HSPA1A, HSPD1 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; NFκB, 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-acetyl-L-cysteine; AMP, adenosine monophosphate; TN, Thermoneutral; OCLN, occludin.
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°C vs. 35°C), 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°C for 8 h/day) condition than the thermoneutral (22°C 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-κB, 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°C–40°C vs. 18°C–21°C), 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°C vs. 32°C) 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 °C vs. 35 °C during 09:00–17:00 h and 28 °C 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 °C vs. 22 °C) 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 °C), but were greater due to supplementation of vitamin C or E either singly or in combination in heat-stressed (34 °C) 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 °C 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...
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.

REFERENCES

1. Varasteh S, Braber S, Akbari P, Garssen J, Fink-Gremmels J. Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galacto-oligosaccharides. PLOS ONE. 2015;10:e0138975. https://doi.org/10.1371/journal.pone.0138975
2. Lara LJ, Rostagno MH. Impact of heat stress on poultry production. Animals. 2013;3:356-69. https://doi.org/10.3390/ani3020356
3. Renaudeau D, Collin A, Yahav S, de Basilio V, Gourdine JL, Collier RJ. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal. 2012;6:707-28. https://doi.org/10.1017/S1751731111002448
4. Becker CA, Collier RJ, Stone AE. Invited review: physiological and behavioral effects of heat stress in dairy cows. J Dairy Sci. 2020;103:6751-70. https://doi.org/10.3168/jds.2019-17929
5. Ratriyanto A, Mosenthin R. Osmoregulatory function of betaine in alleviating heat stress in poultry. J Anim Physiol Anim Nutr. 2018;102:1634–50. https://doi.org/10.1111/jpn.12990
6. Saeed M, Babazadeh D, Naveed M, Arain MA, Hassan FU, Chao S. Reconsidering beta-ine as a natural anti-heat stress agent in poultry industry: a review. Trop Anim Health Prod. 2017;49:1329-38. https://doi.org/10.1007/s11250-017-1355-z
7. Song J, Xiao K, Ke YL, Jiao LF, Hu CH, Diao QY, et al. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult Sci. 2014;93:581-8. https://doi.org/10.3382/ps.2013-03455
8. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol. 2009;9:799-809. https://doi.org/10.1038/nri2653
9. Wallin RPA, Lundqvist A, Moré SH, von Bonin A, Kiessling R, Ljunggren HG. Heat-shock proteins as activators of the innate immune system. Trends Immunol. 2002;23:130-5. https://doi.org/10.1016/s1471-4906(01)02168-8
10. Rea K, Dinan TG, Cryan JF. The microbiome: a key regulator of stress and neuroinflammation. Neurobiol Stress. 2016;4:23–33. https://doi.org/10.1016/j.jynstr.2016.03.001
11. Cholewińska P, Górniak W, Wojnarowski K. Impact of selected environmental factors on microbiome of the digestive tract of ruminants. BMC Vet Res. 2021;17:25. https://doi.org/10.1186/s12917-021-02742-y
12. Burkholder KM, Thompson KL, Einstein ME, Applegate TJ, Patterson JA. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to Salmonella enteritidis colonization in broilers. Poult Sci. 2008;87:1734-41. https://doi.org/10.3382/
13. Slimen IB, Najar T, Ghram A, Abdrrabba M. Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review. J Anim Physiol Anim Nutr. 2016;100:401-12. https://doi.org/10.1111/jpn.12379

14. Kadzere CT, Murphy MR, Silanikove N, Maltz E. Heat stress in lactating dairy cows: a review. Livest Prod Sci. 2002;77:59-91. https://doi.org/10.1016/S0301-6226(01)00330-X

15. Polsky L, von Keyserlingk MAG. Invited review: effects of heat stress on dairy cattle welfare. J Dairy Sci. 2017;100:8645-57. https://doi.org/10.3168/jds.2017-12651

16. Shang Y, Kumar S, Oakley B, Kim WK. Chicken gut microbiota: importance and detection technology. Front Vet Sci. 2018;5:254. https://doi.org/10.3389/fvets.2018.00025

17. Borda-Molina D, Seifert J, Camarinha-Silva A. Current perspectives of the chicken gastrointestinal tract and its microbiome. Comput Struct Biotechnol J. 2018;16:131-9. https://doi.org/10.1016/j.csbj.2018.03.002

18. Yadav S, Jha R. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J Anim Sci Biotechnol. 2019;10:2. https://doi.org/10.1186/s40104-018-0310-9

19. Wang XJ, Feng JH, Zhang MH, Li XM, Ma DD, Chang SS. Effects of high ambient temperature on the community structure and composition of ileal microbiome of broilers. Poult Sci. 2018;97:2153-64. https://doi.org/10.3382/ps/pey032

20. Le Scililour M, Zemb O, Hochu I, Riquet J, Gilbert H, Giorgi M, et al. Effect of chronic and acute heat challenges on fecal microbiota composition, production, and thermoregulation traits in growing pigs. J Anim Sci. 2019;97:3845-58. https://doi.org/10.1093/jas/sky222

21. Xing S, Wang X, Diao H, Zhang M, Zhou Y, Feng J. Changes in the cecal microbiota of laying hens during heat stress is mainly associated with reduced feed intake. Poult Sci. 2019;98:5257-64. https://doi.org/10.3382/ps/pez440

22. Liu G, Zhu H, Ma T, Yan Z, Zhang Y, Geng Y, et al. Effect of chronic cyclic heat stress on the intestinal morphology, oxidative status and cecal bacterial communities in broilers. J Therm Biol. 2020;91:102619. https://doi.org/10.1016/j.jtherbio.2020.102619

23. He J, He Y, Pan D, Cao J, Sun Y, Zeng X. Associations of gut microbiota with heat stress-induced changes of growth, fat deposition, intestinal morphology, and antioxidant capacity in ducks. Front Microbiol. 2019;10:903. https://doi.org/10.3389/fmicb.2019.00903

24. He J, Guo H, Zheng W, Xue Y, Zhao R, Yao W. Heat stress affects fecal microbial and metabolic alterations of primiparous sows during late gestation. J Anim Sci Biotechnol. 2019;10:84. https://doi.org/10.1186/s40104-019-0391-0

25. Xiong Y, Yi H, Wu Q, Jiang Z, Wang L. Effects of acute heat stress on intestinal microbiota in growing-finishing pigs, and associations with feed intake and serum profile. J Appl Microbiol. 2020;128:840-52. https://doi.org/10.1111/jam.14504

26. Shi D, Bai L, Qu Q, Shanshan Z, Meimei Y, Shining G, et al. Impact of gut microbiota structure in heat-stressed broilers. Poult Sci. 2019;98:2405-13. https://doi.org/10.3382/ps/pez026

27. Chen S, Wang J, Peng D, Li G, Chen J, Gu X. Exposure to heat-stress environment affects the physiology, circulation levels of cytokines, and microbiome in dairy cows. Sci Rep. 2018;8:14606. https://doi.org/10.1038/s41598-018-32886-1

28. Zhu L, Liao R, Wu N, Zhu G, Yang C. Heat stress mediates changes in fecal microbiome and functional pathways of laying hens. Appl Microbiol Biotechnol. 2019;103:461-72. https://doi.org/10.1007/s00253-018-9465-8

29. Baek YC, Choi H, Jeong JY, Lee SD, Kim MJ, Lee S, et al. The impact of short-term acute...
heat stress on the rumen microbiome of Hanwoo steers. J Anim Sci Technol. 2020;62:208-17. https://doi.org/10.5187/jast.2020.62.2.208

30. Tajima K, Nonaka I, Higuchi K, Takusari N, Kurihara M, Takenaka A, et al. Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. Anaerobe. 2007;13:57-64. https://doi.org/10.1016/j.anaerobe.2006.12.001

31. Suzuki K, Harasawa R, Yoshitake Y, Mitsuoka T. Effects of crowding and heat stress on intestinal flora, body weight gain, and feed efficiency of growing rats and chicks. Jpn J Vet Sci. 1983;45:331-8. https://doi.org/10.1292/jvms1939.45.331

32. Zhang P, Yan T, Wang X, Kuang S, Xiao Y, Lu W, et al. Probiotic mixture ameliorates heat stress of laying hens by enhancing intestinal barrier function and improving gut microbiota. Ital J Anim Sci. 2017;16:292-300. https://doi.org/10.1080/1828051X.2016.1264261

33. Wang M, Lin X, Jiao H, Uyanga V, Zhao J, Wang X, et al. Mild heat stress changes the microbiota diversity in the respiratory tract and the cecum of layer-type pullets. Poult Sci. 2020;99:7015-26. https://doi.org/10.1016/j.psj.2020.09.024

34. Lee Y, Lee HY. Revisiting the bacterial phylum composition in metabolic diseases focused on host energy metabolism. Diabetes Metab J. 2020;44:658-67. https://doi.org/10.4093/dmj.2019.0220

35. Hu C, Yadnyavalkya P, Gong D, Yu T, Li J, Wu L, et al. Heat stress induced gut microbiota changes activate thr4 / nf- κ b signaling pathway contributing to inflammatory bowel disease in pigs. Res Sq. 2020:1-41. https://doi.org/10.21203/rs.2.21626/v1

36. Uyeno Y, Sekiguchi Y, Tajima K, Takenaka A, Kurihara M, Kamagata Y. An rRNA-based analysis for evaluating the effect of heat stress on the rumen microbial composition of Holstein heifers. Anaerobe. 2010;16:27-33. https://doi.org/10.1016/j.anaerobe.2009.04.006

37. Heida FH, van Zoonen AGJF, Hulscher JBF, te Kiefte BJC, Wessels R, Kooi EMW, et al. A necrotizing enterocolitis-associated gut microbiota is present in the meconium: results of a prospective study. Clin Infect Dis Off Publ Infect Dis. 2016;62:863-70. https://doi.org/10.1093/cid/ciw016

38. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe. 2013;14:207-15. https://doi.org/10.1016/j.chom.2013.07.007

39. Menconi A, Wolfenden AD, Shivaramaiah S, Terraes AJ, Urbano T, Kuttel J, et al. Effect of lactic acid bacteria probiotic culture for the treatment of Salmonella enterica serovar Heidelberg in neonatal broiler chickens and turkey poultis. Poult Sci. 2011;90:561-5. https://doi.org/10.3382/ps.2010-01220

40. Nicola S, Amoruso A, Deidda F, Pane M, Alleina S, Mogna L, et al. Searching for the perfect homeostasis: five strains of Bifidobacterium longum from centenarians have a similar behavior in the production of cytokines. J Clin Gastroenterol. 2016;50:S126-30.

41. Serafini F, Strati F, Ruas-Madiedo P, Turroni F, Foroni E, Duranti S, et al. Evaluation of adhesion properties and antibacterial activities of the infant gut commensal Bifidobacterium bifidum PRL2010. Anaerobe. 2013;21:9-17. https://doi.org/10.1016/j.anaerobe.2013.03.003

42. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015;33:496-503. https://doi.org/10.1016/j.tibtech.2015.06.011

43. Domingo MC, Yansouni C, Gaudreau C, Lamotho S, Lévesque S, Tremblay C, et al. Cloacibacillus sp., a potential human pathogen associated with bacteremia in Quebec and New Brunswick. J Clin Microbiol. 2015;53:3380-3. https://doi.org/10.1128/JCM.01137-15

44. Berkhoff HA. Clostridium colinum sp. nov., nom. rev., the causative agent of ulcer-
ative enteritis (quail disease) in quail, chickens, and pheasants. Int J Syst Evol Microbiol. 1985;35:155-9. https://doi.org/10.1099/00207713-35-2-155
45. Malinen E, Krogius-Kurikka L, Lyra A, Nikkilä J, Jääskeläinen A, Rinttilä T, et al. Association of symptoms with gastrointestinal microbiota in irritable bowel syndrome. World J Gastroenterol. 2010;16:4532-40. https://doi.org/10.3748/wjg.v16.i36.4532
46. Peterson VL, Jury NJ, Cabrera-Rubio R, Draper LA, Crispe F, Cotter PD, et al. Drunk bugs: chronic vapour alcohol exposure induces marked changes in the gut microbiome in mice. Behav Brain Res. 2017;323:172-6. https://doi.org/10.1016/j.bbr.2017.01.049
47. Minamoto Y, Otoni CC, Steelman SM, Büyükleblebici O, Steiner JM, Jergens AE, et al. Alteration of the fecal microbiota and serum metabolite profiles in dogs with idiopathic inflammatory bowel disease. Gut Microbes. 2015;6:33-47. https://doi.org/10.1080/19490976.2014.997612
48. Suchodolski JS, Foster ML, Sohail MU, Leutenegger C, Queen EV, Steiner JM, et al. The fecal microbiome in cats with diarrhea. PLOS ONE. 2015;10:e0127378. https://doi.org/10.1371/journal.pone.0127378
49. Gophna U, Konikoff T, Nielsen HB. Oscillospira and related bacteria: from metagenomic species to metabolic features. Environ Microbiol. 2017;19:835-41. https://doi.org/10.1111/1462-2920.13658
50. Heinken A, Khan MT, Paglia G, Rodionov DA, Harmens HJM, Thiele I. Functional metabolic map of Faecalibacterium prausnitzii, a beneficial human gut microbe. J Bacteriol. 2014;196:3289-302. https://doi.org/10.1128/JB.01780-14
51. Biddle A, Stewart L, Blanchard J, Leschine S. Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. Diversity. 2013;5:627-40. https://doi.org/10.3390/d5030627
52. Meehan CJ, Beiko RG. A phylogenomic view of ecological specialization in the Lachnospiraceae, a family of digestive tract-associated bacteria. Genome Biol Evol. 2014;6:703-13. https://doi.org/10.1093/gbe/evu050
53. Contreras-Jodar A, Nayan NH, Hamzaoui S, Caja G, Salama AAK. Heat stress modifies the lactational performances and the urinary metabolomic profile related to gastrointestinal microbiota of dairy goats. PLOS ONE. 2019;14:e0202457. https://doi.org/10.1371/journal.pone.0202457
54. Sommer F, Bäckhed F. The gut microbiota—masters of host development and physiology. Nat Rev Microbiol. 2013;11:227-38. https://doi.org/10.1038/nrmicro2974
55. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci. 2011;108:16050-5. https://doi.org/10.1073/pnas.1102999108
56. Alhenaky A, Abdelqader A, Abuajamieh M, Al-Fatafah AR. The effect of heat stress on intestinal integrity and Salmonella invasion in broiler birds. J Therm Biol. 2017;70:9-14. https://doi.org/10.1016/j.jtherbio.2017.10.015
57. Quinteiro-Filho WM, Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Sakai M, Sá LRM, et
al. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poult Sci. 2010;89:1905-14. https://doi.org/10.3382/ps.2010-00812

60. Quinteiro-Filho WM, Calefi AS, Cruz DSG, Aloia TPA, Zager A, Astolfi-Ferreira CS, et al. Heat stress decreases expression of the cytokines, avian β-defensins 4 and 6 and Toll-like receptor 2 in broiler chickens infected with Salmonella Enteritidis. Vet Immunol Immunopathol. 2017;186:19-28. https://doi.org/10.1016/j.vetimm.2017.02.006

61. Gabler NK, Koltes D, Schaumberger S, Murugesan GR, Reisinger N. Diurnal heat stress reduces pig intestinal integrity and increases endotoxin translocation. Transl Anim Sci. 2018;2:1-10. https://doi.org/10.1093/tas/ttx003

62. Mayorga EJ, Kvidera SK, Horst EA, Al-Qaisi M, Dickson MJ, Seibert JT, et al. Effects of zinc amino acid complex on biomarkers of gut integrity and metabolism during and following heat stress or feed restriction in pigs. J Anim Sci. 2018;96:4173-85. https://doi.org/10.1093/jas/sky293

63. Alhenaky A, Abdelqader A, Abuajamieh M, Al-Fataftah AR. The effect of heat stress on intestinal integrity and Salmonella invasion in broiler birds. J Therm Biol. 2017;70:9-14. https://doi.org/10.1016/j.jtherbio.2017.10.015

64. Choct M. Managing gut health through nutrition. Br Poult Sci. 2009;50:9-15. https://doi.org/10.1080/00071660802538632

65. Quinteiro-Filho WM, Rodrigues MV, Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Sá LRM, et al. Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: role of acute hypothalamic-pituitary-adrenal axis activation. J Anim Sci. 2012;90:1986-94. https://doi.org/10.2527/jas.2011-3949

66. Abdelqader AM, Abuajamieh M, Hammad HM, Al-Fataftah ARA. Effects of dietary butyrate supplementation on intestinal integrity of heat-stressed cockerels. J Anim Physiol Anim Nutr. 2017;101:1115-21. https://doi.org/10.1111/jpn.12622

67. Quinteiro-Filho WM, Gomes AVS, Pinheiro ML, Ribeiro A, Ferraz-de-Paula V, Astolfi-Ferreira CS, et al. Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with Salmonella Enteritidis. Avian Pathol. 2012;41:421-7. https://doi.org/10.1080/03079457.2012.709315

68. Abdelqader A, Al-Fataftah AR. Effect of dietary butyric acid on performance, intestinal morphology, microflora composition and intestinal recovery of heat-stressed broilers. Livest Sci. 2016;183:78-83. https://doi.org/10.1016/j.livsci.2015.11.026

69. Chen Z, Xie J, Wang B, Tang J. Effect of γ-aminobutyric acid on digestive enzymes, absorption function, and immune function of intestinal mucosa in heat-stressed chicken. Poult Sci. 2014;93:2490-500. https://doi.org/10.3382/ps.2013-03398

70. Chen Z, Xie J, Hu MY, Tang J, Shao ZF, Li MH. Protective effects of γ-aminobutyric acid (GABA) on the small intestinal mucosa in heat-stressed wenchang chicken. J Anim Plant Sci. 2015;25:78-87.

71. Cheng YF, Chen YP, Chen R, Su Y, Zang RQ, He QF, et al. Dietary mannan oligosaccharide ameliorates cyclic heat stress-induced damages on intestinal oxidative status and barrier integrity of broilers. Poult Sci. 2019;98:4767-76. https://doi.org/10.3382/ps/ppez192

72. Cui Y, Gu X. Proteomic changes of the porcine small intestine in response to chronic heat stress. J Mol Endocrinol. 2015;55:277-93. https://doi.org/10.1530/JME-15-0161

73. Ebeid TA, Suzuki T, Sugiyama T. High ambient temperature influences eggshell quality and calbindin-D28k localization of eggshell gland and all intestinal segments of laying hens. Poult Sci. 2012;91:2282-7. https://doi.org/10.3382/ps.2011-01898
74. Garriga C, Hunter RR, Amat C, Planas JM, Mitchell MA, Moretó M. Heat stress increases apical glucose transport in the chicken jejunum. Am J Physiol Regul Integr Comp Physiol. 2006;290:R195-201. https://doi.org/10.1152/ajpregu.00393.2005

75. Goo D, Kim JH, Park GH, Delos Reyes JB, Kil DY. Effect of heat stress and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. Animals. 2019;9:107. https://doi.org/10.3390/ani9030107

76. Gu XH, Hao Y, Wang XL. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. intestinal oxidative stress. Poult Sci. 2012;91:790-9. https://doi.org/10.3382/ps.2011-01628

77. Koch F, Thom U, Albrecht E, Weikard R, Nolte W, Kuhla B, et al. Heat stress directly impairs gut integrity and recruits distinct immune cell populations into the bovine intestine. Proc Natl Acad Sci. 2019;116:10333-8. https://doi.org/10.1073/pnas.1820130116

78. Kpodo KR, Duttlinger AW, Radcliffe JS, Johnson JS. Time course determination of the effects of rapid and gradual cooling after acute hyperthermia on body temperature and intestinal integrity in pigs. J Therm Biol. 2020;87:102481. https://doi.org/10.1016/j.jtherbio.2019.102481

79. Li Q, Wan G, Peng C, Xu L, Yu Y, Li L, et al. Effect of probiotic supplementation on growth performance, intestinal morphology, barrier integrity, and inflammatory response in broilers subjected to cyclic heat stress. Anim Sci J Nihon Chikusan Gakkaiho. 2020;91:e13433. https://doi.org/10.1111/asj.13433

80. Liu F, Yin J, Du M, Pan P, Xu J, Zhu J, et al. Heat-stress-induced damage to porcine small intestinal epithelium associated with downregulation of epithelial growth factor signaling. J Anim Sci. 2009;87:1941-9. https://doi.org/10.2527/jas.2008-1624

81. Liu L, Fu C, Yan M, Xie H, Li S, Yu Q, et al. Resveratrol modulates intestinal morphology and HSP70/90, NF-κB and EGF expression in the jejunal mucosa of black-boned chickens on exposure to circular heat stress. Food Funct. 2016;7:1329-38. https://doi.org/10.1039/c5fo01338k

82. Liu F, Cottrell JJ, Furness JB, Rivera LR, Kelly FW, Wijsesirwardana U, et al. Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. Exp Physiol. 2016;101:801-10. https://doi.org/10.1113/EP085746

83. Porto ML, Giviszé PéN, Saraiva EP, Costa FGP, Moreira Filho ALB, Andrade MSF, et al. Glutamic acid improves body weight gain and intestinal morphology of broiler chickens submitted to heat stress. Braz J Poult Sci. 2015;17:355-62. https://doi.org/10.1590/1516-635x1703355-362

84. Pearce SC, Mani V, Boddicker RL, Johnson JS, Weber TE, Ros JW, et al. Heat stress reduces intestinal barrier integrity and favors intestinal glucose transport in growing pigs. PLOS ONE. 2013;8:e70215. https://doi.org/10.1371/journal.pone.0070215

85. Pearce SC, Mani V, Weber TE, Rhoads RP, Patience JF, Baumgard LH, et al. Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs. J Anim Sci. 2013;91:5183-93. https://doi.org/10.2527/jas.2013-6759

86. Pearce SC, Sanz-Fernandez MV, Hollis JH, Baumgard LH, Gabler NK. Short-term exposure to heat stress attenuates appetite and intestinal integrity in growing pigs. J Anim Sci. 2014;92:5444-5. https://doi.org/10.2527/jas.2014-8407

87. Pearce SC, Lonergan SM, Huff-Lonergan E, Baumgard LH, Gabler NK. Acute heat stress and reduced nutrient intake alter intestinal proteomic profile and gene expression in pigs. PLOS ONE. 2015;10:e0143099. https://doi.org/10.1371/journal.pone.0143099

88. Pearce SC, Sanz Fernandez MV, Torrison J, Wilson ME, Baumgard LH, Gabler NK. Di-
etary organic zinc attenuates heat stress-induced changes in pig intestinal integrity and metabolism. J Anim Sci. 2015;93:4702-13. https://doi.org/10.2527/jas.2015-9018

89. Ruff J, Barros TL, Tellez G, Blankenship J, Lester H, Graham BD, et al. Research Note: evaluation of a heat stress model to induce gastrointestinal leakage in broiler chickens. Poult Sci. 2020;99:1687-92. https://doi.org/10.1016/j.psj.2019.10.075

90. Shakeri M, Cottrell JJ, Wilkinson S, Zhao W, Le HH, McQuade R, et al. Dietary betaine improves intestinal barrier function and ameliorates the impact of heat stress in multiple vital organs as measured by evans blue dye in broiler chickens. Animals. 2019;10:38. https://doi.org/10.3390/ani10010038

91. Sandner G, Mueller AS, Zhou X, Stadlbauer V, Schwarzinger B, Schwarzinger C, et al. Ginseng extract ameliorates the negative physiological effects of heat stress by supporting heat shock response and improving intestinal barrier integrity: evidence from studies with heat-stressed caco-2 cells, c. elegans and growing broilers. Molecules. 2020;25:835. https://doi.org/10.3390/molecules25040835

92. Santos RR, Awati A, Roubos-van den Hil PJ, Tersteeg-Zijderveld MHG, Koolmees PA, Fink-Gremmels J. Quantitative histo-morphometric analysis of heat-stress-related damage in the small intestines of broiler chickens. Avian Pathol. 2015;44:19-22. https://doi.org/10.1080/03079457.2014.988122

93. Sohail MU, Hume ME, Byrd JA, Nisbet DJ, Ljaz A, Sohail A, et al. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult Sci. 2012;91:2235-40. https://doi.org/10.3382/ps.2012-02182

94. Sanz Fernandez MV, Pearce SC, Gabler NK, Patience JF, Wilson ME, Socha MT, et al. Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. Animal. 2014;8:43-50. https://doi.org/10.1017/S1751731113001961

95. Sanz Fernandez MV, Pearce SC, Mani V, Gabler NK, Metzger L, Patience JF, et al. Effects of dairy products on intestinal integrity in heat-stressed pigs. Temperature. 2014;1:128-34. https://doi.org/10.4161/temp.29561

96. Wu QJ, Liu N, Wu KH, Wang GY, Lin L. Glutamine alleviates heat stress-induced impairment of intestinal morphology, intestinal inflammatory response, and barrier integrity in broilers. Poult Sci. 2018;97:2675-83. https://doi.org/10.3382/ps.2018-03922

97. Yu J, Yin P, Liu F, Cheng G, Guo K, Lu A, et al. Effect of heat stress on the porcine small intestine: a morphological and gene expression study. Comp Biochem Physiol A Mol Integr Physiol. 2010;156:119-28. https://doi.org/10.1016/j.cbpa.2010.01.008

98. Yi D, Hou Y, Tan L, Liao M, Xie J, Wang L, et al. N-acetylcysteine improves the growth performance and intestinal function in the heat-stressed broilers. Anim Feed Sci Technol. 2016;220:83-92. https://doi.org/10.1016/j.anifeedsci.2016.07.014

99. Zhang C, Zhao XH, Yang L, Chen XY, Jiang RS, Jin SH, et al. Resveratrol alleviates heat stress-induced impairment of intestinal morphology, microflora, and barrier integrity in broilers. Poult Sci. 2017;96:4325-32. https://doi.org/10.3382/ps/pex266

100. Wang L, Zhu F, Yang H, Li J, Li Y, Ding X, et al. Epidermal growth factor improves intestinal morphology by stimulating proliferation and differentiation of enterocytes and mTOR signaling pathway in weaning piglets. Sci China Life Sci. 2020;63:259-68. https://doi.org/10.1007/s11427-018-9519-6

101. Patra AK. Influence of plant bioactive compounds on intestinal epithelial barrier in poultry. Mini Rev Med Chem. 2020;20:566-77. https://doi.org/10.2174/1389557520666191226111405
102. Patra AK, Amasheh S, Aschenbach JR. Modulation of gastrointestinal barrier and nutrient transport function in farm animals by natural plant bioactive compounds: a comprehensive review. Crit Rev Food Sci Nutr. 2019;59:3237-66. https://doi.org/10.1080/10408398.2018.1486284

103. De Maio A. Heat shock proteins: facts, thoughts, and dreams. Shock. 1999;11:1-12. https://doi.org/10.1097/00024382-199901000-00001

104. Ikwegbue PC, Masamba P, Oyinloye BE, Kappo AP. Roles of heat shock proteins in apoptosis, oxidative stress, human inflammatory diseases, and cancer. Pharmaceuticals. 2017;11:2. https://doi.org/10.3390/ph11010002

105. Xie J, Tang L, Lu L, Zhang L, Xi L, Liu HC, et al. Differential expression of heat shock transcription factors and heat shock proteins after acute and chronic heat stress in laying chickens (Gallus gallus). PLOS ONE. 2014;9:e102204. https://doi.org/10.1371/journal.pone.0102204

106. Zhang WW, Kong LN, Zhang XQ, Luo QB. Alteration of HSF3 and HSP70 mRNA expression in the tissues of two chicken breeds during acute heat stress. Genet Mol Res. 2014;13:9787-94. https://doi.org/10.4238/2014.November.27.6

107. Uerlings J, Song ZG, Hu XY, Wang SK, Lin H, Buyse J, et al. Heat exposure affects jejunal tight junction remodeling independently of adenosine monophosphate-activated protein kinase in 9-day-old broiler chicks. Poult Sci. 2018;97:3681-90. https://doi.org/10.3382/ps.2018.97.300229

108. Hao Y, Gu XH, Wang XL. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 1. intestinal structure and digestive function. Poult Sci. 2012;91:781-9. https://doi.org/10.3382/ps.2011-01627

109. Al Wakeel RA, Shukry M, Abdel Azeez A, Mahmoud S, Saad MF. Alleviation by gamma amino butyric acid supplementation of chronic heat stress-induced degenerative changes in jejenum in commercial broiler chickens. Stress. 2017;20:562-72. https://doi.org/10.1080/10253890.2017.1377177

110. Sun X, Zhang H, Sheikahmadi A, Wang Y, Jiao H, Lin H, et al. Effects of heat stress on the gene expression of nutrient transporters in the jejunum of broiler chickens (Gallus gallus domesticus). Int J Biometeorol. 2015;59:127-35. https://doi.org/10.1007/s00484-014-0829-1

111. Lambert GP. Role of gastrointestinal permeability in exertional heatstroke. Exerc Sport Sci Rev. 2004;32:185-90. https://doi.org/10.1097/00003677-200410000-00011

112. Hai L, Rong D, Zhang ZY. The effect of thermal environment on the digestion of broilers. J Anim Physiol Anim Nutr. 2000;83:57-64. https://doi.org/10.1046/j.1439-0396.2000.00223.x

113. Schreck R, Albermann K, Baueerle PA. Nuclear factor kB: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). Free Radic Res Commun. 1992;17:221-37. https://doi.org/10.3109/10715769209079515

114. Li-Weber M, Krammer PH. Regulation of IL4 gene expression by T cells and therapeutic perspectives. Nat Rev Immunol. 2003;3:534-43. https://doi.org/10.1038/nri1128

115. Alagawany M, Farag MR, EI-Hack MEA, Patra A. Heat stress: effects on productive and reproductive performance of quail. Worlds Poult Sci J. 2017;73:747-56. https://doi.org/10.1017/S0043933917000782

116. Ibtisham F, Nawab A, Niu Y, Wang Z, Wu J, Xiao M, et al. The effect of ginger powder and Chinese herbal medicine on production performance, serum metabolites and antioxidant status of laying hens under heat-stress condition. J Therm Biol. 2019;81:20-4. https://doi.org/10.1016/j.jtherbio.2019.02.002

117. Mack LA, Felver-Gant JN, Dennis RL, Cheng HW. Genetic variations alter production and
behavioral responses following heat stress in 2 strains of laying hens. Poult Sci. 2013;92:285-94. https://doi.org/10.3382/ps.2012-02589

118. Mignon-Graesteau S, Moreri U, Narcy A, Rousseau X, Rodenburg TB, Tixier-Boichard M, et al. Robustness to chronic heat stress in laying hens: a meta-analysis. Poult Sci. 2015;94:586-600. https://doi.org/10.3382/ps.pev028

119. Sahin N, Hayirli A, Orhan C, Tuzcu M, Komorowski JR, Sahin K. Effects of the supplemental chromium form on performance and metabolic profile in laying hens exposed to heat stress. Poult Sci. 2018;97:1298-305. https://doi.org/10.3382/ps.pev435

120. Baumgard LH, Rhoads RP. Effects of heat stress on postabsorptive metabolism and energetics. Annu Rev Anim Biosci. 2013;1:311-37. https://doi.org/10.1146/annurev-animal-031412-103644

121. Johnson JS, Sapkota A, Lay DC. Rapid cooling after acute hyperthermia alters intestinal morphology and increases the systemic inflammatory response in pigs. J Appl Physiol. 2016;120:1249-59. https://doi.org/10.1152/japplphysiol.00685.2015

122. Lovatto PA, Sauvant D, Noblet J, Dubois S, van Milgen J. Effects of feed restriction and subsequent refeeding on energy utilization in growing pigs. J Anim Sci. 2006;84:3329-36. https://doi.org/10.2527/jas.2006-048

123. Puchala R, Patra AK, Animut G, Sahlu T, Goetsch AL. Effects of feed restriction and re-alimentation on mohair fiber growth and tissue gain by growing Angora goats. Livest Sci. 2011;138:180-6. https://doi.org/10.1016/j.livsci.2010.12.022

124. Zhang M, Zou XT, Li H, Dong XY, Zhao W. Effect of dietary γ-aminobutyric acid on laying performance, egg quality, immune activity and endocrine hormone in heat-stressed Roman hens. Anim Sci J. 2012;83:141-7. https://doi.org/10.1111/j.1740-0929.2011.00939.x

125. Osman AM, Tanios NI. The effect of heat on the intestinal and pancreatic levels of amylase and maltase of laying hens and broilers. Comp Biochem Physiol A Physiol. 1983;75:563-7. https://doi.org/10.1016/0300-9629(83)90421-8

126. Mahesh MS, Mohanta RK, Patra AK. Probiotics in livestock and poultry nutrition and health. In: Goel G, Kumar A, editors. Advances in probiotics for sustainable food and medicine. Microorg Sustain. 2021:149-79. https://doi.org/10.1007/978-981-15-6795-7_7

127. Ashraf S, Zaneb H, Yousaf MS, Ljaz A, Sohail MU, Muti S, et al. Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. J Anim Physiol Anim Nutr. 2013;97:68-73. https://doi.org/10.1016/j.jpn.2012.12.041

128. Chen Z, Tang J, Sun YQ, Xie J. Protective effect of γ-aminobutyric acid on antioxidation function in intestinal mucosa of Wenchang chicken induced by heat stress. J Anim Plant Sci. 2013;23:1634-41.

129. Song J, Jiao LF, Xiao K, Luan ZS, Hu CH, Shi B, et al. Cello-oligosaccharide ameliorates heat stress-induced impairment of intestinal microflora, morphology and barrier integrity in broilers. Anim Feed Sci Technol. 2013;185:175-81. https://doi.org/10.1016/j.anifeeds-ci.2013.08.001

130. Al-Fataftah AR, Abdelqader A. Effects of dietary Bacillus subtilis on heat-stressed broilers performance, intestinal morphology and microflora composition. Anim Feed Sci Technol. 2014;198:279-85. https://doi.org/10.1016/j.anifeeds-c.2014.02.012

131. Hajati H, Hassanabadi A, Gollan AG, Nassiri-Moghaddam H, Nassiri MR. The effect of grape seed extract and vitamin C feed supplements on carcass characteristics, gut morphology and ileal microflora in broiler chickens exposed to chronic heat stress. Iranian J Appl Anim Sci. 2015;5:155-65.
132. Humam AM, Loh TC, Foo HL, Samsudin AA, Mustapha NM, Zulkifli I, et al. Effects of feeding different postbiotics produced by Lactobacillus plantarum on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. Animals. 2019;9:644. https://doi.org/10.3390/ani9090644

133. Patra AK. An overview of antimicrobial properties of different classes of phytochemicals. In: Patra AK, editor. Dietary phytochemicals and microbes. Dordrecht, Netherlands: Springer; 2012. p. 1–32.

134. Patra A. Interactions of plant bioactives with nutrient transport systems in gut of livestock. Indian J Anim Health. 2018;57:125-36. https://doi.org/10.36062/ijaah.57.2.2018.125-36

135. Hasheimi SR, Zulkifli I, Somchit MN, Zunita Z, Loh TC, Soleimani, et al. Dietary supplementation of Zingiber officinale and Zingiber zerumbet to heat-stressed broiler chickens and its effect on heat shock protein 70 expression, blood parameters and body temperature. J Anim Physiol Anim Nutr. 2013;97:632-8. https://doi.org/10.1111/j.1439-0396.2012.01302.x

136. Tuzcu M, Sahin N, Karatepe M, Cikim G, Kilinc U, Sahin K. Epigallocatechin-3-gallate supplementation can improve antioxidant status in stressed quail. Br Poult Sci. 2008;49:643-8. https://doi.org/10.1080/00071660802298336

137. Onderci M, Sahin K, Sahin N, Gursu MF, Doerge D, Sarkar FH, et al. The effect of genistein supplementation on performance and antioxidant status of Japanese quail under heat stress. Arch Anim Nutr. 2004;58:463-71. https://doi.org/10.1080/00039420400020017

138. Bello AU, Sulaiman JA, Aliyu MS. Acute phase protein mRNA expressions and enhancement of antioxidant defense system in black-meated Silkie fowls supplemented with clove (Eugenia caryophyllus) extracts under the influence of chronic heat stress. J Anim Sci Technol. 2016;58:39. https://doi.org/10.1186/s40781-016-0122-4

139. Jimoh OA, Ayedun ES, Oyelade WA, Oloruntola OD, Daramola OT, Ayodele SO, et al. Protective effect of soursop (Annona muricata Linn.) juice on oxidative stress in heat stressed rabbits. J Anim Sci Technol. 2018;60:28. https://doi.org/10.1186/s40781-018-0186-4

140. Xie Y, Chen Z, Wang D, Chen G, Sun X, He Q, et al. Effects of fermented herbal tea residues on the intestinal microbiota characteristics of holstein heifers under heat stress. Front Microbiol. 2020;11:1014.https://doi.org/10.3389/fmicb.2020.01014

141. Habibi R, Sadeghi GH, Karimi A. Effect of different concentrations of ginger root powder and its essential oil on growth performance, serum metabolites and antioxidant status in broiler chicks under heat stress. Br Poult Sci. 2014;55:228-37. https://doi.org/10.1080/00071660.2014.887830

142. Habibian M, Sadeghi G, Ghazi S, Moeini MM. Selenium as a feed supplement for heat-stressed poultry: a review. Biol Trace Elem Res. 2015;165:183-93. https://doi.org/10.1007/s12011-015-0275-x

143. Chauhan SS, Celi P, Leury BJ, Clarke IJ, Clarke IJ, Dunshea FR. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. J Anim Sci. 2014;92:3364-74. https://doi.org/10.2527/jas.2014-7714

144. Zhu YW, Lu L, Li WX, Zhang LY, Ji C, Lin X, et al. Effect of dietary manganese on antioxidant status and expression levels of heat-shock proteins and factors in tissues of laying broiler breeders under normal and high environmental temperatures. Br J Nutr. 2015;114:1965-74. https://doi.org/10.1017/S0007114515003803

145. Liao X, Li W, Zhu Y, Zhang L, Lu L, Lin X, et al. Effects of environmental temperature and dietary zinc on egg production performance, egg quality and antioxidant status and expression of heat-shock proteins in tissues of broiler breeders. Br J Nutr. 2018;120:3-12. https://
146. Zhu Y, Liao X, Lu L, Li W, Zhang L, Ji C, et al. Maternal dietary zinc supplementation enhances the epigenetic-activated antioxidant ability of chick embryos from maternal normal and high temperatures. Oncotarget. 2017;8:19814-24. https://doi.org/10.18632/oncotarget.15057

147. Zhu Y, Lu L, Liao X, Li W, Zhang L, Ji C, et al. Maternal dietary manganese protects chick embryos against maternal heat stress via epigenetic-activated antioxidant and anti-apoptotic abilities. Oncotarget. 2017;8:89665-80. https://doi.org/10.18632/oncotarget.20804

148. Ghazi Sh, Habibian M, Moeini MM, Abdolmohammadi AR. Effects of different levels of organic and inorganic chromium on growth performance and immunocompetence of broilers under heat stress. Biol Trace Elem Res. 2012;146:309-17. https://doi.org/10.1007/s12011-011-9260-1

149. Sahin N, Tuzcu M, Onderci M, Eroksuz Y, Sahin K. The effects of vitamin C and E supplementation on heat shock protein 70 response of ovary and brain in heat-stressed quail. Br Poult Sci. 2009;50:259-65. https://doi.org/10.1080/00071660902758981