CONSEQUENCES OF HEAT STRESS ON BROILERS EXPERIMENTALLY CHALLENGED WITH EIMERIA MAXIMA OOCYSTS AND TREATED WITH MONENSIN OR NICARBAZIN

by

EDUARDO ORTEGA

(Under the Direction of Samuel E. Aggrey)

ABSTRACT

Heat stress has been shown to both reduce *Eimeria acervulina* oocyst output and to cause nicarbazin-induced heat stress (HS) toxicity. Here, we examined the effect constant chronic HS has on *Eimeria maxima* oocyst production. It was concluded that in broilers subjected to constant chronic HS, the production of *E. maxima* oocysts is significantly reduced. mRNA expression experiments suggest that exposing nicarbazin-fed broilers to constant chronic HS likely leads to oxidative stress. This may induce a protective response by suppressing appetite, increasing antioxidant capacity, increasing protection against hepatic lipogenesis, inhibiting ROS production and inhibiting intestinal motility. This may explain why HS-acclimated broilers are less susceptible to nicarbazin-induced HS toxicity when compared non-acclimated birds. Furthermore, mRNA expression results also suggest that constant chronic HS improves antioxidant capacity, reduces ROS production, and reduces motility of parasitized epithelial cells. These changes may influence *E maxima* multiplication, reducing *Eimeria* oocyst output.

INDEX WORDS: Nicarbazin, Heat Stress, Coccidiosis, *Eimeria maxima*

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CHAPTER 1

INTRODUCTION

The global production of chicken meat has increased between the years 2000 and 2012 at an average rate of about 3 percent, with an astounding 62 billion chickens being processed for meat in the year 2012 alone (FAOSTAT, 2017). This consistent increase in poultry meat production has been evident since the early 1960s. One of the major factors that are hindering production efficiency worldwide is coccidiosis. Coccidiosis is believed to be responsible for a global annual economic loss of about $3 billion USD per year, resulting from the costs of treatment and prevention as well as losses in production (Blake and Tomley, 2014). One of the most effective and widely used anticoccidials in the industry is nicarbazin (Nicarb®), which has been effective in preventing and/or suppressing coccidiosis since the drug was introduced (Cuckler et al., 1956). The molecular mechanisms by which nicarbazin acts against *Eimeria* remain unknown (Chapman, 1994). Another widely used anticoccidials is monensin (Shumard and Callender, 1967; Chapman et al., 2010).

Under heat stress (HS) conditions, nicarbazin has been associated with lower growth, productivity, and survivability and has therefore been suggested to induce HS toxicity and mortality (Farny, 1965; Sammelwitz, 1965a,b; Buys and Rasmussen, 1978; McDougald and McQuistion, 1980; Wiernusz and Teeter, 1995). Because of this, the use of nicarbazin is usually limited to the cooler months of the year. However, upon exposure to HS, nicarbazin-fed broilers that are acclimated to HS have an a lower susceptibility to

HS toxicity when compared to broilers not acclimated to HS (Wiernusz and Teeter, 1995).

HS is another factor which is also problematic for the productivity of the poultry industry, leading to economic losses and to concerns for animal welfare (Lara and Rostagno, 2013). This is especially important in broilers since genetic selection has produced broilers that are more susceptible to HS (Soleimani et al., 2011). HS has been attributed to economic losses ranging from $128 to165 million (St-Pierre et al., 2003), although, when broilers subjected to HS are infected with *Eimeria acervulina* (Reid et al., 1976; Banfield et al., 1998) or *E. tenella* (Anderson et al., 1976; Reid et al., 1976), a decrease in the severity of coccidiosis has been reported. However, it is yet to be examined if such effect occurs with E. maxima infections. Also, the mechanisms responsible for this phenomenon, at the molecular and genetic levels, have yet to be examined.

The first objective of this study was to examine the effect constant chronic HS has on performance, oocyst shedding, and cloacal temperature in broilers experimentally infected with *E. maxima* oocysts and treated with nicarbazin or monensin. The second objective of this study was is to identify the relationship between nicarbazin and heat shock toxicity through the expression of the heat shock response- and energy homeostasis-related genes. The relationship between HS and reduced oocyst production through the expression of heat shock response- and energy homeostasis-related genes was also investigated.

References

Anderson, W. I., Reid, W. M., and Johnson, J. K. Effects of high environmental temperatures on cecal coccidiosis. Poultry Science 55:1429-1435. 1976.

Banfield, M. J., Ten Doeschate, R. A., and Forbes, J. M. Effect of whole wheat and heat stress on a coccidial infection in broiler chickens. British Poultry Science 39 Suppl:S25-S26. 1998.

Blake, D. P., and Tomley, F. M. Securing poultry production from the ever-present Eimeria challenge. Trends in Parasitology 30:12-19. 2014.

Buys, S. B., and Rasmussen, R. W. Heat stress mortality in nicarbazine fed chickens. Journal of the South African Veterinary Association 49:127-128. 1978.

Chapman, H. D. A review of the biological activity of the anticoccidial drug nicarbazin and its application for the control of coccidiosis in poultry. Poultry Science Reviews (United Kingdom) 5. 1994.

Chapman, H. D., Jeffers, T. K., and Williams, R. B. Forty years of monensin for the control of coccidiosis in poultry. Poultry Science 89:1788-1801. 2010.

Cuckler, A. C., Malanga, C. M., and Ott, W. H. The antiparasitic activity of nicarbazin. Poultry Science 35:98-109. 1956.

FAOSTAT. Livestock primary. In. FAOSTAT, Rome, Italy. 2017.

Farny, D. G. O. Heat stress studies in nicarbazin treated chickens. In: Dissertation Abstracts. pp 7413-7413. 1965.

Lara, L. J., and Rostagno, M. H. Impact of heat stress on poultry production. Animals (Basel) 3:356-369. 2013.

McDougald, L. R., and McQuistion, T. E. Mortality from heat stress in broiler chickens influenced by anticoccidial drugs. Poultry Science 59:2421-2423. 1980.

Reid, W. M., Hines, T. K., Johnson, J., and Stino, K. R. Coccidiosis: effects of high environmental temperatures on anticoccidial protection. Poultry Science 55:1436-1441. 1976.

Sammelwitz, P. H. Factors related to nicarbazin induced heat stress mortality in broilers. Poultry Science 44:1412. 1965a.

Sammelwitz, P. H. Heat stress mortality in broilers. Poultry Science 44:1412. 1965b.

Shumard, R. F., and Callender, M. E. Monensin, a new biologically active compound. VI. Anticoccidial activity. Antimicrob Agents Chemother (Bethesda) 7:369-377. 1967.

Soleimani, A., Zulkifli, I., Omar, A., and Raha, A. Physiological responses of 3 chicken breeds to acute heat stress. Poultry Science 90:1435-1440. 2011.

St-Pierre, N. R., Cobanov, B., and Schnitkey, G. Economic losses from heat stress by US livestock industries. Journal of Dairy Science 86:E52-E77. 2003.

Wiernusz, C. J., and Teeter, R. G. Nicarbazin effects on broiler thermobalance during high ambient temperature stress. Poultry Science 74:577-580. 1995.

CHAPTER 2

LITERATURE REVIEW

Coccidiosis

Chickens (*Gallus gallus domesticus*) are the sole natural hosts to the following seven *Eimeria* species: *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. praecox*, *E. necatrix*, and *E. tenella*. Of these species, *E. acervulina*, *E. maxima*, and *E. tenella* are cause most of the problems related to coccidiosis while it is believed that other species contribute to subclinical coccidiosis (Cervantes, 2008). Following the oral ingestion of an infectious oocyst, termed a sporulated oocyst, the oocyst wall is crushed by the grinding mechanisms of the gizzard, exposing the stieda body to bile salts and pancreatic enzymes, resulting the excystation of the sporozoites (Long, 1981). Each of the four sporocysts then proceed into the upper small intestine where their outer walls are digested by pancreatic secretions, releasing the two sporocysts within each cell. The released sporozoites then enter the cells in the mucosa where 2 to 4 asexual phases take place, followed by a sexual phase and the release of microgametes which, target and merge with macrogametes to form a zygote (McDougald and Fitz-Coy, 2013). The zygote eventually matures into an oocyst that is then shed through the feces and into the environment. This *Eimeria* lifecycle results in intestinal tissue damage that leads to impaired absorption of nutrients, loss of blood, decreased skin pigmentation, and increased susceptibility to other disease-causing agents (McDougald and Fitz-Coy, 2013). Coccidiosis is present wherever chickens are raised and estimated to cost the industry more than $3 billion dollars

worldwide in preventative treatment and production losses (Blake and Tomley, 2014). Most young poultry receive continuous, preventative treatment given that by the time coccidiosis is detected, most of the losses have already occurred (McDougald and Fitz-Coy, 2013).

Prepatent Period and Sporulation

The lifecycle of Eimeria takes 4 to 6 days, depending on the species (McDougald and Fitz-Coy, 2013). The time it takes for sporulation of *E. acervulina*, *E. maxima*, *E. tenella* is 17, 30, and 18 hours. The minimum prepatent period for *E. acervulina*, *E. maxima*, and *E. tenella* is 97, 121, and 115 hours, respectively. With *E. maxima*, the first two asexual phases cause most of the tissue damage in the superficial layer of the epithelium and the sexual stages develop in the deeper tissues from 5 to 8 days post infection (McDougald and Fitz-Coy, 2013). With *E. tenella*, the 2nd generation schizonts begin to mature in the denuded epithelium and result in damage of the of the mucosa and muscularis layer. On days 6 to 7 post infection, the oocysts are then released into the feces (McDougald and Fitz-Coy, 2013).

Elevated Environmental Temperatures Effect Coccidiosis

In coccidia-infected broilers subjected to heat stress, birds have exhibited less severe coccidiosis (Anderson et al., 1976; Reid et al., 1976; Banfield et al., 1998). Anderson et al. (1976) demonstrated that in *E. tenella*-infected broilers, a reduction in coccidiosis severity was observed in broilers subjected to heat stress, as indicated by gross lesion scores, fecal scores, and hematocrit readings. Based on lesion scores and

mortality data, the author also suggested the first asexual generation of *E. tenella* may be the stage most sensitive to elevated environmental temperatures. Reid et al. (1976) also observed less severe coccidiosis in broilers subjected to heat stress in experiments involving either *E. tenella* or *E. acervulina*, as determined by gross lesion scores. Later, a similar observation was made by Banfield et al. (1998) *E. acervulina*-infected broilers as determined by a reduced oocyst output. Since these studies were last published, there has been a lack of work examining the effect heat stress has on coccidiosis. Also, there is yet to be work examining the effect of heat stress on *E. maxima* infections. This is unexpected considering *E. tenella*, *E. acervulina*, and *E. maxima* are the three the most problematic *Eimeria* species in the industry (Cervantes, 2008).

Anticoccidials

Anticoccidial drugs are used for the prevention and treatment of coccidiosis and can be classified as being either coccidiocidal (when the drug destroys the coccidia) or as coccidiostatic (when the drug prevents replication and growth) (McDougald and Fitz-Coy, 2013). Furthermore, anticoccidial drugs can be categorized as either *synthetic compounds*, *polyether antibiotics (*i.e. "*ionophores*"), or as *mixed products* (Peek and Landman, 2011). Ionophores are antibiotics produced through the fermentation of Streptomyces or Actinomadura species. Chemicals act against the metabolism of coccidia while ionophores interrupt the osmotic balance. Two commonly used anticoccidials are nicarbazin (NIC), and monensin (MON). NIC is a synthetic compound and is also referred to by its tradename, Nicarb®. MON is an ionophore and is referred to by its tradename, Coban®.

Monensin

Monensin is a monovalent ionophore that was suggested to have broad-spectrum activity against coccidia (Agtarap et al., 1967). Since its introduction in 1971, monensin has been used to effectively combat coccidiosis due to the slow development of resistance in the field and because of the drug's ability to prevent the development of clinical coccidiosis while allowing the development of immunity against *Eimeria* infections (Chapman et al., 2010). Monensin is also commonly used as a reference anticoccidial in studies comparing it to other anticoccidial agents or anticoccidial vaccines. Furthermore, there is no withdrawal period prior to slaughter in the United States when birds are treated solely with monensin.

Anticoccidial Activity of Monensin

Exposure to monensin stimulates influx of Na^+ ions, causing the activation of (Na^+-K^+)-ATPases that pump out Na^+ ions, resulting in excess extracellular Na^+ concentrations that leads to an excess influx of water into the sporozoites, after the limited energy reserves of the parasite are depleted, causing the parasite to burst (Smith and Galloway, 1983). Another explanation of monensin's mode of action is that monensin obstructs the localization of flotillin-1 within raft structures, resulting in sporozoites and merozoites losing the ability to invade host cells (Chapman et al., 2010).

Monensin and Broiler Performance

It is believed that monensin reduces feed intake during the warmer seasons, leading to monensin being used largely during the winter and spring (Chapman et al.,

2010). However, the author suggests that there is no evidence to support this. When birds subjected to heat stress are fed monensin (100 ppm), no differences were reported in growth, feed efficiency, or mortality when compared to unmedicated birds (McDougald and McQuistion, 1980). At 100 ppm, monensin has been shown to reduce fertility in broiler breeders while having no effect on egg production, egg weight, or hatch (Chapman et al., 2010). However, at the highest approved dietary concentration of 121 ppm monensin has been known to depress growth (Cervantes et al., 1982)

Nicarbazin

NIC is a synthetic drug that was introduced into the industry in 1955 and is a compound consisting of an equimolar complex of 4,4’-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP) (Cuckler et al., 1955). NIC demonstrates effective, broad-spectrum protection against coccidia, providing a useful chemical for preventing or suppressing avian coccidiosis (Cuckler et al., 1956). Among the anticoccidial compounds introduced for the prevention of coccidiosis in broilers, there is yet to be one with a span of sustained efficacy in the field that rivals that of NIC’s (Chapman, 1994). NIC is commonly used against *Eimeria* at a level of 125 ppm (Chapman, 1994). This level provides a highly effective anticoccidial which bears a minimal potential for drug resistance (Bafundo et al., 2008).

When NIC is administered to birds subject to heat stress (HS), reductions in body weight, feed efficiency, and survivability have been reported. Consequently, NIC has been suggested to lead to HS toxicity and mortality (Wiernusz and Teeter, 1995). This is why NIC is generally not used during the warmer seasons or in regions with a hot climate

(Chapman, 1994). When NIC is fed at high levels, DNC, the active component of NIC, has been shown to accumulate in the liver at 10-fold higher concentrations when compared to those of muscle (Cuckler et al., 1956).

Anticoccidial Activity of Nicarbazin

Dinitrocarbanilide (DNC) is the active component of nicarbazin (NIC) (Cuckler et al., 1955). DNC has shown to inhibit succinate-linked NAD+ reduction in beef mitochondria and, in the presence of ATP, to also inhibit energy-dependent transhydrogenase and the accumulation of Ca2+ in rat liver mitochondria, leading to the suggestion that DNC likely affects the energy transduction chain of mitochondria (Wang, 1978). When DNC was administered to chicken erythrocytes, Wang (1978) reported a considerable leakage of K+ ions from the cells which also lacked a tightly coupled influx of H+. DNC is mostly insoluble in water although, with the addition of bovine serum albumin, DNC was shown to be solubilized (Wang, 1978). Consequently, the author suggested DNC may be capable of binding to uncoupling proteins and uncoupling mitochondria. Importantly, when complexed, the potency of DNC increases at least tenfold (Cuckler et al., 1955). Later, Ryley and Wilson (1976) investigated the effect NIC has on *E. tenella*. The investigator showed NIC suppresses the development of 1st generation merozoites and also causes the abnormal, degenerate formation of 2nd generation merozoites. Therefore, Ryley and Wilson (1976) suggested that the obstructed development of the 2nd generation merozoites results from the inhibition of the 1st asexual cycle of the coccidia.

Nicarbazin and Broiler Performance

An increase in the susceptibility to heat stress toxicity has been reported in NIC-fed broilers and shown at levels as low as 30 ppm (Sammelwitz, 1965b,a). Although, when NIC (125 ppm) is removed from feed 4 days before exposure to HS (30°C to 40°C for 6 hours), NIC-induced susceptibility to HS toxicity can be reversed and become completely nonexistent (Sammelwitz, 1965a). This effect of NIC on survivability under HS conditions was later confirmed by Farny (1965), who also observed an increase in mortality. Also, when NIC is fed at levels exceeding the recommended level of 125 ppm, NIC negatively effects growth (Newberne and Buck, 1957; Mushett et al., 1958). Farny (1965) also demonstrated that, at 125 ppm, NIC treatment causes an increase in the general metabolic rate of 6 and 8-week broilers (Farny, 1965).

At the recommended level of 125 ppm, it has been demonstrated that NIC reduces the performance of broilers (McDougald and McQuistion, 1980; Keshavarz and McDougald, 1981; Bartov, 1989). Because Bartov (1989), used diets with low levels of energy and protein, the author concluded that these experimental factors likely caused an increased intake of NIC which may have magnified the effect NIC had on performance. However, Bartov (1989) did not control for environmental temperature as neither did the other studies (McDougald and McQuistion, 1980; Keshavarz and McDougald, 1981). As a result, this prompted Chapman (1994) to suggest there was a lack of convincing evidence supporting the claim that NIC has suppressive effects on performance at 125 ppm.

However, more recently, da Costa et al. (2017) reported that under standard temperatures and in the absence of coccidial challenge, NIC reduces broiler performance

throughout the 28-day growth phase. However, in the presence of coccidial challenge, the author also reported NIC improves broiler performance, irrespective of environmental temperature setting. Importantly, da Costa et al. (2017) reported that under lower environmental temperatures, the performance-suppressing effects of NIC were mitigated. Therefore, the author suggests that under standard growing conditions, the effects of NIC on broiler performance are dependent on both environmental temperature and coccidial infection.

Heat Stress

Another important factor that affects the poultry industry on a worldwide basis is heat stress (HS), which has a significant impact on production and on animal welfare (Lara and Rostagno, 2013). Economic losses from HS are estimated to range from $128 to 165 million (St-Pierre et al., 2003). HS is a physiological and behavioral consequence of an animal's inability to dissipate more heat into the surrounding environment than the amount produced metabolically (Akbarian et al., 2016). Susceptibility to heat stress is more evident in broilers since they are heavy meat-type birds that have been genetically bred to be fast-growing, resulting in broilers more susceptible to HS rather than more resistant (Soleimani et al., 2011).

The Heat Shock Response (HSR)

Heat-induced puffing patterns in chromosomes of *Drosophila* salivary glands were observed by Ritossa (1962). Later, it was determined that these heat shock puffs corresponded to the production of small proteins termed, heat shock proteins (HSPs).

Heat shock proteins are grouped on the basis of molecular weight via electrophoresis on sodium dodecyl gels (Tissiéres et al., 1974). Induction of these HSPs is a characteristic of the heat shock response (HSR) (Morimoto, 2011). From yeast to humans, the HSR is a highly conserved molecular response activated by extreme proteotoxic stresses such as elevated temperatures, oxidative stress, heavy metals, toxins, and bacterial infections (Åkerfelt et al., 2010).

The HSR is mediated by heat shock transcription factors called, heat shock factors (HSFs). HSFs are DNA binding proteins that can bind to multiple copies of the heat shock element (HSE), a highly conserved sequence motif that is present in the promotor regions of HS genes (Tanabe et al., 1997). The HSF-HSE binding activity mediates the transcription of the heat shock genes to increase the synthesis of heat-shock proteins (HSPs), a hallmark of stressed cells, which limit the misfolding and aggregation of proteins through chaperone activity in an effort to maintain protein homeostasis (proteostasis) (Åkerfelt et al., 2010). Given this conservation, heat shock response is suggested as being essential for the survival of organisms subjected to a stressful environment (Åkerfelt et al., 2010). Chicken HSF1, HSF2, and HSF3 were isolated by Nakai et al. (1997) through a mouse HSF1 cDNA probe via cross-hybridization. Fujimoto et al. (2010) later identified HSF4 in the chicken genome.

HSF1

Chicken HSF1 is capable of protecting cells under conditions of thermal stress by regulating other heat-inducible genes called ‘nonclassical heat shock genes’ which, are crucial for protecting cells against heat shock (Fujimoto and Nakai, 2010). For example,

these nonclassical heat shock genes controlled by HSF1 include promenin-2 (encodes for a basal epithelial cell membrane glycoprotein) and PDZK3 (allows cells to overcome the accumulation of proteins through degradation) (Fujimoto and Nakai, 2010; Nakai, 2016). The ability of chicken HSF1 to uniquely regulate only these genes demonstrates chicken HSF1 is crucial for the regulation of nonclassical heat shock genes (Fujimoto and Nakai, 2010).

HSF2

Chicken HSF2 is reported to be a major regulator of proteostasis capacity since it was shown that upon mild heat shock in the physiological range, HSF2 suppresses the accumulation of misfolded proteins (Shinkawa et al., 2011). Consequently, vertebrate HSF2 was suggested to be involved in resistance to mild heat shock and other proteotoxic stress. The investigator also showed HSF2 reduces the activation threshold for chicken HSF3 which results in an increased expression of HSP genes during mild heat shock (Shinkawa et al., 2011). Shinkawa et al. (2011) suggested chicken HSF2 is required for protection against prolonged exposure to heat shock, regardless of HSP expression.

HSF3

In chickens, HSF3, and not HSF1, is the master regulator in cells and is necessary for thermotolerance which involves the expression of classical heat shock genes (Fujimoto and Nakai, 2010). Tanabe et al. (1997) suggests that upon severe stress, chicken HSF3 is involved in the persistent and burst activation of stress genes while chicken HSF1 may be involved in the rapid response to mild and severe stress. Nakai

(1999) also found that HSP-3 was activated by the same stressors that activate HSF1. However, the level of HSF3 activation was consistently lower with each stressor than that of HSF1, demonstrating that HSF3 may be more adapted to extreme stress in comparison. In cells lacking HSF3 and subjected to heat shock, the expression of HSP110, HSP90α, HSP90β, and HSP40 was substantially uninduced (Tanabe et al., 1998). The investigator also reported that under normal conditions, the constitutive expression of HSP110, HSP90α and HSP90β genes were decreased in the absence of HSF3. Consequently, Tanabe et al. (1998) suggested that HSF3 has a significant role in heat shock-induced, basal, and constitutive expression of HSP genes.

HSF4

The last member of the HSF family is HSF4. is ubiquitous among cell types and is important for the expression of heat shock genes and the induction of non-classical genes (Fujimoto et al., 2010). When the organism is heat stressed, Nakai et al. (1997) found that the human HSF4 (hHSF4) was tissue-specific expressed in the heart, skeletal muscle and to a lesser extent, the brain. Furthermore, hHSF4 was shown to lack the HR-C, which is proposed to negatively regulate DNA binding activity for HSF1 and HSF3 (Nakai et al., 1997; Tanabe et al., 1999). HSF4 was shown to suppress the basal expression of heat shock genes, such as HSP90 and HSP70, and was suggested to possibly result from HSE being bound by HSF4 which may produce inhibitory effects on the initiation complex or during elongation (Nakai et al., 1997). HSF4 is expressed in a tissue-specific manner and is important for suppressing the expression of HSP genes.

HSF DNA-Binding activity

Upon heat shock, the DNA-binding activity of HSF1 is acquired by the oligomerization of an inert monomer to a DNA-binding (active) trimer and is translocated to the nucleus (Nakai et al., 1995). In unstressed cells, HSF2 is present both in the cytoplasm and nucleus and has also been suggested to accumulate in the nucleus upon heat shock (Shinkawa et al., 2011). This group also reported that chicken HSF2 is present as a DNA-binding trimer and as a small dimer but upon heat shock, chicken HSF2 is present mostly as a DNA-binding trimer. For chicken HSF3, DNA-binding activity is acquired by the oligomerization of a non-DNA-binding dimer to a DNA-binding trimer (Nakai et al., 1995). They also reported that the heat shock-induced DNA-binding activity of HSP3 was regulated in a cell-type-specific manner and was also delayed when compared to that of HSF1 (Nakai et al., 1995). In unstressed cells, HSF4 remains a DNA-binding trimer in the nucleus and unlike other HSFs, HSF4 lacks the HR-C (carboxyl-terminal heptad repeat) domain, as previously mentioned, which suppresses HSF trimer formation (Nakai et al., 1997; Tanabe et al., 1999). Instead, human HSF4 had a variant heptad repeat which was referred to as DHR (downstream of heptad repeat) (Nakai et al., 1997). Merath et al. (2013) later reported HSF4 has two activator domains and one repression domain, with one of the activator domains being present in the DHR region.

Heat Shock Proteins (HSPs)

HSPs are an important group of molecular chaperones which, based on their molecular weight (kD) class, are commonly classified into the following major families: HSP100/110 ($\geq$ 100 kD), HSP90 (83-90 kD), HSP70 (70 kD), HSP60 or chaperonins

(close to 60 kD), HSP40 (40 kD), and the small HSPs (10-30 kD) (Evgen′ev et al., 2014). Under stress conditions, HSPs interact with affected proteins to properly stabilize their secondary structures to retain their functionality, thereby significantly contributing to cell integrity (Akbarian et al., 2016). Depending on the family, HSPs vary in their functions and expression.

HSP60

HSP60 is highly evolutionarily conserved among distant organisms (van Eden and van der Zee, 2016). HSP60 chaperones exist in mitochondria in single and double ring conformations which provide a central cavity for folding of the enclosed protein (Böttinger et al., 2015). The heptameric ring of HSP10, a co-chaperone of HSP60, forms the lid of the cavity. Upon entry of the non-native substrates into the chamber, which is accommodated in the central cavity of the chaperonin, the subsequent folding processes, associated with the major conformational changes of the central cavity are driven by ATP-hydrolysis (Brandvold and Morimoto, 2015). The survival of cancer cells is associated with HSP60 activity (Cappello et al., 2008), while its overexpression is correlated with evasion of apoptosis via the down-regulation of a p53-promoted pathway (Ghosh et al., 2008). Under standard conditions, HSP60 deficient fibroblasts appear morphologically abnormal and upon heat stress, the structural integrity of the mitochondria declined even more (Huckriede et al., 1995). Furthermore, the author suggested the abnormal appearance of HSP60-deficient cells was likely a result of impaired mitochondrial metabolism while upon heat shock, the increased susceptibility to stress was possibly a direct consequence of HSP60 deficiency.

In broilers, an increase in the expression of HSP60, HSP70, and HSP90 was observed in the heart after 2 hours of HS (Yu et al., 2008). The author suggests that of three upregulated HSPs in the heart, HSP60 is especially crucial for the survival of myocardial cells due to its major role in preserving mitochondrial integrity, function, and capacity for ATP generation. In chicken embryos subjected to thermal manipulation (39°C for 9-18 h), Al-Zghoul et al. (2015) observed an increase in the expression of HSP60, HSP90 and HSF1 in the muscle, heart and brain. Changes in the expression of these genes were suggested to contribute to the chicks acquiring an improved thermotolerance.

HSP70

HSP70 chaperones are inducible under conditions of stress, during which they provide an essential role in preventing aggregation of misfolded proteins and assist in refolding them (Bukau and Horwich, 1998). However, they are also crucial for cell function under normal conditions by helping fold some of the recently translated proteins, directing translocating proteins across organelle membranes via activity at the *cis* and *trans* regions, disassembling oligomeric protein structures, facilitating proteolytic degradation of unsteady proteins and in some cases, they also regulate the biological activity of folded regulatory proteins such as transcription factors (Bukau and Horwich, 1998). These actions depend on the ATP-mediated association of HSP70 with short hydrophobic sections of substrate polypeptides which, can be shielded to further hinder additional folding or aggregation (Bukau and Horwich, 1998). Similarly without ATP, bacterial HSP70 can spontaneously bind misfolding polypeptides and moderately prevent

their aggregation (Finka et al., 2016). HSP70 is also considered to have a protective mechanism by inhibiting the expression of pro-inflammatory cytokines (Yoo et al., 2000; Stocki and Dickinson, 2012).

In broilers subjected to acute HS (35°C for 5 h), Gabriel et al. (1996) observed an amount increase in the amount of HSP70 in the liver at each hour of HS from 1 to 5 h. The investigator also observed an increase in the expression levels of HSP70 which peaked at 3 h of exposure to HS. Gabriel et al. (1996) suggested HS increases the expression and protein levels of HSP70 in the broiler liver which, similar to mammals, occurred through time-dependent mechanisms. In broilers subjected to acute HS, the increase expression of HSP70 is suggested to protect the mucosa from injury by increasing antioxidant capacity by increasing activity of SOD and GSH-Px and to also alleviate oxidative injury by inhibiting lipid peroxidation (Gu et al., 2012). Surai and Kochish (2017) suggest that the observed increases in HSP70 expression in the chicken tissues is crucial for the protective responses that allows chickens to prevent or control the detrimental changes in the structure or functions of proteins resulting from a variety of stressors.

HSP90

The HSP90 family is among the most abundant cellular proteins, and under normal cell growth conditions, about two percent of the cellular proteome is comprised of HSP90 (Brandvold and Morimoto, 2015). Most HSP90 molecules are composed of the following three domains: the N-terminal domain (executes ATP hydrolysis), central domain (provides the interactions with protein substrates), and a C-terminal domain

(required for dimerization) (Evgen′ev et al., 2014). Additionally, HSP90 demonstrates a central chaperone role in the folding, activation, and transportation of numerous regulatory proteins involved in signal transduction pathways such as the normal function of cell cycle. Unlike other molecular chaperones, most of the HSP90 family proteins have chaperoning functions which provide stability for various unstable signal transducers to stabilize them for activation, demonstrating HSP90 is an important factor in cellular signal transduction during normal conditions and following stress (Evgen′ev et al., 2014).

In the heart, liver, and kidney of broilers subjected to acute heat stress, Lei et al. (2009) observed a significantly elevated expression of HSP90 after 2 hours of HS. However, slightly after 3 and 5 hours, the author observed a decrease in the expression of HSP90. Lei et al. (2009) suggested this increase in HSP90 expression, in the initial phase of HS, enhanced the survivability of cells in unfavorable environments. The investigator also reported HSP90 was also found in the endothelial cells of vascular tissues. Furthermore, there was a correlation between HSP90 localization and vasoconstriction which suggested HSP90 expression likely impacts the blood supply of some important organs by vasoconstriction and vessel relaxation in order to protect against organ function exhaustion (Lei et al., 2009). Moreover, Zhang et al. (2018) suggests the HSP90 transcription requires the stable expression of both HSF2 and HSF3 while that of HSF4 was nonessential.

SOD and CAT

Heat stress has been acknowledged as an inducer of oxidative stress, which refers to a severe imbalance between the presence of reactive oxygen species (ROS) and the

available antioxidant capacity of cells (Halliwell and Whiteman, 2004). These highly reactive molecules can modify a variety of biological molecules, and contribute to the development of several metabolic dysfunctions, including cell death by causing "oxidative stress" and "oxidative damage" (Halliwell and Whiteman, 2004). Oxidized molecules can draw electrons from other molecules and result in a chain reaction that can cause serious tissue injury if not controlled (Akbarian et al., 2016). Additionally, oxidative damage may modify the redox equilibrium of several cellular redox couples and can alter the expression of crucial enzymes involved in detoxification, antioxidant defense, cell transitions, and inflammatory responses, among others (Akbarian et al., 2016). Therefore, cells have evolved protective defense systems to protect against the threat of ROS, where the first line of defense consists of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) (Ray and Husain, 2002). SOD catalyzes the dismutation of peroxide radicals to form hydrogen peroxide and oxygen while CAT catalyzes the breakdown of the hydrogen peroxide to water and molecular oxygen (Halliwell, 2006).

Modification of these enzymes (i.e., SOD and CAT) can alter the balance between the production of ROS and the antioxidant system (Azad et al., 2010b). Under conditions of acute HS, the activity of antioxidant enzymes increases in the serum, liver and muscle of broiler chickens (Azad et al., 2010b; Tan et al., 2010). On the other hand, inconsistent observations have been reported with regard to chronic HS. Two forms of SOD were found in the chicken liver (Weisiger and Fridovich, 1973b). One of the SODs was found primarily in the mitochondrial matrix, and identified as Mn-SOD while the other one was found in the intermembrane space and identified as Cu,Zn-SOD (Weisiger and Fridovich,

1973a). Since Mn-SOD is an inducible enzyme, an elevated activity of SOD in tissues could signify an adaptive response to a stressor or demonstrate the capacity of the antioxidant defense under stress conditions (Surai, 2015). The author also suggests that the adaptive responses of SOD may be overwhelmed if stress is too high and result in a decreased SOD activity and lead to the activation of apoptosis.

Energy Homeostasis-Related Genes

Uncoupling Proteins

A significant portion of an animal's resting metabolic rate is attributed to proton leakage (uncoupling) across the inner mitochondrial membrane, illustrating a possible mechanism for energy dissipation or heat production (Dridi et al., 2004). Following the mitochondrial oxidation of fuels, the protons pumped out by the electron transport chain (ETC) generate an electrochemical potential (Dridi et al., 2004). These protons then reenter via F0/F1 ATP synthase to generate ATP from ADP and inorganic phosphate (Pi). However, if the inward proton flow occurs separate of F0/F1 ATP synthase, then this proton leak (uncoupling) will result in heat production rather than ATP synthesis. (Dridi et al., 2004). This proton leak removes excess protons, thereby reducing the production of ROS. In mammals, most of the heat production occurs in brown adipose tissue (BAT) through uncoupling protein 1 (UCP-1), which is located in the inner mitochondrial membrane (Klingenberg and Huang, 1999).

Since the discovery of UCP-1, other closely related UCPs have been discovered such as UCP-2 (Fleury et al., 1997), which is 59% identical to UCP-1. The homologue UCP-3 was also discovered by multiple groups (Boss et al., 1997; Vidal-Puig et al., 1997)

and was found to be 57% identical to UCP-1 and 73% identical to UCP-2. Several years later, Raimbault et al. (2001) identified the avian UCP (avUCP) homologue from chicken skeletal muscle and found it to be 55, 70 and 70% identical with mammalian UCP-1, UCP-2, and UCP-3, respectively.

In humans and rodents, expression of UCP-2 demonstrates an almost ubiquitous pattern (Fleury and Sanchis, 1999; Rousset et al., 2003). On the other hand, the expression of UCP-3 in humans and rodents is generally localized to the BAT, muscle, and heart (Boss et al., 1997). However, in chickens, the expression of avUCP was reported to be exclusive to the muscle (Raimbault et al., 2001). Expression of these uncoupling proteins can be influenced by various factors such as fasting, refeeding, and environmental temperature (Dridi et al., 2004; Mujahid et al., 2007; Dridi et al., 2008; Azad et al., 2010a). For example, in mammalian BAT, fasting decreases the expression of UCP-1 and UCP-3 while refeeding has the opposite effect by increasing the expression of UCP-1 and UCP-3 (Dridi et al., 2004). Unlike mammalian UCP-1 and UCP-3, fasting increases muscle avUCP expression while refeeding decreases the expression of avUCP (Dridi et al., 2004). The expression of muscle avUCP is up-regulated in cold-acclimated chickens (Toyomizu et al., 2002). The author suggested avUCP may be involved in heat production via fatty acid-induced oxidative phosphorylation in the mitochondria of muscle. Furthermore, the exposure of broilers to acute heat stress (34°C for 6, 12 and 18 hours) was reported to reduce muscle avUCP expression and protein content in a time-dependent manner and was suggested to cause the overproduction of mitochondrial ROS and oxidative damage (Mujahid et al., 2007). In contrast, chronic heat stress has been reported to increase the expression of muscle avUCP in broilers (Dridi et al., 2008; Azad

et al., 2010a). Azad et al. (2010a) suggested that a reduction in substrate oxidation, mitochondrial membrane potential, and an elevated expression of muscle avUCP may have led to the increase in muscle concentrations of malondialdehyde (the marker for lipid peroxidation in skeletal muscle). This suggests that the type of heat treatment applied likely determines how avUCP expression will be affected. Therefore, the increased expression of muscle avUCP during chronic heat likely illustrates the broiler's physiological response aimed at maintaining energy homeostasis with the goal of becoming acclimated to the stressor.

Cholecystokinin (CCK)

Another gene affected by heat stress is cholecystokinin (CCK) which, through the activation of the medullary vagal center, is capable of inducing several physiological actions such as pancreatic enzyme secretion, bile secretion and emptying, inhibiting gastric secretion and emptying, inhibiting feed intake, and inducing gastroprotective activities against various irritants (Konturek et al., 2004). CCK also functions as a satiety signal to the brainstem to depress appetite (Richards and Proszkowiec-Weglarz, 2007). It is suggested that under acute heat stress, decreased peripheral and central CCK expression allows intestinal enzymes more time for nutrient digestion due to the lowered passage rate resulting from decreased intestinal mobility and consequently, lower passage rate (Song et al., 2012).

In layers, heat stress (31°C, 7 d) has been demonstrated to significantly decrease the expression of CCK in the duodenum, jejunum, and the hypothalamus tissues while expression in the ileum was not significantly impacted (Song et al., 2012). In broilers

subjected to acute heat stress (35°C, 6 h), the expression of CCK significantly decreased in the duodenum while no significant change in CCK expression was observed for ileum, jejunum, or hypothalamus (Lei et al., 2013). More recently, broilers subjected to chronic heat stress (32°, 14 d) had elevated CCK expressions in duodenum (42 d), jejunum (35 d), and ileum (42 d) tissues (He et al., 2018). Consequently, the reduced feed intake observed under heat stress was attributed to the elevated secretion of anorexigenic hormones, increased expression of appetite-related genes, and the impairment of intestinal integrity (He et al., 2018).

Glucagon-like Peptide 1 (GLP-1)

The amount of work conducted in chickens, regarding GLP-1, is very limited though it has been extensively studied in rodents. GLP-1 is an incretin hormone produce mainly by enteroendocrine L cells in the distal intestine following nutrient digestion (Oh and Jun, 2017). Through its receptor, GLP-1R, GLP-1 affects blood glucose levels to inhibit glucagon secretion, inhibit gastric emptying, suppress feed intake, and to stimulate insulin secretion (Oh and Jun, 2017). GLP-1 is suggested to modulate gut homeostasis by local actions in order to restore gut integrity and may therefore be an early signal that promotes organ protection following disruption of the gut barrier (Lebrun et al., 2017). This suggests that in the small intestine, GLP-1 may provide protection and promote normal barrier function via its anti-inflammatory effects. The anti-inflammatory effects of GLP-1 have also been observed in various organs including the brain, liver, pancreas, kidney, testis, skin, vascular system, and lungs (Oh and Jun, 2017).

In response to various stress factors, it has been demonstrated that GLP-1 or GLP-1R agonists also reduce oxidative stress markers such as SOD and CAT (Oh and Jun, 2017). GLP-1 and GLP-2 were found to also protect against irradiation-induced oxidative damage in rat ileum and colon by alleviating irradiation-induced lipid peroxidation and apoptosis (Deniz et al., 2015). The author suggested the protective roles stemmed from inhibiting neutrophil infiltration and activation of inflammation mediators which induce lipid peroxidation. Therefore, these results suggest GLP-1 also has antioxidant and anti-inflammatory effects which promote normal organ function. It was also suggested that GLP-1 therapies may alleviate fatty liver disease by reducing inflammation in humans and rodents (Lee and Jun, 2016).

In chickens, GLP-1 is secreted by L cells located in the epithelium of crypts and lower part of intestinal villi (Nishimura et al., 2013). GLP-1 has also been demonstrated to suppress feed intake in chickens following intracerebroventricular (ICV) administration (Furuse et al., 1997). In the chicken intestine, GLP-1-immunoreactive cells were found to be distributed only in the jejunum and ileum while they were rarely found in the duodenum (Hiramatsu et al., 2003). When compared to the jejunum, the frequency of GLP-1-immunoreactive cells was significantly higher in the ileum where, they were mostly observed in the lower part of villi and crypts (Hiramatsu et al., 2003; Hiramatsu et al., 2005). Monir et al. (2013) examined the effect restricted feeding has on GLP-1-containing cells. The investigators reported that the occurrence of GLP-1-immunnoreactive cells in the ileum was highest for the 25% feed supply group, medium for the 50% feed supply group, and highest for the control group. This indicates that in the ileum, less GLP-1 was secreted under conditions of reduced feed supply as indicated

by the increased retention of GLP-1 within the cytoplasm of L cells. Monir et al. (2014) later investigated the effect dietary crude protein (CP) level has on the occurrence of GLP-1-containing cells in the chicken ileum and found that as dietary CP level decreased, so did the frequency of GLP-1-containing cells which meant that less GLP-1 was being secreted given more was retained within L cells.

Ghrelin

In rats, ghrelin is involved in regulating fasted motor activity of the gastrointestinal tract (Fujino et al., 2003). In chickens, ghrelin is expressed across various tissue including the liver and the small intestine (Richards et al., 2006). Ghrelin is produced in the proventriculus of chickens and is able to modulate feeding behavior (Richards and Proszkowiec-Weglarz, 2007). Ghrelin was also recognized as being a strong pituitary release factor for growth hormone via the growth hormone sectretagogue receptor (GHS-R) and an inhibitor of feed intake in chickens. In layers, acute heat stress has been demonstrated to significantly increase the expression of ghrelin in the glandular stomach, jejunum, and the hypothalamus tissues while no significant effect was observed in the hypothalamus, or the duodenum (Song et al., 2012). In broilers subjected to acute heat stress (35°C, 6 h), the expression of ghrelin was also significantly increased in the granular stomach, jejunum and duodenum while no significant change in ghrelin expression was observed in the ileum or hypothalamus (Lei et al., 2013). Overall, ghrelin is recognized as a stimulator of growth hormone (GH) and corticosterone (CORT) release, an inhibitor of feed intake following ICV administration, and a promoter of gastrointestinal contraction (Kaiya et al., 2013).

GHS-R1a is recognized as the active receptor for ghrelin in birds and following ligand binding, GHS-R1a is also suggested to regulate ghrelin activity by increasing intracellular Ca^2 (Kaiya et al., 2013). In chickens, ghrelin-mediated contractile activity is gradually increased from the duodenum to the crop and again from the duodenum to the colon. Importantly, it is suggested that gastrointestinal motility is regulated through GHS-R1a since the distribution of GHS-R1a is consistent with contractile activity and also because GHRP-6, an antagonist of GHS-R1a, inhibits ghrelin-induced intestinal contraction (Kitazawa et al., 2007). Additionally, the GH released in response to ghrelin may in turn participate in the negative feedback regulation of the ghrelin-induced GH response (Kaiya et al., 2007).

NUCB2/nesfatin-1

In comparison to the above-mentioned genes, nesfatin-1 has been less studied in avian species though it has been extensively studied in rodents. Nesfatin-1 is a secreted peptide and cleavage product of the N-terminal segment of nucleobindin 2 (NUCB2) however, the secretion and/or existence of the other two putative cleavage products (nesfatin-2 and nesfatin-3) in vivo remains unknown. Oh-I et al. (2006) demonstrated that nesfatin-1 was able to dose-dependently reduce food intake in rodents following ICV injections. This inhibition in feed behavior was confirmed by other investigators (Stengel et al., 2009; Atsuchi et al., 2010; Yosten and Samson, 2010; Wernecke et al., 2014). Additionally, nesfatin-1 was found to decrease water intake (Yosten and Samson, 2010; Könczöl et al., 2012). Nesfatin-1 administration was first found to increase body temperature by Könczöl et al. (2012). This effect on thermogenesis was later

corroborated through calorimetry, a gold standard method of quantifying energy expenditure by directly measuring dry heat loss (Wernecke et al., 2014). In the study, nesfatin-1 increased thermogenesis as illustrated by a significant increase in dry heat loss (+20%). In quail, ICV and peripheral (intraperitoneal) administration of mouse nesfatin-1 also decreased food intake and increased body temperature (Shousha et al., 2015). This demonstrated that nestain-1 plays a role in anorectic and catabolic signaling, confirming that in avian species, nesfatin-1 is also involved in the regulation of energy expenditure.

Nesfatin-1 has been demonstrated to reduce gastric emptying (GE) following ICV administration in both mice and rats (Stengel et al., 2009; Goebel-Stengel et al., 2011). In mice, Atsuchi et al. (2010) demonstrated that ICV administered nesfatin-1 also inhibits gastrointestinal motility. More recently, ICV administration of nesfatin-1 dose-dependently decreased both GE and gastric motility in rats and was suggested to result from the activity of paraventricular nucleus neurons (Guo et al., 2015).

References

Agtarap, A., Chamberlin, J. W., Pinkerton, M., and Steinrauf, L. K. Structure of monensic acid, a new biologically active compound. Journal of the American Chemical Society 89:5737-5739. 1967.

Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A., and Smet, S. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. Journal of Animal Science and Biotechnology 7:37. 2016.

Åkerfelt, M., Morimoto, R. I., and Sistonen, L. Heat shock factors: integrators of cell stress, development and lifespan. Nature Reviews Molecular Cell Biology 11:545-555. 2010.

Al-Zghoul, M.-B., Ismail, Z. B., Dalab, A. E. S., Al-Ramadan, A., Althnaian, T. A., Al-ramadan, S. Y., Ali, A. M., Albokhadaim, I. F., Al Busadah, K. A., Eljarah, A., Jawasreh, K. I., and Hannon, K. M. Hsp90, Hsp60 and HSF-1 genes expression in muscle, heart and brain of thermally manipulated broiler chicken. Research in Veterinary Science 99:105-111. 2015.

Anderson, W. I., Reid, W. M., and Johnson, J. K. Effects of high environmental temperatures on cecal coccidiosis. Poultry Science 55:1429-1435. 1976.

Atsuchi, K., Asakawa, A., Ushikai, M., Ataka, K., Tsai, M., Koyama, K., Sato, Y., Kato, I., Fujimiya, M., and Inui, A. Centrally administered nesfatin-1 inhibits feeding behaviour and gastroduodenal motility in mice. Neuroreport 21:1008-1011. 2010.

Azad, M., Kikusato, M., Maekawa, T., Shirakawa, H., and Toyomizu, M. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 155:401-406. 2010a.

Azad, M. A. K., Kikusato, M., Sudo, S., Amo, T., and Toyomizu, M. Time course of ROS production in skeletal muscle mitochondria from chronic heat-exposed broiler chicken. Comparative Biochemistry and Physiology, Part A 157:266-271. 2010b.

Bafundo, K. W., Cervantes, H. M., and Mathis, G. F. Sensitivity of *Eimeria* field isolates in the United States: responses of nicarbazin-containing anticoccidials. Poultry Science 87:1760-1767. 2008.

Banfield, M. J., Ten Doeschate, R. A., and Forbes, J. M. Effect of whole wheat and heat stress on a coccidial infection in broiler chickens. British Poultry Science 39 Suppl:S25-S26. 1998.

Bartov, I. Lack of effect of dietary factors on nicarbazin toxicity in broiler chicks. Poultry Science 68:145-152. 1989.

Blake, D. P., and Tomley, F. M. Securing poultry production from the ever-present Eimeria challenge. Trends in Parasitology 30:12-19. 2014.

Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P., and Giacobino, J.-P. Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. FEBS Letters 408:39-42. 1997.

Böttinger, L., Oeljeklaus, S., Guiard, B., Rospert, S., Warscheid, B., and Becker, T. Mitochondrial heat shock protein (Hsp) 70 and Hsp10 cooperate in the formation of Hsp60 complexes. Journal of Biological Chemistry 290:11611-11622. 2015.

Brandvold, K. R., and Morimoto, R. I. The chemical biology of molecular chaperones—implications for modulation of proteostasis. Journal of Molecular Biology 427:2931-2947. 2015.

Bukau, B., and Horwich, A. L. The Hsp70 and Hsp60 chaperone machines. Cell 92:351-366. 1998.

Cappello, F., Conway de Macario, E., Marasà, L., Zummo, G., and Macario, A. J. Hsp60 expression, new locations, functions, and perspectives for cancer diagnosis and therapy. Cancer Biology & Therapy 7:801-809. 2008.

Cervantes, H. Coccidiosis control. In: Broiler Supervisor's Short Course. N.C. 2008.

Cervantes, H. M., Jensen, L. S., and Brenes, A. Moderation of monensin-induced growth depression by dietary potassium. Poultry Science 61:1107-1112. 1982.

Chapman, H. D. A review of the biological activity of the anticoccidial drug nicarbazin and its application for the control of coccidiosis in poultry. Poultry Science Reviews (United Kingdom) 5. 1994.

Chapman, H. D., Jeffers, T. K., and Williams, R. B. Forty years of monensin for the control of coccidiosis in poultry. Poultry Science 89:1788-1801. 2010.

Cuckler, A. C., Malanga, C. M., Basso, A. J., and O'Neill, R. C. Antiparasitic activity of substituted carbanilide complexes. Science 122:244-245. 1955.

Cuckler, A. C., Malanga, C. M., and Ott, W. H. The antiparasitic activity of nicarbazin. Poultry Science 35:98-109. 1956.

da Costa, M. J., Bafundo, K. W., Pesti, G. M., Kimminau, E. A., and Cervantes, H. M. Performance and anticoccidial effects of nicarbazin-fed broilers reared at standard or reduced environmental temperatures. Poultry Science 96:1615-1622. 2017.

Deniz, M., Atasoy, B. M., Dane, F., Can, G., Erzik, C., Çetinel, Ş., and Yeğen, B. Ç. Radiation-induced oxidative injury of the ileum and colon is alleviated by glucagon-like peptide-1 and -2. Journal of Radiation Research and Applied Sciences 8:234-242. 2015.

Dridi, S., Onagbesan, O., Swennen, Q., Buyse, J., Decuypere, E., and Taouis, M. Gene expression, tissue distribution and potential physiological role of uncoupling protein in avian species. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 139:273-283. 2004.

Dridi, S., Temim, S., Derouet, M., Tesseraud, S., and Taouis, M. Acute cold-and chronic heat-exposure upregulate hepatic leptin and muscle uncoupling protein (UCP) gene expression in broiler chickens. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 309:381-388. 2008.

Evgen′ev, M. B., Zatsepina, O. G., and Garbuz, D. G. Heat shock proteins and whole body adaptation to extreme environments. Dordrecht : Springer, 2014. 2014.

Farny, D. G. O. Heat stress studies in nicarbazin treated chickens. In: Dissertation Abstracts. pp 7413-7413. 1965.

Finka, A., Mattoo, R. U., and Goloubinoff, P. Experimental milestones in the discovery of molecular chaperones as polypeptide unfolding enzymes. Annual Review of Biochemistry 85:715-742. 2016.

Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., and Ricquier, D. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nature Genetics 15:269. 1997.

Fleury, C., and Sanchis, D. The mitochondrial uncoupling protein-2: current status. The International Journal of Biochemistry & Cell Biology 31:1261-1278. 1999.

Fujimoto, M., Hayashida, N., Katoh, T., Oshima, K., Shinkawa, T., Prakasam, R., Tan, K., Inouye, S., Takii, R., and Nakai, A. A novel mouse HSF3 has the potential to activate nonclassical heat-shock genes during heat shock. Molecular Biology of the Cell 21:106-116. 2010.

Fujimoto, M., and Nakai, A. The heat shock factor family and adaptation to proteotoxic stress. The FEBS journal 277:4112-4125. 2010.

Fujino, K., Inui, A., Asakawa, A., Kihara, N., Fujimura, M., and Fujimiya, M. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. The Journal of Physiology 550:227-240. 2003.

Furuse, M., Matsumoto, M., Okumura, J.-i., Sugahara, K., and Hasegawa, S. Intracerebroventricular injection of mammalian and chicken glucagon-like peptide-1 inhibits food intake of the neonatal chick. Brain Research 755:167-169. 1997.

Gabriel, J. E., Ferro, J. A., Stefani, R. M. P., Ferro, M. I. T., Gomes, S. L., and Macari, M. Effect of acute heat stress on heat shock protein 70 messenger RNA and on heat shock protein expression in the liver of broilers. British Poultry Science 37:443-449. 1996.

Ghosh, J. C., Dohi, T., Kang, B. H., and Altieri, D. C. Hsp60 regulation of tumor cell apoptosis. Journal of Biological Chemistry 283:5188-5194. 2008.

Goebel-Stengel, M., Wang, L., Stengel, A., and Taché, Y. Localization of nesfatin-1 neurons in the mouse brain and functional implication. Brain Research 1396:20-34. 2011.

Gu, X. H., Hao, Y., and Wang, X. L. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. Poultry Science 91:790-799. 2012.

Guo, F. f., Xu, L., Gao, S. l., Sun, X. r., Li, Z. l., and Gong, Y. l. The effects of nesfatin-1 in the paraventricular nucleus on gastric motility and its potential regulation by the lateral hypothalamic area in rats. Journal of Neurochemistry 132:266-275. 2015.

Halliwell, B. Reactive apecies and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiology 141:312. 2006.

Halliwell, B., and Whiteman, M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? British Journal of Pharmacology 142:231-255. 2004.

He, X., Lu, Z., Ma, B., Zhang, L., Li, J., Jiang, Y., Zhou, G., and Gao, F. Effects of chronic heat exposure on growth performance, intestinal epithelial histology, appetite-related hormones and genes expression in broilers. Journal of the Science of Food and Agriculture. 2018.

Hiramatsu, K., Yamasaki, A., and Karasawa, Y. Comparative study on the distribution of glucagon-like peptide-1 (GLP-1)-immunoreactive cells in the intestine of chicken and ostrich. The Journal of Poultry Science 40:39-44. 2003.

Hiramatsu, K., Yamasaki, A., and Shioji, T. Immunohistochemical and morphometrical studies on the distribution of glucagon-like peptide-1 (GLP-1)-immunoreactive cells in the chicken intestine. The Journal of Poultry Science 42:223-229. 2005.

Huckriede, A., Heikema, A., Agsteribbe, E., Sjollema, K., and Briones, P. Morphology of the mitochondria in heat shock protein 60 deficient fibroblasts from mitochondrial myopathy patients. Effects of stress conditions. Virchows Archive 427:159-165. 1995.

Kaiya, H., Darras, V. M., and Kangawa, K. Ghrelin in birds: its structure, distribution and function. The Journal of Poultry Science 44:1-18. 2007.

Kaiya, H., Kangawa, K., and Miyazato, M. Update on ghrelin biology in birds. General and Comparative Endocrinology 190:170-175. 2013.

Keshavarz, K., and McDougald, L. R. Influence of anticoccidial drugs on losses of broiler chickens from heat stress and coccidiosis. Poultry Science 60:2423-2428. 1981.

Kitazawa, T., Kaiya, H., and Taneike, T. Contractile effects of ghrelin-related peptides on the chicken gastrointestinal tract in vitro. Peptides 28:617-624. 2007.

Klingenberg, M., and Huang, S.-G. Structure and function of the uncoupling protein from brown adipose tissue. Biochimica et Biophysica Acta (BBA) - Biomembranes 1415:271-296. 1999.

Könczöl, K., Pintér, O., Ferenczi, S., Varga, J., Kovács, K., Palkovits, M., Zelena, D., and Tóth, Z. Nesfatin-1 exerts long-term effect on food intake and body temperature. International Journal of Obesity 36:1514. 2012.

Konturek, S., Konturek, P., Pawlik, T., and Brzozowski, T. Brain-gut axis and its role in the control of food intake. Journal of Physiology and Pharmacology 55:137-154. 2004.

Lara, L. J., and Rostagno, M. H. Impact of heat stress on poultry production. Animals (Basel) 3:356-369. 2013.

Lebrun, L. J., Lenaerts, K., Kiers, D., Pais de Barros, J.-P., Le Guern, N., Plesnik, J., Thomas, C., Bourgeois, T., Dejong, C. H. C., Kox, M., Hundscheid, I. H. R., Khan, N. A., Mandard, S., Deckert, V., Pickkers, P., Drucker, D. J., Lagrost, L., and Grober, J. Enteroendocrine L cells sense LPS after gut barrier injury to enhance GLP-1 secretion. Cell Reports 21:1160-1168. 2017.

Lee, Y.-S., and Jun, H.-S. Anti-inflammatory effects of GLP-1-based therapies beyond glucose control. Mediators of Inflammation 2016. 2016.

Lei, L., Hepeng, L., Xianlei, L., Hongchao, J., Hai, L., Sheikhahmadi, A., Yufeng, W., and Zhigang, S. Effects of acute heat stress on gene expression of brain-gut neuropeptides in broiler chickens (Gallus gallus domesticus). Journal of Animal Science 91:5194-5201. 2013.

Lei, L., Yu, J., and Bao, E. Expression of heat shock protein 90 (Hsp90) and transcription of its corresponding mRNA in broilers exposed to high temperature. British Poultry Science 50:504-511. 2009.

Long, P. L. The biology of the coccidia. Baltimore: University park press. 1981.

McDougald, L. R., and Fitz-Coy, S. H. Coccidiosis. In: Diseases of Poultry, 13 ed. D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez and V. Nair, eds. Wiley-Blackwell, Ames, Iowa. pp 1148-1166. 2013.

McDougald, L. R., and McQuistion, T. E. Mortality from heat stress in broiler chickens influenced by anticoccidial drugs. Poultry Science 59:2421-2423. 1980.

Merath, K., Ronchetti, A., and Sidjanin, D. J. Functional analysis of HSF4 mutations found in patients with autosomal recessive congenital cataracts functional analysis of HSF4 mutations. Investigative Ophthalmology & Visual Science 54:6646-6654. 2013.

Monir, M. M., Hiramatsu, K., Matsumoto, S., Nishimura, K., Takemoto, C., Shioji, T., Watanabe, T., Kita, K., Yonekura, S., and Roh, S. G. Influences of protein ingestion on glucagon-like peptide (GLP)-1-immunoreactive endocrine cells in the chicken ileum. Animal Science Journal 85:581-587. 2014.

Monir, M. M., Hiramatsu, K., Yamasaki, A., Nishimura, K., and Watanabe, T. The influence of restricted feeding on glucagon-like peptide-1 (GLP-1)-containing cells in the chicken small intestine. Anatomia, Histologia, Embryologia 43:153-158. 2013.

Morimoto, R. I. The heat shock response: systems biology of proteotoxic stress in aging and disease. In: Cold Spring Harbor Symposia on Quantitative Biology. Cold Spring Harbor Laboratory Press. pp 91-99. 2011.

Mujahid, A., Akiba, Y., Warden, C. H., and Toyomizu, M. Sequential changes in superoxide production, anion carriers and substrate oxidation in skeletal muscle mitochondria of heat-stressed chickens. FEBS Letters 581:3461-3467. 2007.

Mushett, C. W., Washko, F. V., Kelley, K. L., and Bloom, A. J. A hematologic-pathologic study in chickens fed graded levels of nicarbazin. Poultry Science 37:580. 1958.

Nakai, A. New aspects in the vertebrate heat shock factor system: Hsf3 and Hsf4. Cell Stress & Chaperones 4:86. 1999.

Nakai, A. Proteostasis and adaptation to high temperature stress. In: Heat Shock Factor. Springer. pp 3-29. 2016.

Nakai, A., Kawazoe, Y., Tanabe, M., Nagata, K., and Morimoto, R. I. The DNA-binding properties of two heat shock factors, HSF1 and HSF3, are induced in the avian erythroblast cell line HD6. Molecular and Cellular Biology 15:5268-5278. 1995.

Nakai, A., Tanabe, M., Kawazoe, Y., Inazawa, J., Morimoto, R. I., and Nagata, K. HSF4, a new member of the human heat shock factor family which lacks properties of a transcriptional activator. Molecular and Cellular Biology 17:469-481. 1997.

Newberne, P. M., and Buck, W. B. Studies on drug toxicity in chicks: 3. The influence of various levels of nicarbazin on growth and development of chicks. Poultry Science 36:304. 1957.

Nishimura, K., Hiramatsu, K., Monir, M. M., Takemoto, C., and Watanabe, T. Ultrastructural study on colocalization of glucagon-like peptide (GLP)-1 with GLP-2 in chicken intestinal L-cells. Journal of Veterinary Medical Science 75:1335-1339. 2013.

Oh, Y. S., and Jun, H.-S. Effects of glucagon-like peptide-1 on oxidative stress and Nrf2 signaling. International Journal of Molecular Sciences 19:26. 2017.

Oh-I, S., Shimizu, H., Satoh, T., Okada, S., Adachi, S., Inoue, K., Eguchi, H., Yamamoto, M., Imaki, T., and Hashimoto, K. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 443:709. 2006.

Peek, H. W., and Landman, W. J. M. Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. Veterinary Quarterly 31:143-161. 2011.

Raimbault, S., Dridi, S., Denjean, F., Lachuer, J., Couplan, E., Bouillaud, F., Bordas, A., Duchamp, C., Taouis, M., and Ricquier, D. An uncoupling protein homologue putatively involved in facultative muscle thermogenesis in birds. Biochemical Journal 353:441-444. 2001.

Ray, G., and Husain, S. A. Oxidants, antioxidants and carcinogenesis. Indian Journal of Experimental Biology 40:1213-1232. 2002.

Reid, W. M., Hines, T. K., Johnson, J., and Stino, K. R. Coccidiosis: effects of high environmental temperatures on anticoccidial protection. Poultry Science 55:1436-1441. 1976.

Richards, M. P., Poch, S. M., and McMurtry, J. P. Characterization of turkey and chicken ghrelin genes, and regulation of ghrelin and ghrelin receptor mRNA levels in broiler chickens. General and Comparative Endocrinology 145:298-310. 2006.

Richards, M. P., and Proszkowiec-Weglarz, M. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. Poultry Science 86:1478-1490. 2007.

Ritossa, F. A new puffing pattern induced by temperature shock and DNP in Drosophila. Cellular and Molecular Life Sciences 18:571-573. 1962.

Rousset, S., Alves-Guerra, M.-C., Ouadghiri-Bencherif, S., Kozak, L. P., Miroux, B., Richard, D., Bouillaud, F., Ricquier, D., and Cassard-Doulcier, A.-M. Uncoupling protein 2, but not uncoupling protein 1, is expressed in the female mouse reproductive tract. Journal of Biological Chemistry 278:45843-45847. 2003.

Ryley, J. F., and Wilson, R. G. Laboratory studies with some older anticoccidials. Parasitology 73:287-309. 1976.

Sammelwitz, P. H. Factors related to nicarbazin induced heat stress mortality in broilers. Poultry Science 44:1412. 1965a.

Sammelwitz, P. H. Heat stress mortality in broilers. Poultry Science 44:1412. 1965b.

Shinkawa, T., Tan, K., Fujimoto, M., Hayashida, N., Yamamoto, K., Takaki, E., Takii, R., Prakasam, R., Inouye, S., Mezger, V., and Nakai, A. Heat shock factor 2 is required for maintaining proteostasis against febrile-range thermal stress and polyglutamine aggregation. Molecular Biology of the Cell 22:3571-3583. 2011.

Shousha, S., Kirat, D., and Naso, T. Effect of central and peripheral Nesfatin-1 on food intake in Japanese quail. AASCIT Journal of Biology 1:1-9. 2015.

Smith, C. K., and Galloway, R. B. Influence of monensin on cation influx and glycolysis of *Eimeria tenella* sporozoites in vitro. The Journal of Parasitology 69:666-670. 1983.

Soleimani, A., Zulkifli, I., Omar, A., and Raha, A. Physiological responses of 3 chicken breeds to acute heat stress. Poultry Science 90:1435-1440. 2011.

Song, Z., Liu, L., Sheikhahmadi, A., Jiao, H., and Lin, H. Effect of heat exposure on gene expression of feed intake regulatory peptides in laying hens. Journal of Biomedicine and Biotechnology 2012. 2012.

St-Pierre, N. R., Cobanov, B., and Schnitkey, G. Economic losses from heat stress by US livestock industries. Journal of Dairy Science 86:E52-E77. 2003.

Stengel, A., Goebel, M., Wang, L., Rivier, J., Kobelt, P., Mönnikes, H., Lambrecht, N. W., and Tache, Y. Central nesfatin-1 reduces dark-phase food intake and gastric emptying in rats: differential role of corticotropin-releasing factor2 receptor. Endocrinology 150:4911-4919. 2009.

Stocki, P., and Dickinson, A. M. The immunosuppressive activity of heat shock protein 70. Autoimmune diseases 2012. 2012.

Surai, P. F. Antioxidant systems in poultry biology: superoxide dismutase. Journal of Animal Research and Nutrition 1. 2015.

Surai, P. F., and Kochish, I. I. Antioxidant systems and vitagenes in poultry biology: Heat shock proteins. In: Heat Shock Proteins in Veterinary Medicine and Sciences. Springer. pp 123-177. 2017.

Tan, G. Y., Yang, L., Fu, Y. Q., Feng, J. H., and Zhang, M. H. Effects of different acute high ambient temperatures on function of hepatic mitochondrial respiration, antioxidative enzymes, and oxidative injury in broiler chickens. Poultry Science 89:115-122. 2010.

Tanabe, M., Kawazoe, Y., Takeda, S., Morimoto, R. I., Nagata, K., and Nakai, A. Disruption of the HSF3 gene results in the severe reduction of heat shock gene expression and loss of thermotolerance. The EMBO Journal 17:1750-1758. 1998.

Tanabe, M., Nakai, A., Kawazoe, Y., and Nagata, K. Different thresholds in the responses of two heat shock transcription factors, HSF1 and HSF3. Journal of Biological Chemistry 272:15389-15395. 1997.

Tanabe, M., Sasai, N., Nagata, K., Liu, X.-D., Liu, P. C., Thiele, D. J., and Nakai, A. The mammalian HSF4 gene generates both an activator and a repressor of heat shock genes by alternative splicing. Journal of Biological Chemistry 274:27845-27856. 1999.

Tissiéres, A., Mitchell, H. K., and Tracy, U. M. Protein synthesis in salivary glands of Drosophila melanogaster: Relation to chromosome puffs. Journal of Molecular Biology 84:389-398. 1974.

Toyomizu, M., Ueda, M., Sato, S., Seki, Y., Sato, K., and Akiba, Y. Cold-induced mitochondrial uncoupling and expression of chicken UCP and ANT mRNA in chicken skeletal muscle. FEBS Letters 529:313-318. 2002.

van Eden, W., and van der Zee, R. Heat shock proteins. In: Compendium of Inflammatory Diseases. M. J. Parnham, ed. Springer Basel, Basel. pp 569-575. 2016.

Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J. S., and Lowell, B. B. UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. Biochemical and Biophysical Research Communications 235:79-82. 1997.

Wang, C. C. Biochemical and nutritional aspects of coccidia. Avian Coccidiosis:135-184. 1978.

Weisiger, R. A., and Fridovich, I. Mitochondrial superoxide simutase. Site of synthesis and intramitochondrial localization. Journal of Biological Chemistry 248:4793-4796. 1973a.

Weisiger, R. A., and Fridovich, I. Superoxide dismutase. Organelle specificity. Journal of Biological Chemistry 248:3582-3592. 1973b.

Wernecke, K., Lamprecht, I., Jöhren, O., Lehnert, H., and Schulz, C. Nesfatin-1 increases energy expenditure and reduces food intake in rats. Obesity 22:1662-1668. 2014.

Wiernusz, C. J., and Teeter, R. G. Nicarbazin effects on broiler thermobalance during high ambient temperature stress. Poultry Science 74:577-580. 1995.

Yoo, C.-G., Lee, S., Lee, C.-T., Kim, Y. W., Han, S. K., and Shim, Y.-S. Anti-inflammatory effect of heat shock protein induction is related to stabilization of IκBα through preventing IκB kinase activation in respiratory epithelial cells. The Journal of Immunology 164:5416-5423. 2000.

Yosten, G. L., and Samson, W. K. The anorexigenic and hypertensive effects of nesfatin-1 are reversed by pretreatment with an oxytocin receptor antagonist. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 298:R1642-R1647. 2010.

Yu, J., Bao, E., Yan, J., and Lei, L. Expression and localization of Hsps in the heart and blood vessel of heat-stressed broilers. Cell Stress & Chaperones:327. 2008.

Zhang, X., Zhang, M., Su, Y., Wang, Z., Zhao, Q., Zhu, H., Qian, Z., Xu, J., Tang, S., Wu, D., Lin, Y., Kemper, N., Hartung, J., and Bao, E. Inhibition of heat stress-related apoptosis of chicken myocardial cells through inducing Hsp90 expression by aspirin administration in vivo. British Poultry Science 59:308-317. 2018.

CHAPTER 3

EFFECTS OF CHRONIC HEAT STRESS ON PERFORMANCE, CLOACAL TEMPERATURE, AND OOCYSTS SHEDDING IN BROILERS EXPERIMENTALLY INFECTED WITH EIMERIA MAXIMA AND TREATED WITH MONENSIN OR NICARBAZIN

Overview

The effects of heat-stress (HS) and *Eimeria* challenge and coccidiostats on the growth performance of broiler chickens were investigated. The study consisted of a 2×2×3 factorial design experiment with two temperature settings (27°C or 34°C), two levels of *Eimeria* challenge (non-challenged or challenged with 2.5 x 10^4 *Eimeria maxima*) and three anticoccidial treatments (untreated, nicarbazin at 125ppm, or monensin at 100ppm). At hatch, 480 Cobb500 chicks were assigned equally to 12 treatments. There were 5 replicates per treatment and 8 birds per replicate. Treatment started when the birds were 14 days-of-age (d) and lasted for 2 weeks. Cloacal temperature and body weight were measured in individual birds and feed intake was measured per pen at 15, 21 and 28 d. Fecal oocysts counts were done using two collection composites consisting of droppings collected from 19-21 and 25-28 d. Irrespective of *Eimeria*-challenge, growth and feed intake were significantly reduced in birds subjected to heat stress. Cloacal temperature was 0.18°C higher in infected birds than non-infected birds. In the thermoneutral environment, oocyst were present in infected birds while none were detected in non-infected birds. Oocyst counts for the MON treatment were about

1.5-fold higher than NIC at 27°C. No oocysts were detected in infected birds raised at 34°C regardless of coccidiostat treatment. Data from this experiment shows that at 27°C, NIC does not significantly depress growth and protects birds against coccidial infection. Importantly, there also appears to be an association between elevated ambient temperatures and obstruction of *E. maxima* oocyst development in coccidial-challenged broilers.

Introduction

The chicken is a natural host to seven species of *Eimeria* protozoan parasites which cause intestinal damage that dissrupts feeding and digestive processes or the absorption of nutrients, dehydration, loss of blood and skin pigmentation. Coccidiosis can make chickens more susceptible to other disease agents (McDougald and Fitz-Coy, 2013). Coccidiosis is a massive economic burden for the poultry industry with global economic losses of about $3 billion (Blake and Tomley, 2014). Another factor which is also an economic burden for the poultry industry is heat stress (HS) which has been attributed to economic losses ranging from $128 to165 million (St-Pierre et al., 2003).

To control coccidiosis, anticoccidials are commonly supplied continuously to young birds as a preventative treatment since it was realized that most of the losses already occur by the time signs of coccidiosis are evident (McDougald and Fitz-Coy, 2013). Nicarbazin (NIC) is one of the most effective and widely used anticoccidials since its introduction in the 1950s and is classified as a ‘chemical’ anticoccidial (Cuckler et al., 1955). In the field, NIC has been effective due to the low potential for the development of drug resistance (Bafundo and Jeffers, 1990; Chapman, 1994). However, NIC has been

reported to increase heat stress (HS)-related mortalities in commercial (Buys and Rasmussen, 1978) and research (Farny, 1965; Sammelwitz, 1965a,b; McDougald and McQuistion, 1980; Wiernusz and Teeter, 1995) settings. Because of this, the use of NIC is tends to be limited to cooler seasons.

Moreover, in the presence of coccidial infection, chickens subjected to elevated environmental temperatures have been reported as having less severe coccidiosis in studies involving *Eimeria acervulina* (Reid et al., 1976; Banfield et al., 1998) and *E. tenella* (Anderson et al., 1976; Reid et al., 1976). Little additional work has been done on the effect heat stress on coccidiosis. Also, none of the previously mentioned studies examined *E. maxima* infections.

The objective of this study is to examine the effect constant chronic HS has on the oocyst shedding, cloacal temperature, and performance of *E. maxima*-infected broilers. The effect constant chronic HS has on the cloacal temperature and performance of nicarbazin-fed broilers is also examined.

Materials and Methods

Design and Animals

Research conducted using live broiler chickens was in accordance with the guidelines approved by the institutional animal care and use committee of the University of Georgia. Male Cobb500 chicks were raised from hatch until 13 d of age under standard husbandry practices. At 14 d of age, 480 chicks of similar body weight were divided equally into 12 treatment groups, each with 5 replicates (*n*=40), and each replicate having 8 birds. A 2×2×3 experimental design was employed, with two temperature settings

(27°C or 34°C), two levels of coccidiosis (0 or 2.5×10^4 sporulated *Eimeria maxima* oocysts), and three levels of feed regimens (untreated; nicarbazin, 125 ppm; or monensin, 100 ppm).

Anticoccidial Treatments

From hatch, all broilers were fed standard broiler starter mashup from hatch to 13 d of age. From 14 to 28 d of age, broilers were then assigned to either of the anticoccidial treatments which consisted of unmedicated; monensin, 100 ppm; and nicarbazin, 125 ppm. Drug premixes were mixed with a standard grower diet (Southern Poultry, Athens, GA).

Temperature Treatments

Two environmentally controlled rooms were used to expose birds to a constant temperature of 27°C (TN) or 34°C (HS) for the duration of the study (14 to 28 d of age). The daily temperature and relative humidity were monitored daily over the duration of the study. The average temperature for the TN and HS environments were 27°C and 34°C, respectively. The average humidity for the TN and HS environments were 68% and 47%, respectively.

Coccidial Assay and Challenge

Six groups of birds in each room were inoculated with either 0 or 2.5×10^4 sporulated *Eimeria maxima* oocysts at 14 d. Three groups within each level of inoculation were assigned to either the untreated, nicarbazin (125 ppm), or monensin (100 ppm) feed

regimens. The coccidia inoculate consisted of 25,000 sporulated *E. maxima* oocysts, which were administered to individual birds through oral inoculation using an electronic pipette. Two composite fecal samples were collected from each battery cage. The first composite consisted of droppings collected between 19 to 21 d while the second composite consisted of droppings collected between 25 to 28 d. The droppings were analyzed by McMaster technique using saturated Na Cl solution. The oocyst counts are presented in oocysts per gram of fecal material.

Performance and Cloacal Temperature Measurements

Feed consumption and individual body weights were measured at 14, 15, 21, and 28 d of age. Individual cloacal temperatures were taken using a medical thermometer at 14, 15, 21, and 28 d of age.

Statistical Analysis

The data was analyzed statistically using the general linear models procedure, followed by Tukey's multiple comparisons test (version 9.4, SAS Institute Inc., Cary, NC). A *P*-value of less than 5% is assumed to be statistically significant.

Results

Cloacal Temperature

The cloacal temperature results are presented in Table 3.1. Birds reared at 34°C had a significantly elevated cloacal temperature at 15, 21, and 28 d of age when compared to groups reared at 27°C. Moreover, birds receiving NIC had a significantly

reduced (*P* < 0.05) cloacal temperature at 15 d of age when compared to MON and unmedicated groups. At 21 d of age, NIC significantly (*P* < 0.0001) increased cloacal temperature when compared to MON and unmedicated. At 15 d of age, the coccidial challenge significantly (*P* = 0.008) elevated the cloacal temperature of broilers when compared to the non-challenged groups, irrespective of environmental temperature or anticoccidial treatments.

A significant temperature x challenge interaction was recorded for cloacal temperature at 15, 21, and 28 d of age (*P* = 0.002, *P* = 0.006, and *P* = 0.009, respectively). This interaction indicates that at measurement period, irrespective of anticoccidial treatment, the coccidial infection tended to increase and decrease cloacal temperatures of birds raised at 27°C and 34°C, respectively. A significant temperature x medication interaction was also recorded for cloacal temperature at 15 d of age (P=0.012). This interaction indicates that in the 34°C environment, NIC-fed birds had significantly lower cloacal temperatures at 15 d of age, however this was not the observed results for the NIC group raised in the 27°C environment, regardless of the presence of coccidial infection. A significant challenge x medication interaction was recorded for cloacal temperature at day 15 (*P* = 0.044). This interaction indicates that, irrespective of the environmental temperature, NIC-fed birds had significantly lower cloacal temperatures at 15 d of age when compared to those of the MON and unmedicated groups.

Performance Parameters

The BWG, FCR, and feed intake (FI) results are presented in Table 3.2, Table 3.3, and Table 3.4, respectively. Raising birds at 34°C significantly (P<.0001) impaired BWG, FCR, and FI through each of the experimental periods when compared to those reared at 27°C. When compared to MON and un–medicated groups, birds receiving NIC had a significantly reduced BWG from 14 to 15, 15 to 21, 21 to 28 and 14 to 28 d (P < 0.0001, P = 0.025, P = 0.023, and P = 0.002, respectively) (Table 3.2). Additionally, NIC also reduced FCR but only from 14 to 15 and 15 to 21 d of age (P = 0.012, P = 0.025, respectively) (Table 3.3). A significant temperature x medication interaction was recorded for BWG during the periods 21 to 28 and 14 to 28 d (P = 0.013 and P = 0.023, respectively). These interactions indicate that the growth suppressive effects of NIC were evident in the HS environment and not in those raised in the TN environment. The only significant challenge x medication interaction for BWG was recorded during the period from 15 to 21 days (P < 0.0001). This interaction indicated that the suppressive effect of coccidial challenge on growth were less evident among broilers fed NIC which exhibited lower weight gains than the other groups.

Overall, subjecting birds to coccidial infection impaired BWG almost significantly from 14 to 15 d (P = 0.053), and significantly impaired BWG from 15 to 21 d (P = 0.007). A significant temperature x challenge interaction was recorded in BWG from 14 to 15, 15 to 21, and 14 to 28 d of age (P < 0.001, P < 0.0001, and P = 0.031, respectively) (Table 3.2). This interaction demonstrates that in comparison to birds free of coccidial challenge, subjecting birds to coccidial infection impaired BWG for birds reared in the 27°C environment however, when birds were raised in the 34°C

environment, the effects of coccidial infection on BWG were significantly decreased. The only significant temperature x challenge interaction observed for FCR was in the 14 to 15 d time period ($P = 0.022$) (Table 3.3). This suggests that in the 34°C environment, feed efficiency was better in broilers subjected to coccidial challenge than those free of infection however, in the 27°C environment, those free of coccidial challenge exhibited the better performance.

When compared to birds reared at 27°C, FCR from 14 to 15, 21 to 28, and 14 to 28 d of age was significantly reduced ($P < 0.0001$) among birds raised in the 34°C environment. When compared to MON and un–medicated groups, NIC reduced FI from 14 to 15, 21 to 28 and 14 to 28 d of age ($P < 0.0001$, $P = 0.002$, and $P = 0.001$, respectively). The only significant challenge x medication interaction for FI was recorded during the period from 14 to 15 days ($P = 0.009$). This interaction indicates that while coccidial infection did not increase FI for MON and NIC groups, coccidial infection largely impaired feed intake among birds that received unmedicated feed.

E. Maxima Oocysts Output

The fecal oocyst output data, as determined by fecal oocyst counts, is presented in on Table 3.5. The sample composites were collected between 19 to 21 and 25 to 28 d of age (5 to 7 and 11 to 14 days post infection, respectively). Environmental temperature, coccidial challenge, and medication each had a significant effect on oocyst output from 5 to 7 d post infection ($P = 0.008$, $P < 0.0001$, and $P = 0.001$, respectively) and on oocyst output from 11 to 14 d post infection ($P = 0.008$, $P < 0.0001$, and $P < 0.0001$, respectively). Additionally, significant temperature x medication, challenge x medication,

temperature x challenge, and temperature x medication x challenge interactions were recorded for oocyst output from 5 to 7 d post infection ($P = 0.008$, $P = 0.008$, $P = 0.001$, and $P = 0.008$, respectively) and for oocyst output from 11 to 14 d post infection ($P = 0.008$, $P = 0.008$, $P < 0.0001$, and $P = 0.008$, respectively).

These interactions indicate that exposing *E. maxima*-infected broilers to constant chronic HS (34°C) completely diminished oocyst output when compared to birds reared in TN (27°C) environment. With regard to birds reared at 27°C, the oocyst output from challenged birds was consistently higher from 5 to 7 and 11 to 14 d post infection when compared to non-challenged groups. The temperature x medication x challenge interaction indicates that treatment with coccidiostats significantly ($P < 0.05$) reduced the oocyst output of challenged birds from 5 to 7 and 11 to 14 d post infection. Of the two coccidiostats, oocyst output for NIC–treated birds was 97% and 70% lower than MON groups from 5 to 7 and 11 to 14 d post infection, respectively.

Discussion

In the current study, broilers reared in the 34°C environment had significantly ($P<0.05$) reduced weight gain (–58%), feed intake (–38%), and a significantly ($P<0.05$) higher feed conversion ratio (+46%) from 14 to 28 d of age. This observed decrease in performance agrees with observations consistently described with regard to broilers subjected to heat stress (Geraert et al., 1996; Altan et al., 2000; Lara and Rostagno, 2013). Additionally, rearing birds at 34°C significantly elevated ($P<0.05$) cloacal temperature measured at 15, 21, and 28 d of age (+1.7, +1.0, and +1.7°C, respectively). This observed relationship between elevated environmental temperatures and increased

cloacal temperatures agrees with those of prior studies (Donkoh, 1989; Cooper and Washburn, 1998; Soleimani et al., 2011).

When broilers are raised at elevated environmental temperatures, the ensuing reduction in feed intake is intended to reduce the broiler's production of metabolic heat and maintain homeothermy (Ryder et al., 2004). This agrees with the significant differences we observed for the cloacal temperatures of NIC-fed broilers (at 15 and 21 d of age) which also exhibited the corresponding changes to feed intake. In this study, the coccidial infection significantly increased the cloacal temperatures of birds raised in the TN environment at 15 and 21 d of age, irrespective of anticoccidial treatment. This observation is in agreement with a recent study which also recorded significantly higher cloacal temperatures for coccidia-challenged broilers (da Costa et al., 2017).

In heat stress conditions, NIC reduced weight gain and feed efficiency, which are in agreement with prior studies observing a reduction in performance when NIC-fed birds are subjected to elevated environmental temperatures (McDougald and McQuistion, 1980; Keshavarz and McDougald, 1981; Harris and Macy, 1988). When broilers are raised at elevated environmental temperatures, the subsequent reduction in feed intake is intended to reduce the broiler's production of metabolic heat and maintain homeothermy (Ryder et al., 2004). This explains the likely reason NIC-fed broilers exhibited significantly lower and higher cloacal temperatures after 24 and 48 h of HS exposure, respectively, which coincided with their daily feed intake.

Importantly, in this study we demonstrate that in broilers subjected to constant chronic HS, the oocyst shedding of *E. maxima* oocysts is significantly curtailed, regardless of anticoccidial treatment. Accordingly, the administered coccidial challenge

only reduced weight gains in the TN environment, irrespective of anticoccidial treatment. In a previous study, Banfield et al. (1998) subjected broilers to 35°C for 5 h daily (from 21 to 58 d of age). These HS-broilers were inoculated with *E. acervulina* oocysts at 21 d of age for the primary challenge and re-infected at 42 d of age for the secondary challenge. In agreement with our finding, Banfield et al. (1998) observed a significant reduction in daily oocyst counts and total oocysts output during the primary challenge. Importantly, during the secondary coccidial infection, the author also reported a significantly lower oocyst yield both in the control and HS groups where, those of the HS group were noticeably lower, numerically. The author indicated the establishment of immunity in both control and HS groups, suggesting the phenomenon is associated with a cell-mediated response in the broiler. When nicarbazin was fed to broilers in the TN environment, growth was not significantly reduced although, under HS, nicarbazin did significantly growth.

Conclusion

The effect elevated environmental temperatures have on broiler performance and body temperature were investigated in this study. This study shows that regardless of coccidial challenge status, NIC did not significantly reduce growth when compared to unmedicated birds at 27°C. Feeding NIC under HS significantly reduce growth when compared to unmedicated birds at 34°C. In broilers reared at 34°C concurrently for 14 days, coccidial challenge had no significant effect on broiler performance, when compared to the groups raised at 27°C.

This study demonstrated that at elevated environmental temperatures (34 ° from 14 to 24 d of age) the production of *E. maxima* oocysts is significantly curtailed. Consequently, we report that the concurrent exposure of broilers to HS (34°C) significantly reduced the severity of *E. maxima* coccidiosis as determined by oocyst counts and performance parameters.

Table 3.1. Cloacal temperature measured at 3 time periods for birds raised at 2 environmental temperatures and fed 3 anticoccidial treatments in the presence and absence of coccidial infection.

Temperature Setting (°C)	Coccidial challenge	Anticoccidial	Cloacal Temperature (°C)		
			15 d	21 d	28 d
TN			41.5^{b}	41.2^{b}	41.4^{b}
HS			43.3^{a}	42.2^{a}	42.5^{a}
	Non-challenged		42.4^{b}	41.7	42.0^{a}
	Challenged		42.5^{a}	41.7	41.9^{b}
		Unmedicated	42.5^{a}	41.6^{b}	42.0
		Nicarbazin	42.3^{b}	41.8^{a}	42.0
		Monensin	42.5^{a}	41.6^{b}	42.0
TN	Non-challenged		41.5^{c}	41.1^{b}	41.4^{c}
	Challenged		41.6^{b}	41.2^{b}	41.4^{c}
HS	Non-challenged		43.3^{a}	42.2^{a}	42.6^{a}
	Challenged		43.3^{a}	42.1^{a}	42.4^{b}
TN		Unmedicated	41.6^{c}	41.1	41.4
		Nicarbazin	41.5^{c}	41.2	41.4
		Monensin	41.6^{c}	41.2	41.5
HS		Unmedicated	43.4^{a}	42.1	42.5
		Nicarbazin	43.2^{b}	42.4	42.5
		Monensin	43.4^{a}	42.1	42.5
	Non-challenged	Unmedicated	42.4^{abc}	41.7^{abc}	42.0
		Nicarbazin	42.3^{c}	41.7^{ab}	42.1
		Monensin	42.4^{bc}	41.6^{bc}	42.0
	Challenged	Unmedicated	42.5^{ab}	41.5^{c}	41.9
		Nicarbazin	42.3^{c}	41.9^{a}	41.9
		Monensin	42.6^{a}	41.7^{ab}	41.9
TN	Non-challenged	Unmedicated	41.5	41.1^{c}	41.3
		Nicarbazin	41.5	41.1^{c}	41.5
		Monensin	41.4	41.2^{c}	41.5
	Challenged	Unmedicated	41.6	41.1^{c}	41.5
		Nicarbazin	41.6	41.4^{c}	41.4
		Monensin	41.7	41.2^{c}	41.4
HS	Non-challenged	Unmedicated	43.4	42.3^{a}	42.6
		Nicarbazin	43.2	42.3^{a}	42.7
		Monensin	43.4	42.0^{ab}	42.6
	Challenged	Unmedicated	43.4	41.9^{b}	42.4
		Nicarbazin	43.1	42.4^{a}	42.4
		Monensin	43.4	42.2^{a}	42.4
SEM			0.04	0.03	0.04
Source of Variation			*P*–values		
Environmental temperature			<.0001	<.0001	<.0001
Coccidial challenge			0.008	0.646	0.004
Medication			<.0001	<.0001	0.8383
temperature × challenge			0.002	0.006	0.009
temperature × medication			0.012	0.111	0.515
challenge × medication			0.044	0.001	0.237
temperature × medication × challenge			0.432	0.017	0.188

[a–b]Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

Table 3.2. Body weight gain (BWG) measured at 4 time periods for birds raised at 2 environmental temperatures and fed 3 anticoccidial treatments in the presence and absence of coccidial infection.

Temperature Setting (°C)	Coccidial challenge	Anticoccidial	BWG (g) 14 to 15 d	15 to 21 d	21 to 28 d	14 to 28 d
TN			67^{a}	337^{a}	454^{a}	861^{a}
HS			53^{b}	149^{b}	157^{b}	359^{b}
	Non-challenged		61^{a}	248^{a}	307	617
	Challenged		59^{a}	238^{b}	305	603
		Unmedicated	63^{a}	248^{a}	314^{a}	626^{a}
		Nicarbazin	54^{b}	236^{b}	292^{b}	582^{b}
		Monensin	63^{a}	246^{ab}	311^{ab}	622^{a}
TN	Non-challenged		71^{a}	350^{a}	456	878^{a}
	Challenged		64^{b}	323^{b}	452	843^{a}
HS	Non-challenged		51^{c}	146^{c}	158	355^{b}
	Challenged		54^{c}	153^{c}	157	363^{b}
TN		Unmedicated	69	338	450^{a}	858^{a}
		Nicarbazin	62	329	450^{a}	845^{a}
		Monensin	71	342	462^{a}	879^{a}
HS		Unmedicated	57	157	179^{b}	393^{b}
		Nicarbazin	46	142	134^{c}	320^{c}
		Monensin	55	149	160^{bc}	364^{bc}
	Non-challenged	Unmedicated	64	265^{a}	310	636
		Nicarbazin	56	232^{b}	293	583
		Monensin	63	248^{ab}	318	631
	Challenged	Unmedicated	62	231^{b}	318	616
		Nicarbazin	53	239^{b}	291	582
		Monensin	62	244^{b}	304	613
TN	Non-challenged	Unmedicated	71	368	441	873
		Nicarbazin	67	335	461	871
		Monensin	73	348	467	890
	Challenged	Unmedicated	66	309	458	843
		Nicarbazin	57	323	440	819
		Monensin	69	336	458	867
HS	Non-challenged	Unmedicated	57	162	178	398
		Nicarbazin	44	129	125	296
		Monensin	53	147	170	371
	Challenged	Unmedicated	57	152	179	388
		Nicarbazin	48	156	143	344
		Monensin	56	152	150	358
SEM			1	5	9	15

Source of Variation	*P*–values 14 to 15 d	15 to 21 d	21 to 28 d	14 to 28 d
Environmental temperature	<.0001	<.0001	<.0001	<.0001
Coccidial challenge	0.053	0.007	0.701	0.183
Medication	<.0001	0.025	0.023	0.002
temperature × challenge	0.000	<.0001	0.735	0.031
temperature × medication	0.153	0.477	0.013	0.023
challenge × medication	0.676	<.0001	0.246	0.754
temperature × medication × challenge	0.215	0.220	0.206	0.178

$^{a–b}$Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

Table 3.3. Feed conversion ratio (FCR) measured at 4 time periods for birds raised at 2 environmental temperatures and fed 3 anticoccidial treatments in the presence and absence of coccidial infection.

Temperature Setting (°C)	Coccidial challenge	Anticoccidial	FCR (g:g)			
			14 to 15 d	15 to 21 d	21 to 28 d	14 to 28 d
TN			1.15[b]	1.48[b]	1.21[b]	1.30[b]
HS			1.28[a]	2.20[a]	1.90[a]	1.90[a]
	Non-challenged		1.21	1.86	1.54	1.61
	Challenged		1.22	1.81	1.57	1.60
		Unmedicated	1.20[ab]	1.73[b]	1.53[a]	1.57[a]
		Nicarbazin	1.27[a]	2.00[a]	1.66[a]	1.69[a]
		Monensin	1.17[b]	1.79[ab]	1.48[a]	1.56[a]
TN	Non-challenged		1.11[c]	1.43[b]	1.15	1.27
	Challenged		1.18[bc]	1.53[b]	1.27	1.34
HS	Non-challenged		1.30[a]	2.30[a]	1.92	1.95
	Challenged		1.25[ab]	2.10[a]	1.87	1.86
TN		Unmedicated	1.14	1.45	1.20	1.30
		Nicarbazin	1.19	1.52	1.29	1.35
		Monensin	1.10	1.46	1.15	1.27
HS		Unmedicated	1.25	2.00	1.85	1.84
		Nicarbazin	1.35	2.47	2.03	2.03
		Monensin	1.23	2.12	1.81	1.85
	Non-challenged	Unmedicated	1.21	1.69	1.59	1.59
		Nicarbazin	1.24	2.13	1.57	1.69
		Monensin	1.16	1.77	1.45	1.54
	Challenged	Unmedicated	1.18	1.77	1.46	1.55
		Nicarbazin	1.29	1.87	1.75	1.68
		Monensin	1.17	1.81	1.51	1.57
TN	Non-challenged	Unmedicated	1.15	1.38	1.24	1.30
		Nicarbazin	1.11	1.45	1.08	1.25
		Monensin	1.06	1.46	1.13	1.26
	Challenged	Unmedicated	1.14	1.52	1.17	1.29
		Nicarbazin	1.27	1.59	1.49	1.44
		Monensin	1.15	1.46	1.16	1.28
HS	Non-challenged	Unmedicated	1.27	1.99	1.94	1.88
		Nicarbazin	1.37	2.81	2.06	2.13
		Monensin	1.26	2.09	1.77	1.83
	Challenged	Unmedicated	1.22	2.01	1.75	1.80
		Nicarbazin	1.32	2.14	2.00	1.92
		Monensin	1.20	2.15	1.86	1.86
SEM			0.02	0.07	0.06	0.05
Source of Variation			*P*–values			
Environmental temperature			<.0001	<.0001	<.0001	<.0001
Coccidial challenge			0.708	0.542	0.634	0.876
Medication			0.012	0.025	0.135	0.100
temperature × challenge			0.022	0.086	0.249	0.145
temperature × medication			0.746	0.133	0.844	0.562
challenge × medication			0.533	0.190	0.270	0.864
temperature × medication × challenge			0.473	0.084	0.339	0.270

[a–b]Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

Table 3.4. Feed intake (FI) measured at 4 time periods for birds raised at 2 environmental temperatures and fed 3 anticoccidial treatments in the presence and absence of coccidial infection.

Temperature Setting (°C)	Coccidial challenge	Anticoccidial	FI (g)			
			14 to 15 d	15 to 21 d	21 to 28 d	14 to 28 d
TN			77[a]	496[a]	525[a]	1096[a]
HS			66[b]	325[b]	298[b]	683[b]
	Non-challenged		72	412	408	893
	Challenged		71	408	415	886
		Unmedicated	75[a]	404	435[a]	918[a]
		Nicarbazin	67[b]	419	385[b]	853[b]
		Monensin	72[a]	407	415[ab]	897[a]
TN	Non-challenged		78	501	515	1103
	Challenged		75	491	536	1089
HS	Non-challenged		66	324	302	684
	Challenged		66	326	294	682
TN		Unmedicated	79	491	540	1111
		Nicarbazin	74	499	502	1064
		Monensin	78	498	534	1113
HS		Unmedicated	71	318	330	725
		Nicarbazin	61	340	268	642
		Monensin	67	316	297	681
	Non-challenged	Unmedicated	77[a]	416	440	939
		Nicarbazin	67[d]	415	364	837
		Monensin	72[bc]	406	420	904
	Challenged	Unmedicated	72[bc]	393	429	898
		Nicarbazin	68[cd]	424	406	869
		Monensin	73[b]	408	410	890
TN	Non-challenged	Unmedicated	82	510	535	1132
		Nicarbazin	75	488	479	1061
		Monensin	78	505	531	1116
	Challenged	Unmedicated	75	472	544	1091
		Nicarbazin	72	509	525	1067
		Monensin	78	491	537	1109
HS	Non-challenged	Unmedicated	73	322	345	746
		Nicarbazin	60	341	249	613
		Monensin	66	308	310	692
	Challenged	Unmedicated	69	314	314	705
		Nicarbazin	63	339	286	672
		Monensin	67	324	283	671
SEM			1	12	16	28
Source of Variation			*P*–values			
Environmental temperature			<.0001	<.0001	<.0001	<.0001
Coccidial challenge			0.132	0.657	0.535	0.556
Medication			<.0001	0.356	0.002	0.001
temperature × challenge			0.078	0.495	0.201	0.620
temperature × medication			0.155	0.560	0.537	0.336
challenge × medication			0.009	0.314	0.085	0.073
temperature × medication × challenge			0.419	0.382	0.830	0.537

[a–b]Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's *t* or Tukey's test.

Table 3.5. Fecal oocyst counts obtained from composite samples collected at 2 time periods from birds raised at 2 environmental temperatures and fed 3 anticoccidial treatments in the presence and absence of coccidial infection.

Temperature Setting (°C)	Coccidial challenge	Anticoccidial	Oocyst counts (oocysts/g) 19 to 21 d	25 to 28 d
TN			1542[a]	2717[a]
HS			0[b]	0[b]
	Non-challenged		0[b]	0[b]
	Challenged		1542[a]	2717[a]
		Unmedicated	1676[a]	2751[a]
		Nicarbazin	17[b]	307[b]
		Monensin	620[ab]	1017[ab]
TN	Non-challenged		0[b]	0[b]
	Challenged		3084[a]	5434[a]
HS	Non-challenged		0[b]	0[b]
	Challenged		0[b]	0[b]
TN		Unmedicated	3352[a]	5503[a]
		Nicarbazin	33[b]	614[b]
		Monensin	1241[ab]	2034[b]
HS		Unmedicated	0[b]	0[b]
		Nicarbazin	0[b]	0[b]
		Monensin	0[b]	0[b]
	Non-challenged	Unmedicated	0[b]	0[b]
		Nicarbazin	0[b]	0[b]
		Monensin	0[b]	0[b]
	Challenged	Unmedicated	3352[a]	5503[a]
		Nicarbazin	33[b]	614[b]
		Monensin	1241[b]	2034[b]
TN	Non-challenged	Unmedicated	0[b]	0[b]
		Nicarbazin	0[b]	0[b]
		Monensin	0[b]	0[b]
	Challenged	Unmedicated	6703[a]	11006[a]
		Nicarbazin	67[b]	1227[b]
		Monensin	2481[b]	4069[b]
HS	Non-challenged	Unmedicated	0[b]	0[b]
		Nicarbazin	0[b]	0[b]
		Monensin	0[b]	0[b]
	Challenged	Unmedicated	0[b]	0[b]
		Nicarbazin	0[b]	0[b]
		Monensin	0[b]	0[b]
SEM			328	519
Source of Variation			*P*–values	
Environmental temperature			<.0001	<.0001
Coccidial challenge			0.001	<.0001
Medication			0.008	0.008
temperature × challenge			0.001	<.0001
temperature × medication			0.008	0.008
challenge × medication			0.008	0.008
temperature × medication × challenge			0.008	0.008

[a–b]Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student's t or Tukey's test.

References

Altan, O., Altan, A., Oguz, I., Pabuçcuoglu, A., and Konyalioglu, S. Effects of heat stress on growth, some blood variables and lipid oxidation in broilers exposed to high temperature at an early age. British Poultry Science 41:489-493. 2000.

Anderson, W. I., Reid, W. M., and Johnson, J. K. Effects of high environmental temperatures on cecal coccidiosis. Poultry Science 55:1429-1435. 1976.

Bafundo, K. W., and Jeffers, T. K. Selection for resistance to monensin, nicarbazin, and the monensin plus nicarbazin combination. Poultry Science 69:1485-1490. 1990.

Banfield, M. J., Ten Doeschate, R. A., and Forbes, J. M. Effect of whole wheat and heat stress on a coccidial infection in broiler chickens. British Poultry Science 39 Suppl:S25-S26. 1998.

Blake, D. P., and Tomley, F. M. Securing poultry production from the ever-present Eimeria challenge. Trends in Parasitology 30:12-19. 2014.

Buys, S. B., and Rasmussen, R. W. Heat stress mortality in nicarbazine fed chickens. Journal of the South African Veterinary Association 49:127-128. 1978.

Chapman, H. D. A review of the biological activity of the anticoccidial drug nicarbazin and its application for the control of coccidiosis in poultry. Poultry Science Reviews (United Kingdom) 5. 1994.

Cooper, M., and Washburn, K. The relationships of body temperature to weight gain, feed consumption, and feed utilization in broilers under heat stress. Poultry Science 77:237-242. 1998.

Cuckler, A. C., Malanga, C. M., Basso, A. J., and O'Neill, R. C. Antiparasitic activity of substituted carbanilide complexes. Science 122:244-245. 1955.

da Costa, M. J., Bafundo, K. W., Pesti, G. M., Kimminau, E. A., and Cervantes, H. M. Performance and anticoccidial effects of nicarbazin-fed broilers reared at standard or reduced environmental temperatures. Poultry Science 96:1615-1622. 2017.

Donkoh, A. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. International Journal of Biometeorology 33:259-265. 1989.

Farny, D. G. O. Heat stress studies in nicarbazin treated chickens. In: Dissertation Abstracts. pp 7413-7413. 1965.

Geraert, P. A., Padilha, J. C. F., and Guillaumin, S. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: biological and endocrinological variables. British Journal of Nutrition 75:205-216. 1996.

Harris, G. C., Jr., and Macy, L. B. Effect of nicarbazin and maxiban on broiler performance when fed to different age broiler males subjected to environmental stress. Poultry Science 67:17-17. 1988.

Keshavarz, K., and McDougald, L. R. Influence of anticoccidial drugs on losses of broiler chickens from heat stress and coccidiosis. Poultry Science 60:2423-2428. 1981.

Lara, L. J., and Rostagno, M. H. Impact of heat stress on poultry production. Animals (Basel) 3:356-369. 2013.

McDougald, L. R., and Fitz-Coy, S. H. Coccidiosis. In: Diseases of Poultry, 13 ed. D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez and V. Nair, eds. Wiley-Blackwell, Ames, Iowa. pp 1148-1166. 2013.

McDougald, L. R., and McQuistion, T. E. Mortality from heat stress in broiler chickens influenced by anticoccidial drugs. Poultry Science 59:2421-2423. 1980.

Reid, W. M., Hines, T. K., Johnson, J., and Stino, K. R. Coccidiosis: effects of high environmental temperatures on anticoccidial protection. Poultry Science 55:1436-1441. 1976.

Ryder, A. A., Feddes, J. J. R., and Zuidhof, M. J. Field study to relate heat stress index to broiler performance. The Journal of Applied Poultry Research 13:493-499. 2004.

Sammelwitz, P. H. Factors related to nicarbazin induced heat stress mortality in broilers. Poultry Science 44:1412. 1965a.

Sammelwitz, P. H. Heat stress mortality in broilers. Poultry Science 44:1412. 1965b.

Soleimani, A., Zulkifli, I., Omar, A., and Raha, A. Physiological responses of 3 chicken breeds to acute heat stress. Poultry Science 90:1435-1440. 2011.

St-Pierre, N. R., Cobanov, B., and Schnitkey, G. Economic losses from heat stress by US livestock industries. Journal of Dairy Science 86:E52-E77. 2003.

Wiernusz, C. J., and Teeter, R. G. Nicarbazin effects on broiler thermobalance during high ambient temperature stress. Poultry Science 74:577-580. 1995.

CHAPTER 4

THE EFFECT CONSTANT CHRONIC HEAT STRESS HAS ON THE EXPRESSION OF HEAT SHOCK RESPONSE- AND ENERGY HOMEOSTASIS-RELATED GENES IN BROILERS TREATED WITH MONENSIN OR NICARBAZIN

Overview

This study evaluates the gene expression of energy homeostasis-, appetite- and heat shock response-related genes to characterize nicarbazin-induced heat stress (HS) toxicity and HS-induced obstruction of *Eimeria* oocyst production. The study consisted of a 2×3 factorial design experiment with two temperature settings (27°C or 34°C), and three anticoccidial treatments (untreated, nicarbazin at 125ppm, or monensin at 100ppm). At hatch, 240 Cobb500 chicks were assigned equally to 6 treatments. There were 5 replicates per treatment and 8 birds per replicate. Birds were assigned to treatments at day 14 of age until day 28. Liver, ileum and *P. major* were collected at days 15 and 28. The results indicate that when broilers fed nicarbazin are subjected to constant chronic HS, there is a subsequent increase in antioxidant capacity, decrease in intestinal motility, attenuation of appetite, and increased protection against tissue injury when compared to those fed nicarbazin in the absence of HS. These differences may explain the increased survivability observed when NIC is administered to HS-acclimated broilers. Moreover, when broilers are subjected to constant chronic HS, there is an observed increase in antioxidant capacity to protect against HS-induced ROS production which may provide cross-protection against *Eimeria*-infections by obstructing ROS-induced intercellular

adhesion which may lead to obstructing coccidia from multiplying resulting in a curtailed oocyst output.

Introduction

Heat stress (HS) is a physiological and behavioral consequence resulting from the inability of an animal to dispel more heat into the surrounding environment than is generated metabolically (Akbarian et al., 2016). This thermal imbalance hinders bird performance in the broiler industry and consequently, attributes to economic losses in the U.S. ranging from $128 to 165 million (St-Pierre et al., 2003). Broilers are genetically selected for growth, which has generated broilers that are increasingly more susceptible to stress rather than more resistant (Soleimani et al., 2011). This demonstrates why HS has become increasingly important in the broiler industry, especially in warmer regions.

Another factor which also disrupts productivity in the poultry industry is coccidiosis, which has been estimated to contribute to global economic losses of about $3 billion (Blake and Tomley, 2014). Nicarbazin, is one of the most effective and widely used anticoccidials in the poultry industry owing to its efficacy in preventing and controlling coccidiosis. However, the use of NIC is generally not appropriate in regions with a hotter climate since NIC is known to increase broiler susceptibility to HS-toxicity and mortality when fed to birds subjected to elevated environmental temperatures (Farny, 1965; Sammelwitz, 1965; Buys and Rasmussen, 1978; McDougald and McQuistion, 1980; McDougald and Fitz-Coy, 2013). However, the molecular mechanisms underlying this increased susceptibility to HS-toxicity remain elusive. Furthermoe, upon exposure to HS, when NIC is fed to HS-acclimated broilers, susceptibility to HS-mediated toxicity IS

reduced when compared to broilers not acclimated to HS (Wiernusz and Teeter, 1995). This suggests that upon HS, broilers not acclimated to HS are more susceptible to NIC-induced HS toxicity in comparison to those acclimated to HS.

In chickens, coccidiosis is caused by protozoan parasites belonging to the genus *Eimeria*. Of the seven *Eimeria* species that the chicken is the natural host to, *Eimeria acervulina*, *E. maxima*, and *E. tenella* are generally the most problematic in the industry (Cervantes, 2014). Importantly, subjecting coccidia-challenged broilers to elevated environmental temperatures significantly interrupts the cycling of *E. acervulina* (Banfield et al., 1998). Our previous study demonstrated that the cycling of *E. maxima* too can be interrupted when broilers are subjected to elevated environmental temperatures. However, the mechanisms underlying these observations have yet to be elucidated.

The objective of this study is to identify the relationship between NIC and heat shock toxicity and between HS and reduced oocyst production through the expression of the genes involved with the heat shock response (catalase , CAT; superoxide dismutase, SOD; heat shock factors, HSFs; and heat shock proteins, HSPs) and energy homeostasis (avian uncoupling proteins, avUCP; cholecystokinin, CCK; ghrelin; growth hormone sectretagogue receptor, GHS-R; glucagon-like peptide 1, GLP-1; nesfatin-1; and insulin).

Materials and Methods

Design and Animal Management

Research conducted using live broiler chickens was in accordance with the guidelines approved by the institutional animal care and use committee of the University of Georgia. Male Cobb500 chicks were raised from hatch until 13 d of age under standard

husbandry practices. At 14 d of age, 240 chicks of similar body weight were divided equally into 6 treatment groups, each with 5 replicates (*n*=40), and each replicate having 8 birds. A 2×3 experimental design was employed, with two temperature settings (27°C or 34°C), and three levels of feed regimens (untreated; nicarbazin, 125 ppm; or monensin, 100 ppm). Broilers had unlimited access to water and feed. Two environmentally controlled rooms were used to expose birds at a constant temperature of 27°C (HS) or 34°C (TN) for the duration of the study (14 to 28 d of age).

Anticoccidial Treatments

From hatch, all broilers were fed standard broiler starter mashup from hatch to 13 d of age. From 14 to 28 d of age, broilers were then assigned to either of the anticoccidial treatments which consisted of unmedicated; monensin, 100 ppm; and nicarbazin, 125 ppm. Drug premixes were mixed with a standard grower diet (Southern Poultry, Athens, GA).

Temperature Treatments

Two environmentally controlled rooms were used to expose birds to a constant temperature of 27°C (TN) or 34°C (HS) for the duration of the study (14 to 28 d of age). The daily temperature and relative humidity were monitored daily over the duration of the study. The average temperature for the TN and HS environments were 27°C and 34°C, respectively. The average humidity for the TN and HS environments were 68% and 47%, respectively.

Tissue Sampling and RNA Extraction.

For gene expression analysis, ileum, liver, and *P. major* tissues were collected at 15 and 28 d of age from 5 birds per each treatment and then immediately placed in cryogenic containers and stored in liquid nitrogen and later stored frozen at -86°C. TRIzol reagent (Invitrogen; Carlsbad, CA USA) was used for RNA isolation according to the manufacturer's instructions, then cleaned with RNeasy Mini Kit (Quiagen, Valencia, CA, USA) according to the manufacturer's protocol. The RNA samples were treated with RNase-Free DNase (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, then suspended in DEPC water. The quality and concentration of the samples were then measured using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE), then stored at -86°C. For cDNA synthesis, 10 µl of RNA sample were reversed transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA) in accordance to the manufacturer's protocol. cDNA was then diluted to a concentration of 20 ng/µl. One µl of cDNA served as a template in a 20 µl PCR mixture containing PowerUp SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA) and each forward and reverse primer at a concentration of 150 nM. The RT-PCR conditions were 50°C for 2 minutes, 95°C for 2 followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. At the end of each reaction, melting curves were determined to ensure a single PCR product for each gene.

To examine the mRNA expression of HS-related genes, the expressions of catalase (CAT), superoxide dismutase (SOD), heat shock factor 1 (HSF1), HSF2, HSF3, HSF4, heat shock protein-60 (HSP60), HSP70, and HSP90 were examined in the liver and *P. major*. Moreover, to examine the mRNA expression of feed intake- and energy

metabolism-related genes, the expressions of avian uncoupling protein (avUCP), cholecystokinin (CCK), ghrelin, growth hormone sectretagogue receptor 1 (GHS-R1), glucagon, and nucleobindin 2 (NUCB2), were examined in the liver and ileum while insulin was examined in the liver measured. The RT-PCR data was analyzed according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and were normalized to β-actin in each sample.

Statistical Analysis

The data was analyzed statistically using the general linear models procedure, followed by Tukey's multiple comparisons test (version 9.4, SAS Institute Inc., Cary, NC). A *P*-value of less than 5% is assumed to be statistically significant.

Results

Effect of Nicarbazin on the Expression of CAT, SOD, HSFs, and HSPs

In the liver of HS broilers fed nicarbazin, expression of CAT, SOD, HSF1, HSF2, and HSF3 significantly increased at day 15 when compared to the control (Figure 4.1). In the liver of TN broilers fed nicarbazin, CAT, SOD, HSF2 and HSF3 expressions were significantly increased at day 15 when compared to the control (Figure 4.1). In contrast to TN broilers fed nicarbazin, the liver expressions of CAT, SOD, and HSF3 were significantly down-regulated at day 15 in TN broilers fed monensin when compared to the control, respectively (Figure 4.1 and 4.2). In the *P. major* of HS broilers fed nicarbazin, expressions of HSF1, HSF2, HSF3, and HSF4 were significantly up-regulated at day 15 when compared to the control (Figures 4.1 and 4.2).

In the liver of HS broilers fed nicarbazin, the HSP60, HSP70, and HSP90 expressions were significantly up-regulated at day 15 when compared to the control (Figure 4.3). In the liver of TN broilers fed nicarbazin, expressions of HSP60 and HSP90 were significantly down-regulated at day 28 when compared to the control (Figure 4.3). In the *P. major* of HS broilers fed nicarbazin, the expressions of HSP60 and HSP90 significantly increased at day 15 when compared to the control (Figure 4.3). In the *P. major* of TN broilers fed nicarbazin, the expressions of HSP60 and HSP70 were significantly down-regulated and up-regulated, respectively, at day 15 when compared to the control (Figure 4.3).

Effect of Nicarbazin on the Expression of avUCP, CCK, Ghrelin, GHS-R1a, GLP-1, Nesfatin-1

In the liver of HS broilers fed nicarbazin, expressions of avUCP, GHS-R1a, NUCB2, and insulin were significantly up-regulated at day 15 when compared to the controls (Figure 4.4 and 4.5). In the liver of TN broilers fed nicarbazin, expressions of GHS-R1a and NUCB2 significantly increased at day 15 when compared to the control (Figure 4.5). In the liver of TN broilers fed nicarbazin, the CCK and GLP-1 expressions significantly increased at day 28 when compared to the control (Figure 4.4 and 4.5). In the liver of HS broilers fed nicarbazin, the expressions of CCK, ghrelin, GHS-R1a, and NUCB2 significantly increased at day 28 when compared to the control (Figure 4.4 and 4.5).

In the ileum of TN broilers fed nicarbazin, expressions of CCK and NUCB2 significantly increased at day 15 when compared to the control (Figure 4.4 and 4.5). In

the ileum of HS broilers fed nicarbazin, the CCK, GLP-1, and NUCB2 expressions were significantly up-regulated while ghrelin and GHS-R1a expressions were significantly down-regulated at day 15 when compared to the control (Figure 4.4 and 4.5). In the ileum of HS broilers fed nicarbazin at day 28, the expressions of avUCP, ghrelin, and GHS-R1a were significantly increased when compared to the control (Figure 4.4 and 4.5).

Effect of Heat Stress on the Expression of CAT, SOD, HSFs, and HSPs

The results of mRNA expression studies of HSFs are shown in Figure 4.6 and those of CAT, SOD, and HSPs are shown in Figure 4.7. In the liver, the HSF3, HSF4, CAT and SOD expressions were significantly up-regulated at day 15 in broilers subjected to chronic HS when compared to the control. Additionally, the expression of HSP90 at day 15 in the liver was significantly down-regulated in broilers subjected to HS when compared to the control. In the liver, expression of HSP70 and all the HSFs were significantly increased at day 28 in broilers subjected to HS when compared to the control.

In the *P. major*, the HSF4, CAT and SOD expressions were significantly up-regulated at day 15 in broilers subjected to concurrent HS when compared to the control. Moreover, the expression of HSF2 in the *P. major* was significantly down-regulated at day 15 in broilers subjected to constant chronic HS when compared to the control. In the *P. major*, expression of CAT, SOD, and all HSFs and HSPs were significantly elevated by the chronic HS when compared to the control at day 28

Effect of Heat Stress on Expression of avUCP, CCK, Ghrelin, GHS-R1a, GLP-1, NUCB2

The results of mRNA expression studies of avUCP, CCK, Ghrelin, GHS-R1a, GLP-1, and NUCB2 are shown in Figure 4.8. In the liver, expressions of CCK, GLP-1 and NUCB2 were significantly elevated at day 15 in broilers subjected to HS when compared to the control. At day 28 in the liver, Ghrelin, GHS-R1a and HLP-1 expressions were significantly up-regulated in broilers subjected to chronic HS when compared to the control.

In the ileum, the avUCP, Ghrelin, GHS-R1a and NUCB2 expressions were significantly up-regulated when broilers were subjected to chronic HS when compared to the control. On the other hand, the expression of GLP-1 in the ileum was significantly down-regulated in broilers subjected to heat stress at day 15 and day 28.

Discussion

Heat stress is identified as an inducer of oxidative stress and if not controlled, it can result in severe tissue damage (Akbarian et al., 2016). Oxidative stress is defined as the presence of reactive oxygen species (ROS) at a level which exceeds the capacity of animal cells and can lead to oxidative damage (Halliwell and Whiteman, 2004). To combat this, cells have antioxidant enzymes as a first line of defense which includes catalase (CAT) and superoxide dismutase (SOD) (Ray and Husain, 2002). SOD converts $O_2^{\bullet -}$ to H_2O_2 which is then converted to H_2O with the aid of CAT (Ray and Husain, 2002). In this study, feeding broilers nicarbazin up-regulated the mRNA expression of SOD and CAT in the liver at day 15, regardless of temperature treatment (Figure 4.1). However, no significant changes in the expressions of CAT or SOD were observed in the

P. major of broilers fed nicarbazin. These observations suggest that within 24 hours, treatment with nicarbazin likely increases the production of ROS in the liver causing an increased expression of SOD to increase the conversion of superoxide to H_2O_2, and an increased expression of CAT to overcome the elevated production of H_2O_2. However, at day 28, SOD was no longer up-regulated among broilers fed nicarbazin and CAT was only up-regulated in broilers fed nicarbazin which were subjected to persistent chronic heat stress. This shows that among broilers fed nicarbazin, those acclimated to heat stress are better capable of protecting against nicarbazin-induced oxidative stress by maintaining an increased expression of the antioxidant enzyme CAT. This is in agreement to a prior study reporting that acclimating chicks to heat stress can reduce nicarbazin-induced heat stress toxicity (Wiernusz and Teeter, 1995).

Moreover, when examining the absorption and elimination of nicarbazin's components, Cuckler et al. (1956) reported that in chickens fed nicarbazin, the liver contained 10-fold more dinitrocarbanilide (DNC) than the muscle. Also, feeding nicarbazin at high levels was shown to cause liver damage, which was determined histologically (Newberne and Buck, 1957). These reports explain our observation that in the liver, nicarbazin has a greater effect on the expression of antioxidant genes when compared to muscle. Moreover, in the present study, when heat stressed broilers were treated with either coccidiostat (monensin or nicarbazin), the expressions of HSF1, HSF2, HSP70 and HSP90 at day 15 were significantly up-regulated in the liver while the upregulated expressions of HSF3 and HSP60 were unique to HS birds fed nicarbazin, which were also the only group which exhibited an up-regulated expression of CAT and SOD. This suggests that in addition to HSF1, HSF2, HSP70 and HSP90 expressions in

the liver, HSF3 and HSP60 may play a major role in the up-regulated expression of CAT and SOD genes. Although, when broilers were kept in the thermoneutral environment, the up-regulation of these genes in nicarbazin-fed birds was, typically, either less-evident or not observed at all at day 15.

In an in vitro study conducted with rat testes, the effect of monensin on antioxidant enzyme activities was investigated by Singh et al. (2006). The group suggested that monensin treatment causes peroxidative damage, possibly through inhibiting the activities of antioxidant enzymes which, include SOD and CAT.

In HSP60 deficient fibroblasts, the reduced integrity of mitochondria is further reduced upon heat stress and suggested to directly result from the lack HSP60 (Huckriede et al., 1995). Upon exposure to HS Lei et al. (2009) reported the up-regulation of HSP90 in the heart, liver and kidney in broilers during the early phase of HS. The authors reported that HSP90 functioned primarily in the nucleus of these cells and suggested it is important for the survival of cells in disadvantageous environments. Al-Zghoul et al. (2015) reported that in broiler embryos subjected to thermal manipulation (39°C, 9-18 hours), the increased expressions of HSP60 and HSP90 were suggested to be associated with in the acquirement of improved thermotolerance (Al-Zghoul et al., 2015). Therefore, HSP60 and HSP90 appear to be important in cell survival, especially upon thermal stressors. In the present study, at day 28, broilers kept in the thermoneutral environment and fed nicarbazin exhibited a significant down-regulation in the expressions of HSF4, HSP60, and HSP90 genes in the liver while a similar but non-significant trend was also observed in the muscle (Figure 4.2 and 4.3). This suggests that the when nicarbazin is fed to broilers in the absence of constant

chronic heat stress, the down-regulated expression of HSF4, HSP60, and HSP90 genes may be a major factor involved in the increased susceptibility that is observed with nicarbazin in broilers not acclimated to HS (Wiernusz and Teeter, 1995). Therefore, in broilers which are not acclimated to HS, nicarbazin may cause severe tissue damage by hindering mitochondrial integrity/function and decreasing survivability of cells resulting in a broiler that is more susceptible to heat stress toxicity. Additionally, this is evidence that HSF4 may be involved in suppressing the expressions of both HSP60 and HSP90. In the present study, broilers fed nicarbazin and subjected to constant chronic heat stress exhibited an up-regulated expression of HSP70 in the liver at day 28 (Figure 4.3). In chickens, the expression of HSP70 has been suggested as being crucial in protecting chicken tissues against various stressors by preserving the structure and functions of proteins (Surai and Kochish, 2017). Consequently, this suggests that up-regulated expression of HSP70 in the liver may be crucial for the decreased susceptibility to nicarbazin-induced toxicity observed with HS-acclimatized broilers.

Nesfatin-1 is a secreted anorexigenic peptide, derived from its precursor nucleobindin-2, which has been shown to reduce ROS production during the acute phase of intestinal ischemia-reperfusion, which was found to be protective by balancing oxidant capacity (Ceylan et al., 2015). In rats, nesfatin-1 was also shown to have anti-inflammatory and antioxidant effects in the liver by alleviating cholestatic liver damage caused by obstructive jaundice through reducing edema in the portal area, neutrophil infiltration, bile duct proliferation, hepatocyte necrosis, basement membrane damage, and parenchymal necrosis (Solmaz et al., 2016). In rats, nesfatin-1 treatment also alleviates chronic gastric ulcers through the modulation of oxidant-antioxidant balance by

increasing the levels of SOD and CAT (Kolgazi et al., 2017). The investigator also reported that nesfatin-1 decreased inflammatory mediators and neutrophil migration through cyclooxygenase (COX)-dependent mechanism, mainly through COX-2. In the present study, at day 28, broilers fed nicarbazin experienced an up-regulated expression of nesfatin-1 in the liver and ileum at day 15, regardless of temperature treatment, while at day 28, nesfatin-1 was up-regulated only in nicarbazin-fed broilers subjected to constant chronic heat stress. (Figure 4.5). This suggests that the expression of nesfatin-1 may play a major role in protecting against tissue injury through its antioxidant and anti-inflammatory effects. Also, in the present study, the expression pattern of nesfatin-1 in the liver was very similar to those of SOD and, especially, CAT. Additionally, at day 28, both CAT and nesfatin-1 were up-regulated in the liver of nicarbazin-fed broilers which were subjected to constant chronic heat stress. Taken together, this suggests that in the broiler liver, nesfatin-1 may play a major role in increasing antioxidant capacity by up-regulating the expression of antioxidant enzymes to protect against the proteotoxic or oxidative effects of nicarbazin and that nicarbazin may also inhibit intestinal motility by up-regulating the expression of nesfatin-1.

In chickens, ghrelin is an anorexigenic peptide and the ligand of growth hormone sectretagogue-receptor (GHS-R) where, GHS-R1a is recognized as the active variant of the receptor (Kaiya et al., 2007). In this study, broilers fed nicarbazin and subjected to constant chronic heat stress exhibited a reduction in feed intake from day 14 to day 15 (Table 4.1). Accordingly, at day 15, the expression of ghrelin was significant down-regulated in the liver and ileum (Figure 4.4). An explanation for this observation is that the reduced intake (days 14 to 15) that occurred shortly after treatment, likely lead to a

momentary decrease in ghrelin expression to promote hunger and increase feed intake shortly after nicarbazin-fed broilers were subjected to HS. Indeed, these birds exhibited a numerically higher feed intake from day 15 to day 21 when compared to monensin or the control (Table 4.1). Moreover, at day 28, broilers fed nicarbazin and subjected to constant chronic heat stress exhibited an increased expression of ghrelin and GHS-R1a (Figures 4.4 and 4.5). Accordingly, the same groups also exhibited significantly lower cumulative feed intake from day 21 to day 28 and over the duration of the study (days 14 to 28) (Table 4.1). This observation agrees with the suggestion that ghrelin is capable of modulating feeding behavior in chickens and is as an inhibitor of feed intake (Richards and Proszkowiec-Weglarz, 2007). This also suggests that in nicarbazin-fed broilers subjected to constant chronic heat stress, the expression of ghrelin may play a major role in altering appetite and feed intake.

In chickens, ghrelin has been shown to mediate contractile activity in the gastrointestinal tract and this is suggested to be mediated through its receptor, GHS-R1a (Kitazawa et al., 2007). Furthermore, ghrelin was also shown to significantly reduce the expression of fatty acid synthase in the liver of broiler chicks, suggesting ghrelin reduced hepatic lipogenesis (Buyse et al., 2009). In chickens, administration of ghrelin also stimulates corticosterone (CORT) release, gastrointestinal contraction, and the use of lipids as an energy source (Kaiya et al., 2013). It has been previously reported that feeding high levels of nicarbazin to broilers induces liver damage and exhibit fatty degeneration in the liver, as determined histologically (Newberne and Buck, 1957). Chronic heat stress too has been shown to cause fatty degeneration in the broiler liver, as determined histologically (Aengwanich and Simaraks, 2004). In the present study, at day

28, we observed an increase in the expression of ghrelin and its receptor, GHS-R1a, in the liver of broilers fed nicarbazin and subjected to constant chronic heat stress when compared to the control (Figures 4.4 and 4.5). This suggests that when broilers subjected to constant chronic heat stress are fed nicarbazin, the expression of ghrelin and GHS-R1a in the liver may play a major role in preventing lethal liver injury by protecting against hepatic lipogenesis.

Mitochondrial anion carrier proteins including uncoupling proteins (UCP) are suggested to have an inhibitory effect on the overproduction of mitochondrial superoxide (Mujahid et al., 2007). Acute stress has been demonstrated to down-regulate avUCP expression and protein level which correlated with an increased superoxide production (Mujahid et al., 2006). However, chronic HS has been demonstrated to up-regulate the expression of avUCP in the muscle (Dridi et al., 2008; Azad et al., 2010). In this study, the expression of avUCP was up-regulated solely in the liver and ileum of broilers fed nicarbazin and subjected to acute heat stress at day 15 (Figure 4.4). However, at day 28, this group exhibited an up-regulated expression of avUCP solely in the ileum. This suggests that in broilers fed nicarbazin and subjected to constant chronic heat stress the expression of avUCP may protect against injury by inhibiting ROS production which may occur in a tissue-specific manner.

The expression of CCK has been reported to be significantly down-regulated in the intestine of chickens subjected to acute HS (Song et al., 2012; Lei et al., 2013) and was suggested to decrease intestinal mobility which results in a lower passage rate in order to allow more time for intestinal enzymes to digest nutrients. However, when broilers are subjected to chronic HS, He et al. (2018) reported CCK is up-regulated in the

intestine and suggested it contributes to the observed reduction in feed intake due to its appetite-inhibiting effect. In the liver, CCK expression has also been shown to be up-regulated following exposure of broilers to cyclic elevated environmental temperatures (Coble et al., 2014). In the present study, the expression of CCK in broilers treated with nicarbazin, regardless of temperature treatment, was significantly up-regulated in the ileum and liver at day 15 and day 28, respectively (Figure 4.4). However, when nicarbazin-fed broilers were subjected to constant chronic heat stress, the fold-increase in CCK expression was enhanced. This suggests that nicarbazin increases the expression of CCK which suppresses appetite possibly by decreasing intestinal motility and increasing satiety and this effect is enhanced in conditions of constant chronic heat stress.

It has previously been reported, that during coccidial infection, there is a decreased in SOD activity in the blood which suggests the bird's antioxidant status is impaired during both *E. tenella* (Georgieva et al., 2006) and *E. acervulina* (Koinarski et al., 2005) infections, followed by oxidative damage. It was also recently reported treating *E. tenella*-infected broilers treated with IL17A antibody reduced ROS production, which was suggested to inhibit infected epithelial cells in the crypt from migrating due to intercellular adhesion being obstructed (del Cacho et al., 2014). Importantly, del Cacho et al. (2014) also observed a reduction in *E. tenella* oocyst shedding. In this study, genes associated with increased antioxidant capacity (avUCP, nesfatin-1, CAT, SOD) were up-regulated among broilers subjected to constant chronic heat stress (Figures 4.7 and 4.8) which indicates they were more protected against increased ROS production when compared to those in the thermoneutral environment. Therefore, when *Eimeria*-infected chickens are subjected to elevated environmental temperatures, the subsequent increase

in antioxidant capacity, in response to HS-induced ROS production, may provide cross-protection against *Eimeria* infections by protecting against the oxidative stress that precedes coccidial infections. Moreover, Zhang et al. (2018) reported that exposure to HS (40°C. 0-24 hours) had no significant effect on HSF-4 expression and suggested there is a lack of evidence demonstrating the association between HSF4 and stress-induced transcriptional activity. However, in the present study, the expression of HSF4 was significantly upregulated in the liver and muscle in broilers at day 15 and day 28 (Figure 4.6). This demonstrates that HSF4 transcriptional activity is influenced in broilers upon exposure to constant chronic heat stress.

Conclusion

Among broilers fed nicarbazin, those subjected concurrently to heat stress are better capable of protecting against nicarbazin-induced injury in the liver by maintaining an increased expression of CAT, HSP70, ghrelin, and GHS-R1a in the liver. Furthermore, when nicarbazin is fed to broilers in the absence of heat stress, a subsequent event of acute HS may cause an overproduction of ROS, decreased antioxidant capacity, induce oxidative damage, and induce fatty degeneration in the liver when compared to those acclimated to HS. Moreover, this study also suggests that when *Eimeria*-infected broilers are subjected to HS, the subsequent increase in antioxidant capacity may provide protection against coccidial infections by hindering ROS-induced obstruction of intercellular adhesion.

Table 4.1. Feed intake (FI) measured at 4 time periods for birds raised at 2 environmental temperatures and fed 3 anticoccidial treatments in the absence of coccidial infection.

Temperature Setting (°C)	Anticoccidial	FI (g)			
		14 to 15 d	15 to 21 d	21 to 28 d	14 to 28 d
TN	Unmedicated	82[a]	510[a]	535[a]	1132[a]
	Nicarbazin	75[b]	488[a]	479[a]	1061[a]
	Monensin	78[ab]	505[a]	531[a]	1116[a]
HS	Unmedicated	73[bc]	322[b]	345[b]	746[b]
	Nicarbazin	60[d]	341[b]	249[c]	613[c]
	Monensin	66[c]	308[b]	310[bc]	692[bc]
SEM		72	409	404	885
Source of Variation		*P*–values			
Environmental temperature		<0.0000	<0.0000	<0.0000	<0.0000
Medication		<0.0000	0.5296	0.0017	0.0002
temperature × medication		0.175	0.0277	0.5629	0.3495

[a–b]Means in a column not sharing a common superscript are significantly different ($P < 0.05$) b Student's *t* or Tukey's test.

Table 4.2. Primer pairs used to analyze gene expression by quantitative RT-PCR.

[1]Gene Symbol	GenBank Accession No.	Forward Primer (5'→3')	Reverse Primer (5'→3')
CAT	NM_001031215.2	GAGATGGTGAGGGCAGTTATT	GCCAATGTATGAGGAGGTTAGT
SOD	NM_205064.1	GGCTTGTCTGATGGAGATCAT	GCTTGCCTTCAGGATTAAAGTG
HSF1	L06098.1	CAGGGAAGCAGTTGGTTCACTACACG	CCTTGGGTTTGGGTTGCTCAGTC
HSF2	NM_001167764.1	CGCTGCTCGCATTCCT	TGTGGCCTCACTTGCTTCT
HSF3	NM_001305041.1	TCCACCTCTCCTCTCGGAAG	CAACAGGACTGAGGAGCAGG
HSF4	NM_001172374.1	TGCCAGCCTTCCTAACCAAG	TGGTGCCATTCGTACTCCAG
HSP60	NM_001012916.1	AGCCAAAGGGCAGAAATG	TACAGCAACAACCTGAAGACC
HSP70	NM_001006685.1	CGGGCAAGTTTGACCTAA	TTGGCTCCCACCCTATCTCT
HSP90	NM_001109785.1	GGCTACCCTATTAGGCTCTTTG	TCTTCTCCTCCTTCTCCTCTTT
avUCP	Q8AYM4	GAGGAGAATGGAGAGAGGAGAA	CCATCTGGAGAACCTCAACAC
CCK	NM_001001741	CCTAAAGAACAGCAGGCGATAG	CACTGATTCGAAGAGGACAACA
Ghrelin	Q8AV73	GTCTGGTCCAGTCATTACATCTC	GCTGAGAAGGAGAATTCCTAGC
GHS-R1a	NM_204394	GTGGATCTCCAGCATCTTCTT	GGACCGATGTTCTTCCTCTTC
GLP-1	NM_205260.4	CCGGATCAGCTCTCAAGTAATG	GCAGCTTGACCTTCCAAATAAG
Nesfatin-1	NP_001006468.1	GTGAGAACACGACTGGATGAA	CCTGATGGTCTATGCCAGTATC
β-actin	NM_205518.1	AGACATCAGGGTGTGATGGTTGGT	TCCCAGTTGGTGACAATACCGTGT

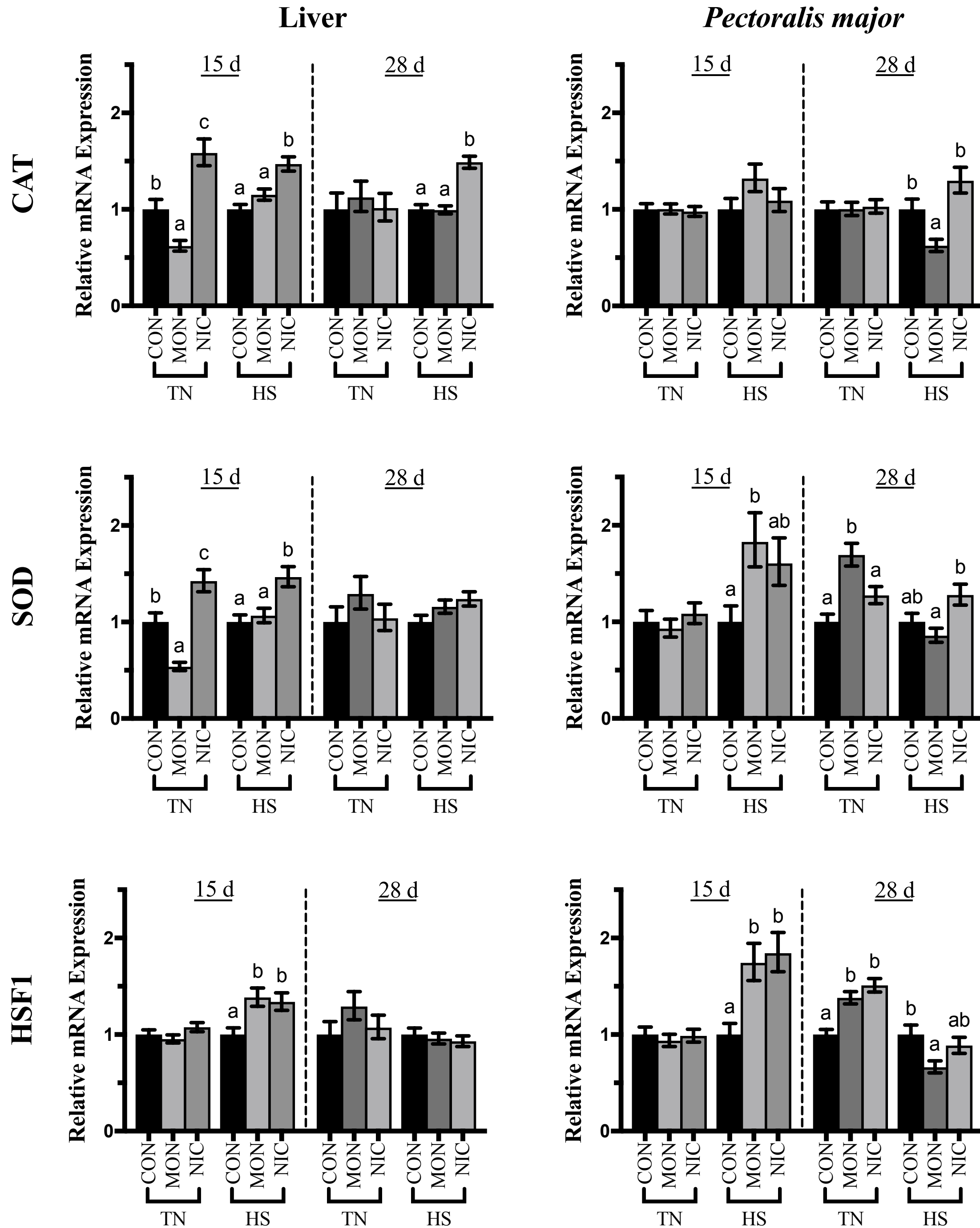

Figure 4.1. mRNA expression levels of catalase (CAT), superoxide dismutase (SOD), and heat shock factor 1 (HSF1) in the liver and *P. major* in the absence of coccidial infection.
[a-c] Gene expression means within the same cluster with no common super script differ significantly (*P*<0.05).

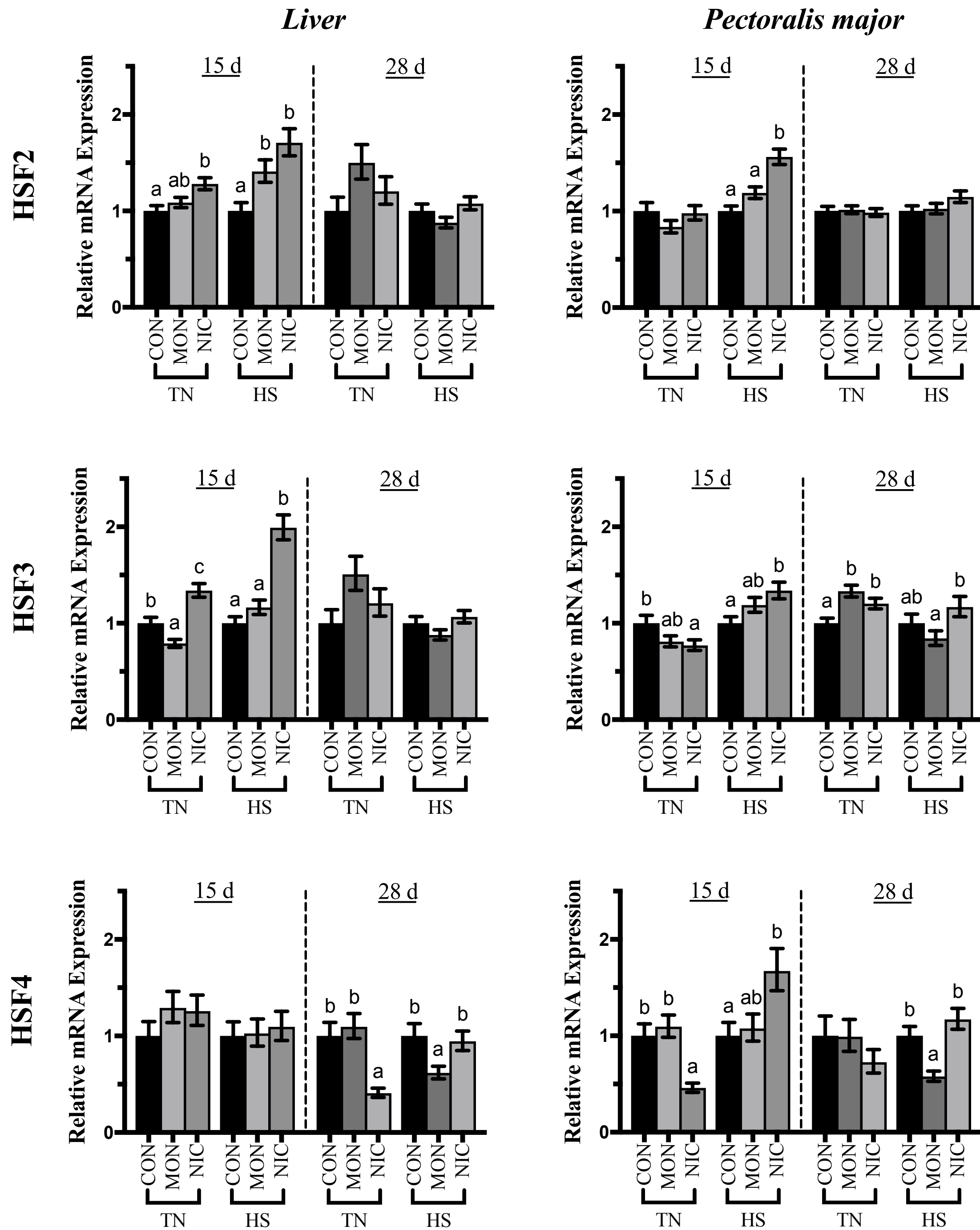

Figure 4.2. mRNA expression levels of heat shock factor 2 (HSF2), HSF3, and HSF4 in the liver and *P. major* in the absence of coccidial infection.
[a-c] Gene expression means within the same cluster with no common super script differ significantly (P<0.05).

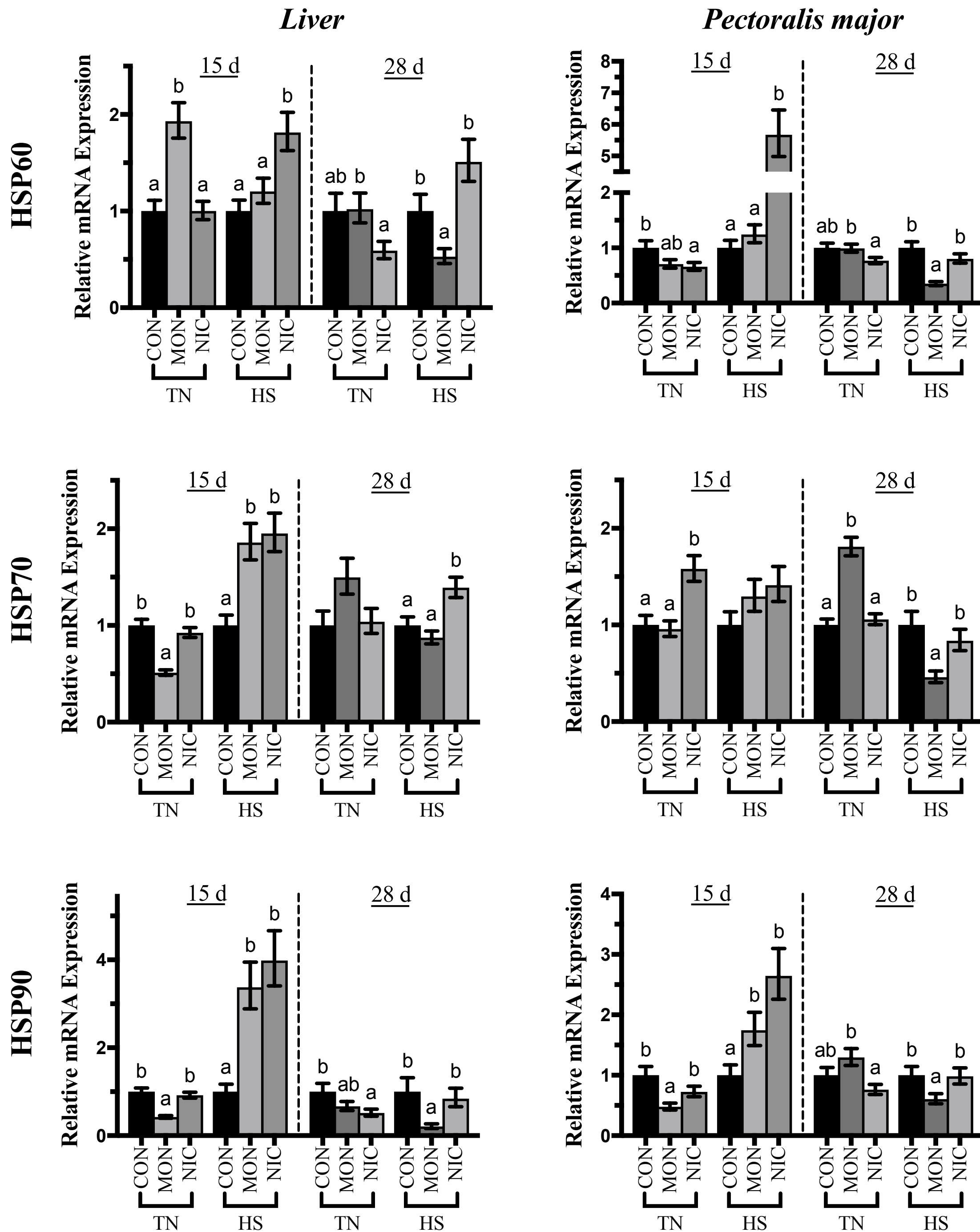

Figure 4.3. mRNA expression levels of heat shock protein 60 (HSP60), HSP70, and HSP90 in the liver and *P. major* in the absence of coccidial infection.
[a-c] Gene expression means within the same cluster with no common super script differ significantly (P<0.05).

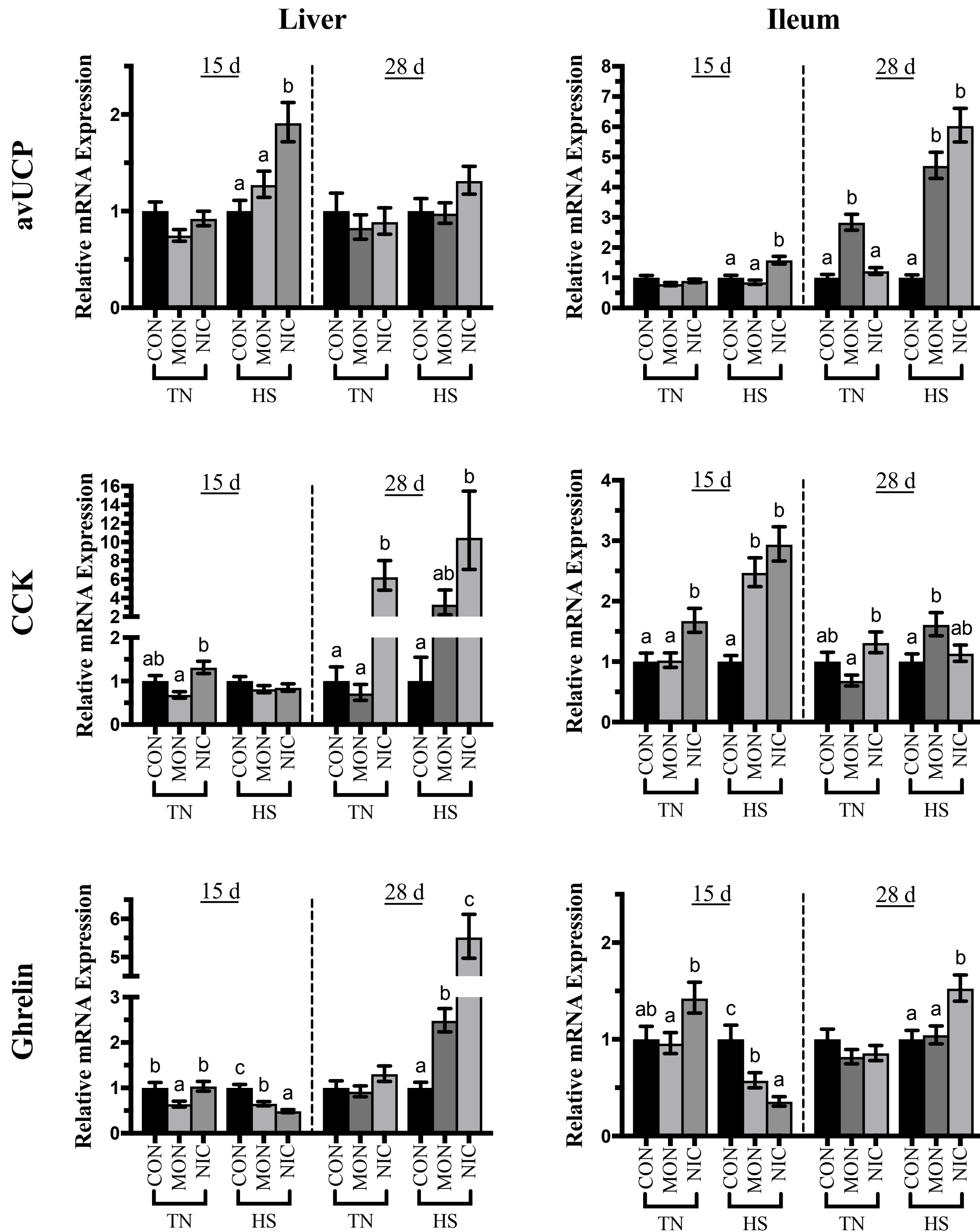

Figure 4.4. mRNA expression of avian uncoupling proteins (avUCP), cholecystokinin (CCK), and ghrelin in the liver and ileum in the absence of coccidial infection.
[a-c] Gene expression means within the same cluster with no common super script differ significantly (*P*<0.05).

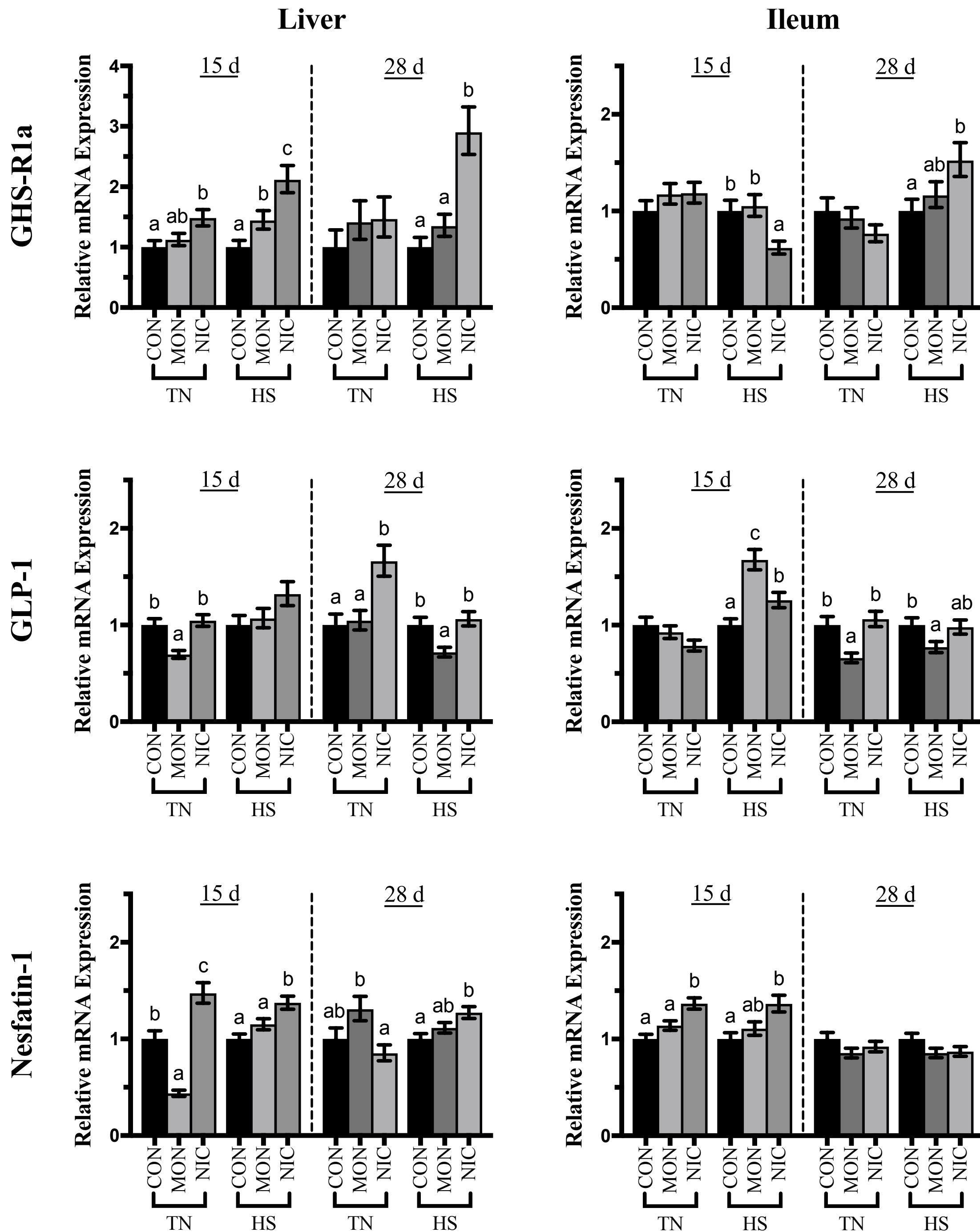

Figure 4.5. mRNA expression of growth hormone sectretagogue receptor (GHS-R), glucagon-like peptide 1 (GLP-1), and nesfatin-1 in the liver and ileum in the absence of coccidial infection.
[a-c] Gene expression means within the same cluster with no common super script differ significantly (P<0.05).

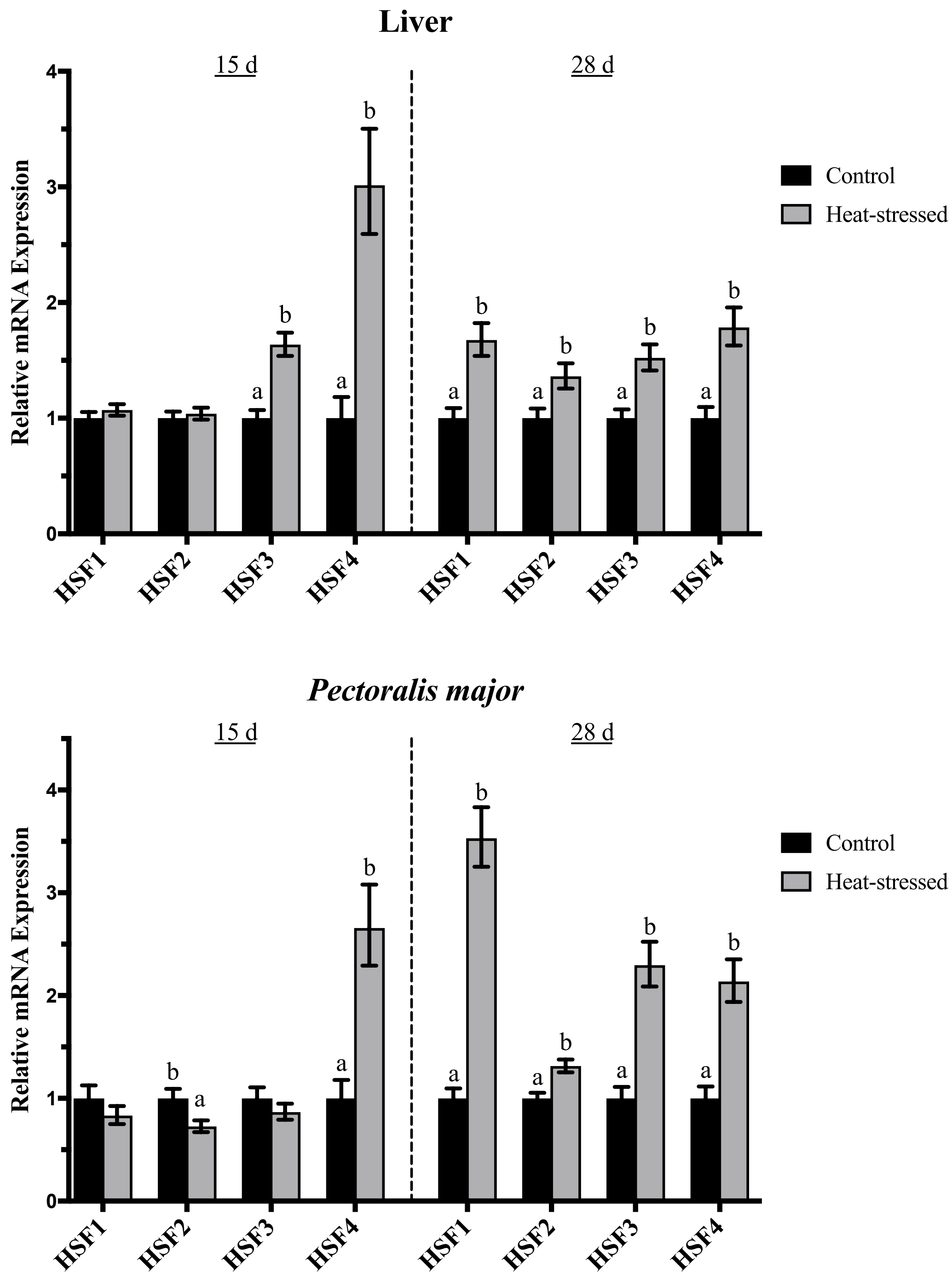

Figure 4.6. mRNA expression levels of heat shock factor 1 (HSF1), HSF2, HSF3, and HSF4 in the liver and *P. major* in broilers subjected to heat stress in the absence of coccidial infection.
[a-c] Gene expression means within the same cluster with no common super script differ significantly (*P*<0.05).

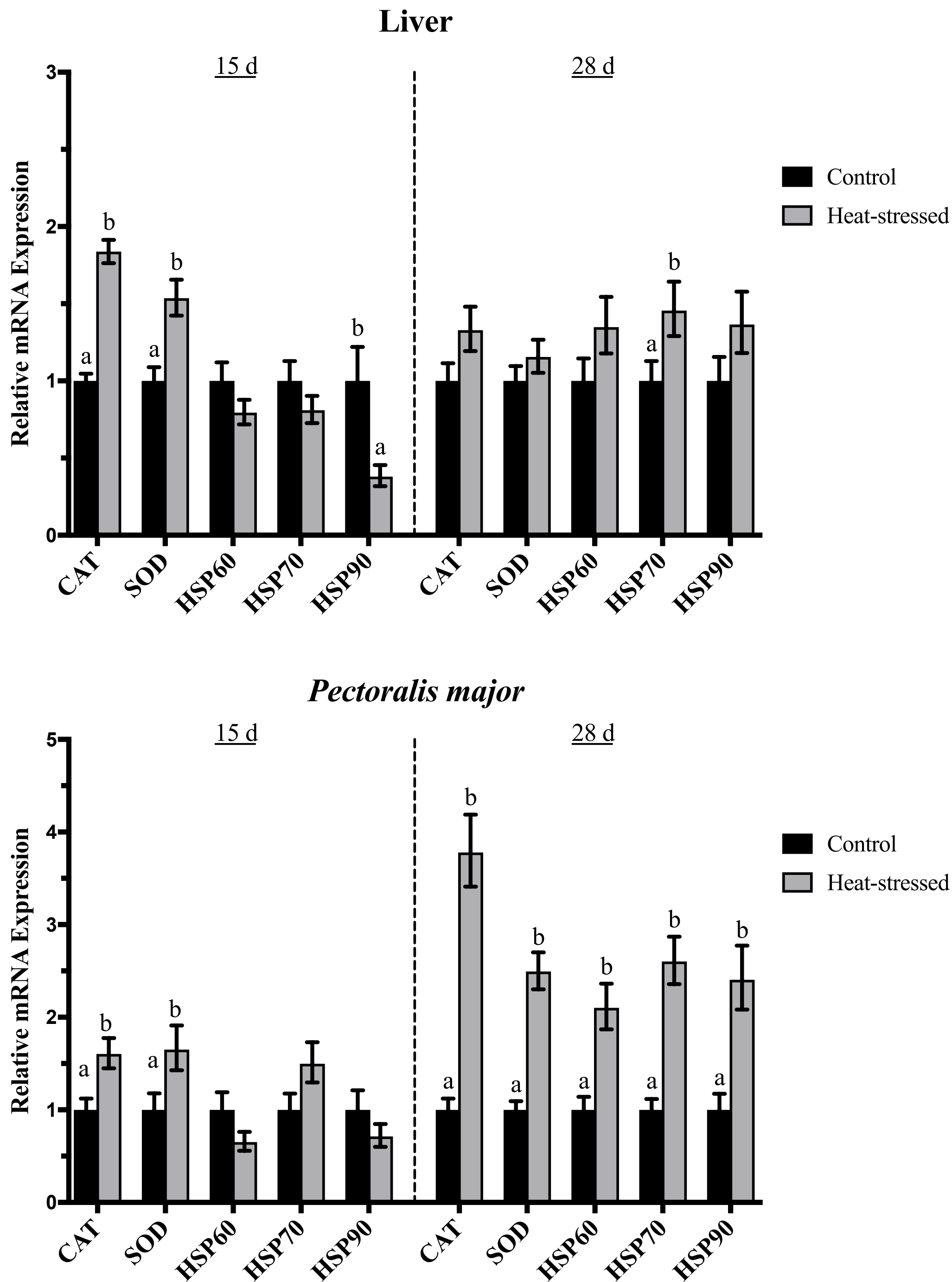

Figure 4.7. mRNA expression levels of catalase (CAT), superoxide dismutase (SOD), heat shock protein 60 (HSP60), HSP70, and HSP90 in the liver and *P. major* in broilers subjected to heat stress in the absence of coccidial infection.

[a-c] Gene expression means within the same cluster with no common super script differ significantly ($P<0.05$).

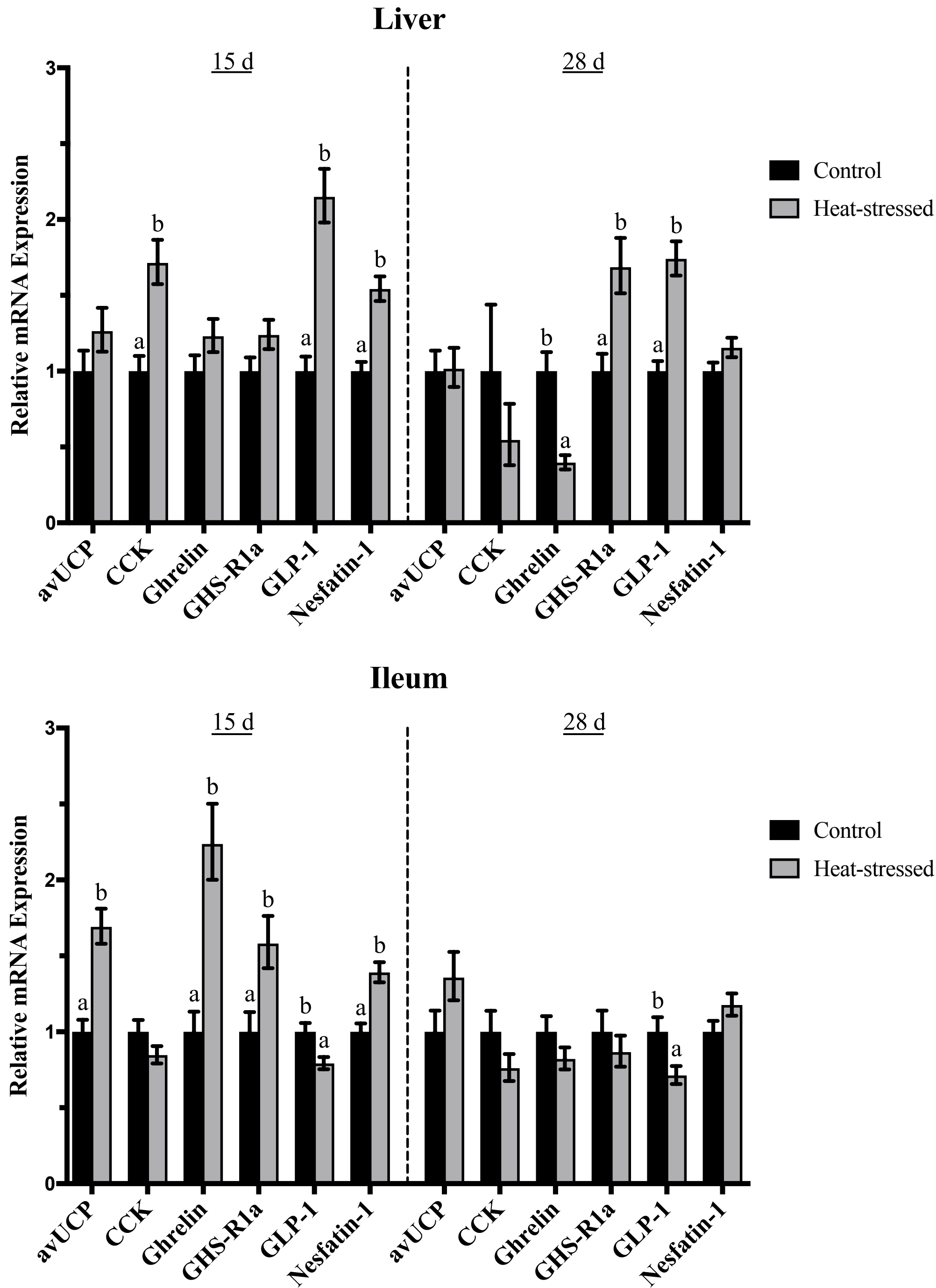

Figure 4.8. mRNA expression levels of avian uncoupling protein (avUCP), cholecystokinin (CCK), ghrelin, growth hormone sectretagogue receptor 1 (GHS-R1), glucagon, and nesfatin-1 in the liver and ileum in broilers subjected to heat stress, in the absence of coccidial infection.

$^{a\text{-}c}$ Gene expression means within the same cluster with no common super script differ significantly (P<0.05).

References

Aengwanich, W., and Simaraks, S. Pathology of heart, lung, liver and kidney in broilers under chronic heat stress. Pathology 26:418. 2004.

Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A., and Smet, S. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. Journal of Animal Science and Biotechnology 7:37. 2016.

Al-Zghoul, M.-B., Ismail, Z. B., Dalab, A. E. S., Al-Ramadan, A., Althnaian, T. A., Al-ramadan, S. Y., Ali, A. M., Albokhadaim, I. F., Al Busadah, K. A., Eljarah, A., Jawasreh, K. I., and Hannon, K. M. Hsp90, Hsp60 and HSF-1 genes expression in muscle, heart and brain of thermally manipulated broiler chicken. Research in Veterinary Science 99:105-111. 2015.

Azad, M., Kikusato, M., Maekawa, T., Shirakawa, H., and Toyomizu, M. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 155:401-406. 2010.

Banfield, M. J., Ten Doeschate, R. A., and Forbes, J. M. Effect of whole wheat and heat stress on a coccidial infection in broiler chickens. British Poultry Science 39 Suppl:S25-S26. 1998.

Blake, D. P., and Tomley, F. M. Securing poultry production from the ever-present Eimeria challenge. Trends in Parasitology 30:12-19. 2014.

Buys, S. B., and Rasmussen, R. W. Heat stress mortality in nicarbazine fed chickens. Journal of the South African Veterinary Association 49:127-128. 1978.

Buyse, J., Janssen, S., Geelissen, S., Swennen, Q., Kaiya, H., Darras, V. M., and Dridi, S. Ghrelin modulates fatty acid synthase and related transcription factor mRNA levels in a tissue-specific manner in neonatal broiler chicks. Peptides 30:1342-1347. 2009.

Cervantes, H. Managing coccidiosis in broiler breeders. In: Broiler Breeder & Hatchery Conference. N.C. 2014.

Ceylan, A., Ümran, T., Genç, O., Akcılar, R., and Şahin, S. Balanced oxidative status by nesfatin-1 in intestinal ischemia-reperfusion. International journal of clinical and experimental medicine 8:3318. 2015.

Coble, D. J., Fleming, D., Persia, M. E., Ashwell, C. M., Rothschild, M. F., Schmidt, C. J., and Lamont, S. J. RNA-seq analysis of broiler liver transcriptome reveals novel responses to high ambient temperature. BMC Genomics 15:1084-1084. 2014.

Cuckler, A. C., Malanga, C. M., and Ott, W. H. The antiparasitic activity of nicarbazin. Poultry Science 35:98-109. 1956.

del Cacho, E., Gallego, M., Lillehoj, H. S., Quílez, J., Lillehoj, E. P., Ramo, A., and Sánchez-Acedo, C. IL-17A regulates *Eimeria tenella* schizont maturation and migration in avian coccidiosis. Veterinary Research 45:25. 2014.

Dridi, S., Temim, S., Derouet, M., Tesseraud, S., and Taouis, M. Acute cold-and chronic heat-exposure upregulate hepatic leptin and muscle uncoupling protein (UCP) gene expression in broiler chickens. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 309:381-388. 2008.

Farny, D. G. O. Heat stress studies in nicarbazin treated chickens. In: Dissertation Abstracts. pp 7413-7413. 1965.

Georgieva, N. V., Koinarski, V., and Gadjeva, V. Antioxidant status during the course of *Eimeria tenella* infection in broiler chickens. The Veterinary Journal 172:488-492. 2006.

Halliwell, B., and Whiteman, M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? British Journal of Pharmacology 142:231-255. 2004.

He, X., Lu, Z., Ma, B., Zhang, L., Li, J., Jiang, Y., Zhou, G., and Gao, F. Effects of chronic heat exposure on growth performance, intestinal epithelial histology, appetite-related hormones and genes expression in broilers. Journal of the Science of Food and Agriculture. 2018.

Huckriede, A., Heikema, A., Agsteribbe, E., Sjollema, K., and Briones, P. Morphology of the mitochondria in heat shock protein 60 deficient fibroblasts from mitochondrial

myopathy patients. Effects of stress conditions. Virchows Archive 427:159-165. 1995.

Kaiya, H., Darras, V. M., and Kangawa, K. Ghrelin in birds: its structure, distribution and function. The Journal of Poultry Science 44:1-18. 2007.

Kaiya, H., Kangawa, K., and Miyazato, M. Update on ghrelin biology in birds. General and Comparative Endocrinology 190:170-175. 2013.

Kitazawa, T., Kaiya, H., and Taneike, T. Contractile effects of ghrelin-related peptides on the chicken gastrointestinal tract in vitro. Peptides 28:617-624. 2007.

Koinarski, V., Georgieva, N., Gadjeva, V., and Petkov, P. Antioxidant status of broiler chickens, infected with *Eimeria acervulina*. Revue de medecine veterinaire 156:498. 2005.

Kolgazi, M., Ozdemir-Kumral, Z. N., Cantali-Ozturk, C., Demirci, E. K., Yuksel, M., Sirvanci, S., and Yegen, B. C. Anti-inflammatory effects of nesfatin-1 on acetic acid-induced gastric ulcer in rats: involvement of cyclo-oxygenase pathway. Journal of Physiology and Pharmacology 68:765-777. 2017.

Lei, L., Hepeng, L., Xianlei, L., Hongchao, J., Hai, L., Sheikhahmadi, A., Yufeng, W., and Zhigang, S. Effects of acute heat stress on gene expression of brain-gut neuropeptides in broiler chickens (Gallus gallus domesticus). Journal of Animal Science 91:5194-5201. 2013.

Lei, L., Yu, J., and Bao, E. Expression of heat shock protein 90 (Hsp90) and transcription of its corresponding mRNA in broilers exposed to high temperature. British Poultry Science 50:504-511. 2009.

Livak, K. J., and Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408. 2001.

McDougald, L. R., and Fitz-Coy, S. H. Coccidiosis. In: Diseases of Poultry, 13 ed. D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez and V. Nair, eds. Wiley-Blackwell, Ames, Iowa. pp 1148-1166. 2013.

McDougald, L. R., and McQuistion, T. E. Mortality from heat stress in broiler chickens influenced by anticoccidial drugs. Poultry Science 59:2421-2423. 1980.

Mujahid, A., Akiba, Y., Warden, C. H., and Toyomizu, M. Sequential changes in superoxide production, anion carriers and substrate oxidation in skeletal muscle mitochondria of heat-stressed chickens. FEBS Letters 581:3461-3467. 2007.

Mujahid, A., Sato, K., Akiba, Y., and Toyomizu, M. Acute heat stress stimulates mitochondrial superoxide production in broiler skeletal muscle, possibly via downregulation of uncoupling protein content. Poultry Science 85:1259-1265. 2006.

Newberne, P. M., and Buck, W. B. Studies on drug toxicity in chicks: 3. The influence of various levels of nicarbazin on growth and development of chicks. Poultry Science 36:304. 1957.

Ray, G., and Husain, S. A. Oxidants, antioxidants and carcinogenesis. Indian Journal of Experimental Biology 40:1213-1232. 2002.

Richards, M. P., and Proszkowiec-Weglarz, M. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. Poultry Science 86:1478-1490. 2007.

Sammelwitz, P. H. Factors related to nicarbazin induced heat stress mortality in broilers. Poultry Science 44:1412. 1965.

Singh, M., Kalla, N. R., and Sanyal, S. N. Effect of monensin on the enzymes of oxidative stress, thiamine pyrophosphatase and DNA integrity in rat testicular cells in vitro. Experimental and Toxicologic Pathology 58:203-208. 2006.

Soleimani, A., Zulkifli, I., Omar, A., and Raha, A. Physiological responses of 3 chicken breeds to acute heat stress. Poultry Science 90:1435-1440. 2011.

Solmaz, A., Gülçiçek, O. B., Erçetin, C., Yiğitbaş, H., Yavuz, E., Arıcı, S., Erzik, C., Zengi, O., Demirtürk, P., and Çelik, A. Nesfatin-1 alleviates extrahepatic cholestatic damage of liver in rats. Bosnian Journal of Basic Medical Sciences 16:247. 2016.

Song, Z., Liu, L., Sheikhahmadi, A., Jiao, H., and Lin, H. Effect of heat exposure on gene expression of feed intake regulatory peptides in laying hens. Journal of Biomedicine and Biotechnology 2012. 2012.

St-Pierre, N. R., Cobanov, B., and Schnitkey, G. Economic losses from heat stress by US livestock industries. Journal of Dairy Science 86:E52-E77. 2003.

Surai, P. F., and Kochish, I. I. Antioxidant systems and vitagenes in poultry biology: Heat shock proteins. In: Heat Shock Proteins in Veterinary Medicine and Sciences. Springer. pp 123-177. 2017.

Wiernusz, C. J., and Teeter, R. G. Nicarbazin effects on broiler thermobalance during high ambient temperature stress. Poultry Science 74:577-580. 1995.

Zhang, X., Zhang, M., Su, Y., Wang, Z., Zhao, Q., Zhu, H., Qian, Z., Xu, J., Tang, S., Wu, D., Lin, Y., Kemper, N., Hartung, J., and Bao, E. Inhibition of heat stress-related apoptosis of chicken myocardial cells through inducing Hsp90 expression by aspirin administration in vivo. British Poultry Science 59:308-317. 2018.

CHAPTER 5

CONCLUSION

Three main conclusions can be made from the results reported on oocyst counts and gene expression. First, subjecting *E. maxima*-infected broilers to constant chronic heat stress reduces the severity of coccidiosis as determined by oocyst production, which is significantly curtailed. Second, subjecting broilers to constant chronic heat stress causes to the obstruction of *E. maxima* oocyst production possibly by inhibition of ROS production, intestinal motility, and improved antioxidant capacity. Third, HS-acclimated broilers fed nicarbazin are less susceptible to nicarbazin-induced heat stress toxicity, at least in part, through increased protection against hepatic lipogenesis, inhibited ROS production, inhibited intestinal motility, suppressed inflammation and increased antioxidant capacity.