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Heat stress-induced physiological changes in pigs, and its mitigation by dietary treatments

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LIST OF ABBREVIATIONS AND ACRONYMS

ACC	Acetyl coenzyme A carboxylase
ADFI	Average daily feed intake
ADG	Average daily gain
AJ	Adherens junction
ANOVA	Analysis of variance
AT	Ambient temperature
ATP	Adenosine triphosphate
BW	Body weight
CAT	Catalase
CKII-α	Casein kinase II-α
CP	Crude protein
cPKC	conventional protein kinase C
DM	Dry matter
FASS	Federation of Animal Science Societies
FCR	Feed conversion ratio
g	Gram
g	Relative centrifugal force
GIT	Gastrointestinal tract
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
h	Hour
HI	Heat increment
HIF-1α	Hypoxia-inducible factor 1-alpha
HS	Heat stress
HSP	Heat shock protein
IE	Intestinal epithelium
IEC	Intestinal epithelial cell
IL	Interleukin
IPCC	Intergovernmental Panel on Climate Change
ISC	Intestinal stem cells

IU	International unit
kDA	Kilo Daltons
kg	Kilogram
LPS	Lipopolysaccharides
ME	Metabolizable energy
MLCK	Myosin light chain kinase
MUC	Mucin glycoproteins
NFE	Nitrogen-free extract
OM	Organic matter
OS	Oxidative stress
ppm	Parts per million
PSE	Pale soft exudative
RE	Retained energy
RH	Relative humidity
ROS	Reactive oxygen species
SFK	c-Src family kinases
SOD	Superoxide dismutase
T3	Triiodothyronine
T4	Thyroxine
TER	Transepithelial resistance
TJ	Tight junction
TNF-α	Tumor necrosis factor alpha
TNZ	Thermo-neutral zone
USA	United States of America
ZnAA	Zinc amino acid
ZO-1	Zonula occludin-1

1. INTRODUCTION

The increase in global population, human activities, and the continuous use of natural resources to satisfy human interest and food production have led to global warming faster than in the last century. The Intergovernmental Panel on Climate Change (IPCC) reported that from 2001-2020, the earth's surface warmed by 0.99 °C more than it did between 1850-1900. The last decade (2011-2020) was the warmest, with temperatures rising by about 1.09 °C above that period and is expected to increase by 1.5 °C in the following decades (IPCC, 2021). Temperature is a significant environmental variable that influences domesticated animals' health, welfare, and productivity (KUCZYNSKI et al., 2011). Hence, changes in environmental temperature brought by climate change can compromise agricultural and livestock production (MAYORGA et al., 2018*a*; PASQUI and DI GUISEPPE, 2019; THORNTON et al., 2021). Extreme climate events are responsible for significant material losses globally, and extreme heat has negatively influenced agricultural productivity in many countries, including the USA and Europe (BATTISTI et al., 2009; KUCZYNSKI et al., 2011). One of the primary issues restricting production efficiency in the swine industry is the climate, particularly when the ambient temperature (AT) is above the pigs' thermal comfort level. A direct result of climate change, thermal stress, particularly heat stress (HS), can have a negative effect on animal welfare, nutrition, and animal health by disrupting metabolism, generating oxidative stress (OS), and suppressing the immune system (BABINSZKY et al., 2011; CUI and GU, 2015; LACETERA, 2018; LACETERA, 2019; BERNABUCCI et al., 2019; CUI et al., 2019; BORGES et al., 2020). Moreover, its impact on the animals' feed intake, nutrient utilization, reproduction, and production performance can potentially cause significant economic losses (ABDUREHMAN and AMEHA, 2018). Pigs' performance suffers as a result of physiological, metabolic (alteration of plasma metabolites concentration), and behavioral changes brought on by thermoregulatory responses to HS (RENAUDEAU et al., 2013; GABLER and PEARCE, 2015). Such response includes an increase in water intake, a reduction in feed and nutrient intake, and an increase in respiration rate, which makes pigs less able to utilize the dietary energy in the diet and uses most of the diet's metabolizable energy for maintenance (BABINSZKY et al., 2012; COTRELL et al., 2020). HS can also impair the integrity and functionality of the gastrointestinal tract by inflicting intestinal damage and inducing hypoxia and OS (GABLER and PEARCE, 2015; COLLIN et al., 2001*b*; CUI and GU, 2015; YU et al., 2010). HS can also promote electrolyte losses through excessive urination and evaporation, leading to electrolyte imbalance which is also

exacerbated by the stressors' influence on respiratory alkalosis and renal failure (GABLER and PEARCE, 2015; TANG et al., 2018; COTTRELL et al., 2020; HEO et al., 2005; AGRIC.WA.GOV., 2020).

Although research reported that HS could be detrimental to pig performance, several studies also noted that the duration of the pigs' exposure to HS could influence the susceptibility and adaptability of pigs to HS consequences (RENAUDEAU et al., 2008; RENAUDEAU et al., 2010: PEARCE et al., 2014), and some dietary antioxidants (vitamins and micro-minerals) are capable of alleviating HS adverse effects. Various nutritional interventions have been studied, particularly on the supplementation of feed additives, and have shown potential in alleviating the impact of HS on the animal's performance (RHOADS et al., 2013). However, HS will remain a significant threat to the swine industry with the expected global temperature rise. Therefore, it is necessary to understand its induced-physiological changes and explore a practical and economic nutritional approach to address it. Vitamins (C and E) and micro-minerals (selenium (Se) and zinc (Zn)) are potential nutritional tools to mitigate the HS adverse effects. The antioxidant function of vitamin C is to protect cellular structures from the damaging effects of free radicals. Vitamin E, on the other hand, as a lipid-soluble antioxidant, can neutralize free radicals caused by HS (TRABER and STEVENS, 2011). Se and Zn, have their distinct antioxidant function. To prevent cell damage and OS, Se incorporates into proteins in the form of selenoproteins (antioxidant enzymes). While Zn slows the oxidative process by inducing the expression of metallothionein (responsible for zinc-related cell homeostasis and protection against oxidative stress) and acts as a powerful electrophilic scavenger and cytoprotective agent (KIELCZYKOWSKA et al., 2018; JAROSZ et al., 2017).These substances capability of neutralizing the excess reactive oxygen species (ROS) produced during HS-induced OS and protect cells against the toxic effects of free radicals could improve the nutrient utilization and performance of heat-stressed pigs (PHAM-HUY et al., 2008; LOBO et al., 2010; COTTRELL et al., 2015). There are several studies regarding the use of several dietary antioxidants to mitigate the effect of HS (LIU et al., 2016; PEARCE et al., 2015*a*; MANI et al., 2019), but to our knowledge, supplementation of vitamins C and E, and micro-minerals Se and Zn at elevated levels and in combination to address chronic HS in pigs is not yet explored. Also limited information is available on the influence of the length of chronic HS on the physiological changes in pigs.

1.1. Research aims

The main aim of the research program was to determine the effects of chronic HS and the mitigation capacity of combined dietary antioxidants supplementation in the diet (Se, Zn, vitamin E, and C) on the physiological responses of high genetic potential fattening pigs (DanBred), especially on the:

- Production performance (growth performance and meat quality)
- Nutrient and mineral digestibility and retention (faecal and ileal)
- Blood biochemical parameters and electrolyte balance
- Immune response (gene expression of cytokines and heat shock proteins)

2. LITERATURE REVIEW

2.1. Effect of heat stress on pigs

The progressive increase in global temperature engendered by climate change and the ensuing heat stress (HS) intensification is a severe threat to food security that is expected to stay throughout the 21st century (FAO, 2016; WANG and ZHANG, 2019). HS is a physiological condition when a hotter environment compromises an animal's ability to control their internal temperature. In this condition, animals receive more heat from their surroundings than it is transferring from their body to the environment and at the same time, produce more heat from metabolism. Although high ambient temperature is a huge factor in causing HS in pigs, it is not the sole culprit in inducing HS to pigs, as studies show that it is also influenced by relative humidity (RH) and the index that combines these factors is called heat index (HI). HS index charts released by Iowa State University and Ontario Ministry of Agriculture show that pigs are in danger of experiencing HS at AT of 26 °C with an RH of 75 to 90%. This indicates that even at temperatures within the desirable limits for pigs, it still makes them susceptible to HS when the RH is high. This can be supported by the fact that, under such temperatures, pigs tend to loss more heat through evaporation (panting); however, it is insufficient as higher RH makes it less effective since less moisture can be evaporated. However, when the AT is at 30 °C and above, RH under 50% is enough to cause HS to pigs (XIN and HARMON, 1998; EASTWOOD, 2020). Pigs are negatively affected by HS, and it was suggested that modern genotypes are more sensitive to its adverse effects than older genotypes; as modern genotypes produce more metabolic heat (BROWN-BRANDL et al., 2001; BROWN-BRANDL et al., 2004; RENAUDEAU et al., 2011; RAUW et al., 2017).

Pigs are homeothermic animals, they can maintain a relatively constant body temperature within narrow limits despite a wide variation in the surrounding climatic environment. The physiological process that pigs perform to balance heat production and heat loss mechanism is called thermoregulation (COLLIER et al., 2019). Pigs have several means of regulating body temperature, pigs lose heat through sensible (conduction, thermal radiation, and convection) and latent heat loss (evaporation). Conduction, thermal radiation, and convection each requires a temperature gradient between the animal and its surroundings. Thus, as the ambient temperature rises, animals redistribute blood to the skin in an effort to increase radiant heat loss. Even so, evaporation is the only effective route of heat loss when the ambient temperature rises above the pigs' thermal comfort. This is because the rise of ambient temperature makes the temperature gradient between the animal and the environment

smaller, subsequently it decreases the transfer of heat by conduction, radiation, and convection. Furthermore, the pigs' thick subcutaneous adipose tissue layer affects their thermoregulatory ability, which hinders sensible heat loss. Considering their lack of functional sweat glands their evaporative heat dissipation is more likely dependent on the respiratory route (panting). The pigs increase in respiration rate under HS condition promotes an increase in the airflow and evaporation of water from the lungs, subsequently releasing heat (COLLIER and GEBREMEDHIN, 2015; SMITH and EASTWOOD, 2017; MAYORGA et al., 2018*a*). The ambient temperature range that suits the pigs' comfort is called thermo-neutral zone (Table 1). As the ambient temperature (AT) rises above it, production efficiency suffers because nutrients are diverted to maintain euthermia, as maintaining a safe body temperature becomes the top priority (RENAUDEAU et al., 2012; MAYORGA et al., 2018*a*). Maintaining euthermia under HS through increasing heat loss can lead to physiological strains (reduced nutrient intake, digestibility, retention, etc.). If heat loss is inadequate, it can force the pig to initiate various strategies (behavior) to minimize heat production that could negatively affect the animal's production synthesis and performance (JOHNSON et al., 2013; HAO et al., 2014; PEARCE et al., 2015*b*).

Table 1.

Thermal comfort of growing and finish pigs

Class of pigs	Thermal comfort	Lower extreme	Upper extreme
Growing 35 to 70 kg	15 to 25°C (59 to 77°F)	−5°C (23°F)	35°C (95°F)
Finishing 70 to 100 kg	10 to 25°C (50 to 77°F)	−20°C (4°F)	35°C (95°F)

Source: FASS, 2010

2.1.1. HS effect on feed intake and growth

Pigs exposed to high environmental temperatures activate numerous physiological, metabolic, and behavioral mechanisms to reduce heat production and increase heat dissipation to maintain euthermia (ARTUSO-PONTE, 2018). The amount of voluntary feed intake and how livestock animals use their metabolizable energy depend on the environment. Animals exposed to high external temperatures make more effort to expel heat from their bodies by breathing more quickly, drinking more water, and eating less food (ALEENA et al., 2016). The pig’s voluntary feed intake reduction attenuates the thermic effect of feeds (TEF) and heat production (CERVANTES et al., 2018). It also facilitates the necessary heat loss

(HUYUNH and AARNIK, 2005). HS directly affects metabolic activity by increasing the secretion of two adipokines: leptin and adiponectin, and the expression of their receptors (BERNABUCCI et al., 2009). Leptin increases hypothalamic axis stimulation and decreases feed intake (RABE et al., 2008; MORERA et al., 2012). Adiponectin regulates feeding behavior via peripheral and central mechanisms and acts as a hunger signal. Hence, by raising leptin and adiponectin levels and decreasing feed intake, HS stimulates the hypothalamic axis. Because of the calorie restriction, hyperthermic animals can produce less heat (HOYDA et al., 2012; SLIMEN et al., 2016). However, this could lead to consequences on the animals' production performance and product quality as it will also lower their nutrient uptake (RENAUDEAU et al., 2011; SONG et al., 2011; YANG et al., 2014).

Growing to finish pigs are more prone to HS than young pigs; as the pig gets older and heavier, its optimal temperature decreases (18-21 °C). Pigs' exposure to acute HS (37 °C for 2-6 hours) and long-term HS above 27°C for more than 2 to 4 days can cause substantial losses in their production performance (MYER and BUCLIN, 2001; PEARCE et al., 2014; MORALES et al., 2014). Several pieces of research have confirmed this. COLLIN et al. (2001*a*) reported that the voluntary feed intake of pigs was reduced when exposed to a temperature of 33 °C, which lowered body weight gain and negatively affected the pigs' feed efficiency. LE BELLEGO et al. (2002) also confirmed that increasing AT above thermoneutrality reduced the average daily feed intake (ADFI) of pigs to 55 g per degree of increase in temperature over the entire duration of the pigs growing period (27-100kg). In another study, OLIVEIRA et al. (2018) observed a reduction of average daily gain (ADG) and ADFI of 24.7% on growing to finish pigs reared under HS conditions compared to pigs in thermal comfort (22 °C). It was also observed that pigs under HS are the least efficient feed converters. Moreover, RENAUDEAU et al. (2011); CAMPOS et al. (2017) revealed that when the pig's body weight increases, the AT impact becomes more pronounced. A degree increase in AT between 24 and 30 °C results in a 50-gram reduction in feed intake in pigs weighing 60 kg, and the corresponding reduction would be an average of 80 grams in pigs weighing 90 kg. However, the reported decrease in ADFI and ADG is also influenced by the duration of exposure, as SANTOS et al. (2018) observed a 31% reduction of the said parameters in pigs exposed to HS (30 °C) for 27 days (1st period of their trial) compared to those pigs in thermal comfort (23 °C). However, on 28 to 55 days (2nd period of their trial), the said reduction was lesser (26 and 18%, respectively). The decrease in ADFI in this period was around 100 g/°C; the ADG of heat-stressed pigs in the 1st period and 2nd period was 310 and 175 grams per

day lower than pigs under thermal comfort, respectively. It indicates that the growth performance of pigs exposed to long-term HS is still negatively affected.

2.1.2. The intestinal epithelium

The function of the gastrointestinal tract (GIT) is vast. Feed consumption, digestion, subsequent active or passive nutrient absorption, barrier function, and host interactions with GIT microbiota are all included in this. Additionally, it regulates immunological and epithelial processes necessary for the body's normal biological processes and homeostasis, both in the GIT and the body (PLUSKE et al., 2018). Its optimal and effective functionality and health are essential in determining animal performance (CELI et al., 2017). The intestines' digestive, absorptive and protective functions depend on an intact and functional intestinal epithelium (IE) (UMAR, 2010). As an active barrier, it serves both the uptake of nutrients and prevents harmful substances and potential pathogens from entering the bloodstream (STROMBERG et al., 2004; OSWALD, 2006). The intestine is inhabited by many microorganisms, which provides an avenue for nutrition, metabolism, and immunity (OKUMURA and TAKEDA, 2017). A single layer of intestinal epithelial cells (IECs) bound together by tight junctions (TJs) (composed of transmembrane proteins: occludin, claudins, and junctional adhesion molecules) makes up the IE (SCHNEEBERGER and LYNCH, 2004; SUZUKI, 2020). This unique composition of the IE prevents harmful microorganisms, antigens and toxins from the gut lumen from entering the circulation (WILLIAMS et al., 2014). This is achieved through the IEC's function in creating a mucosal barrier (physical and chemical barrier) which maintains symbiosis between the gut microbiota and the host. The barriers maintain homeostasis by segregating gut microbiota and host immune cells, which prevents inflammation due to excessive immune response (OKUMURA and TAKEDA, 2018). IECs also influence the recruitment and activation of immune cells through the production of cytokines and chemokines (OSWALD, 2006; STADNYK, 2002), thus being appreciated as a central component of innate immunity (ONYIAH and COLGAN, 2016). As a single cell layer, IE is selectively permeable through transcellular (nutrients passing through the cell) and paracellular (via TJ) pathways (DOKLADNY et al., 2016). The IEC maintains barrier integrity through weak protein-protein bonding of junctional complexes such as TJ, adherens junction (AJ), and desmosomes, all of which have occlusive properties (CAICEDO et al., 2016). Proteins that link adjacent epithelial cells to the actin cytoskeleton are present in the AJ and desmosomes, which are essential for the mechanical linking of cells (GABLER and

PEARCE, 2015; GROSCHWITZ and HOGAN, 2009). The TJ regulates the formation of intestinal barriers by modulating cell proliferation, differentiation, and polarization (MATTER et al., 2005). It is also responsible for regulating ions, solutes, and water across the intestinal epithelium through paracellular movement (LEE et al., 2018). The pigs' IE renews every two to three days, compelled by the intestinal stem cells (ISCs) (VERDILLE et al., 2019). This ensures that only the fittest and metabolically able cells comprise the IE and maintain an impermeable barrier to gut microbiota and luminal contents, as well as for nutrient digestion, absorption, and secretion of antimicrobial peptides (BLANDER, 2016). Another positive influence of ISCs is the generation of highly proliferative transit-amplifying cells. These cells then differentiate into enterocytes and secretory cells upon migration to the villi and Paneth cells (secrete antimicrobial peptides) upon migration towards the crypt. Although Paneth cells' existence in the pig's intestine is debatable (MYER, 1982), several researchers have confirmed that it exists (GONZALEZ et al., 2013; VAN DER HEE et al., 2018). The stability of ISC's self-renewal and differentiation controls intestinal epithelial homeostasis and is essential for ensuring intestinal epithelial integrity (VERDILLE et al., 2019).

2.1.3. Pigs' intestinal integrity and function and immune response under heat stress challenge

Harmonized regulation of the mucus layer, TJs, IECs, and the enteric immune system influences the intestine's integrity and function (GABLER and PEARCE, 2015; GROSCHWITZ and HOGAN, 2009). The first line of defense against intestinal injury is provided by the intestinal mucus layer (JOHANSSON et al., 2013), as it serves as a physical barrier against bacteria and antigenic substances in the lumen by coating the interior surface of the intestine and lubricating its luminal contents (HERATH et al., 2020). It is composed of mucin glycoprotein (MUC2) and bioactive molecules such as epithelial-bound mucins (MUC1, MUC3, and MUC17), which are synthesized by the intestinal goblet cells (KIM and HO, 2010) that are confined to the crypts of Lieberkühn and on the small intestinal villi. In the colon, goblet cells amass at the opening of the colonic crypts and are also found deep within the crypts and on the surface of the colon (BIRCHENOUGH et al., 2016; SCHROEDER, 2019). The high polymeric protein backbone structure of these mucins linked to numerous hygroscopic and hydrophilic oligosaccharide side-chains contributes to the mucus layers' gel-like structure (KIM and HO, 2010; ANDRIANIFAHANANA et al., 2006). The intestinal mucus layer establishes an active semi-permeable barrier that allows

passage of nutrients from the gut lumen towards the epithelium (MACIERZANKA et al., 2006) while promoting clearance that separates bacteria from the epithelial cells that inhibit inflammation and infection (HANSSON, 2012). HS can directly and indirectly (via reduced feed intake) compromise the intestinal integrity of pigs. The former takes effect as HS induces hypoxia (through shifting the splanchnic blood flow to the peripheral blood circulation, which results in reduced blood flow to the intestinal epithelium, subsequently decreases its oxygen supply) and OS in the intestine, as assessed by an acute increase of Hypoxia-inducible factor 1-alpha (HIF-1α mRNA) abundance and increase in lipid oxidation (4-HNE abundance). Moreover, intestinal structures of proteins responsible for cell structure and motility (such as alpha-actinin-1 and myosin regulatory light chain) as well as for cellular proliferation and apoptosis tumor necrosis factor (TNF) receptor-associated protein 1 and Erlin-2) are altered by HS along with reduction of endogenous antioxidants (GPx and GSH). This leads to the deprivation of oxygen and nutrients to the enterocytes and the loosening of TJs. Nutrient restriction due to reduced feed intake also causes alterations in intestinal function and morphology of pigs, thus accompanied by HS's direct effects exacerbating intestinal permeability (COLLIN et al., 2001*b*; CUI and GU, 2015; PEARCE et al., 2013*a*; LAMBERT, 2009; ZEITOUNI et al., 2016; GUPTA et al., 2017; LIAO and NYACHOTI, 2017; LIAN et al., 2020) with an emphasis on intestinal morphology parameters, such as reduced villus height and crypt depth, villus to crypt ratio and decrease in the mucosal surface, sloughing of the intestinal villi as well as undergoing autolysis, regardless of the duration of exposure (YU et al., 2010; DONG et al., 2012; PEARCE et al., 2012; PEARCE et al., 2013*a*; PEARCE et al., 2014; KUMAR et al., 2017; LAN and KIM, 2018; ABUAJAMIEH et al., 2018). This then influences changes in cellular proliferation and membrane function (SONNA et al., 2002). The intact morphological structure of the intestine is important for nutrient utilization and absorption, and it is associated with longer villi which is a good indicator of a healthy gut; thus, its reduction and damage can be cautiously used to represent increasing intestinal permeability and infiltration of endotoxins (ABUAJAMIEH et al., 2018; YU et al., 2010). The translocation of endotoxins into the blood can instigate an inflammatory response and production of cytokines (interleukin 1β (*IL-1β*) and tumor necrosis factor-alpha (*TNF-α*)) (CUI et al., 2019; GANESAN et al., 2016). Nevertheless, these consequences on intestinal integrity can be influenced by the duration and intensity of the pigs' exposure to HS. During the first 2 to 4 h of HS (37 °C and 40 % RH), the pigs' intestinal integrity in the ileum declined,

associated with a decrease in transepithelial resistance (TER), while the colon remained unaffected during this period. Between 6 and 12 h TER rebounds as it increases; however, it ultimately declines after 24 h of exposure signifying intestinal permeability (PEARCE et al., 2014; PEARCE et al., 2012; PEARCE et al., 2015*b*). Under such conditions, PEARCE et al. (2014) observed an increase in ileum MUC2 after 6h of exposure to HS, which can act as a protective barrier for the intestine and may combat the decrease in intestinal integrity. However, in another study, pigs exposed to HS for 3 h showed a reduction of goblet cells in the jejunum and ileum (KPODO et al., 2020), and this was also observed even during the recovery period of 7 days after constant exposure to HS for 3 days (ABUAJAMIEH et al., 2018). This suggests that mucin glycoprotein (MUC2) production and activity can be reduced as this is produced by the goblet cells, which can then potentially compromise intestinal function; which could lead to a higher risk of infection caused by increased bacterial adhesion to the epithelium (KPODO et al., 2020; BROOM et al., 2018). PEARCE et al. (2013*b*) observed decreased resistance across the intestinal epithelium of pigs under HS, as assessed by reduced TER, which favors increasing intestinal permeability and allows the translocation of lipopolysaccharides (LPS) into the systemic circulation; that leads to endotoxemia and increases inflammation (DOKLADNY et al., 2006; GABLER et al., 2018).

TJ, which is responsible for the intestine's physical barrier integrity, can be compromised under HS with evidence of its altered expression and localization. Pigs under 24 h of exposure to HS showed increased claudin3 and occludin in the ileum (PEARCE et al., 2013*b*). Claudin3, acts as a sealing component of TJ, and occludin is essential for TJ integrity (GÜNZEL and FROMM et al., 2012). An increase in the TJ proteins may indicate improvement of the intestinal barrier during HS as it strives to relieve the stress-induced permeability; this is often associated with heat-induced expression of heat shock proteins (HSPs) (DOKLADNY et al., 2006). Recovery of the intestine from HS is influenced by HSPs (HSP27, 70, and 90). These molecular chaperones aid in protein folding and cell survival under stress conditions. Expression of these proteins was upregulated in the ileum and colon of pigs within 2 to 4 h of exposure to HS; such upregulation might influence the partial recovery from HS. LIU et al. (2009) observed severe damage in the small intestine (epithelium shedding at the tips of the intestinal villi, and shorter villus height and shallower crypt depth for the duodenum and jejunum) of Chinese mini pigs exposed to HS (40 °C) 5 h a day for 3 days. However, they also observed gradual

recovery from the pigs exposed to the same condition on the 6th day of the exposure until day 10; still, it is incomparable to those pigs in the thermo-neutral group. This gradual recovery is possibly influenced by HSPs' ability to prevent the activation of conventional protein kinase C (cPKC), resulting in reduced myosin light chain kinase (MLCK) protein phosphorylation of the actin cytoskeleton (PEARCE et al., 2014; LIU et al., 2009). Noticeably, TJ protein distribution was altered; as PEARCE et al. (2013*b*) observed, the upregulation of claudin protein was more in the membrane fraction, while occludin was more in the cytosolic fraction. TJ complexes' regulation is disturbed with emphasis on key kinases, particularly in a c-Src family kinases (SFKs) manner (PEARCE et al., 2013*b*; BASUROY et al., 2003). Multiple kinases' influence on occludin phosphorylation is believed to contribute to the regulation and modification of TJ; involvement of SFKs in the assembly and integrity of TJ is evident by regulating cell proliferation, migration, differentiation, and adhesion (DÖRFEL and HUBER, 2012; OKADA, 2012). Disassociation of TJ protein complexes was observed upon activation and upregulation of Casein kinase II-α (CKII-α) in the ileum of pigs under HS, which impaired its barrier function (PEARCE et al., 2013*b*). Indeed, as observed in mice, CKII-α plays significant roles: in occludin phosphorylation and as a regulator of zonula occludin-1 (ZO-1), claudin-1, and claudin-2proteins (RALEIGH, 2011). Moreover, increased expression and activation of MLCK were observed and associated with reduced intestinal integrity (PEARCE et al., 2013*b*). MLCK primarily mediates the actin cytoskeleton regulation in the epithelial cells, which affects its role in TJ physiology and intestinal integrity (TURNER, 2006). In a different study, where finishing pigs were subjected to cyclical HS (35 ˚C for 12 h and 22 ˚C for 12 h) for 30 days, reduced expression of TJ proteins ZO-1 and occludin were observed compromising their epithelial barrier function (YI et al., 2020). Despite the varied intensity of HS, the intestinal integrity and function of pigs are compromised as assessed by HS's effects on the intestinal mucus layer, TJs, enteric immune, and antioxidant system, which are condensed in *Table 2*. The condition of the gut under thermo-neutral zone (TNZ) and HS is illustrated in *Figure 1*, along with the role of endogenous antioxidants in suppressing ROS.

Table 2.

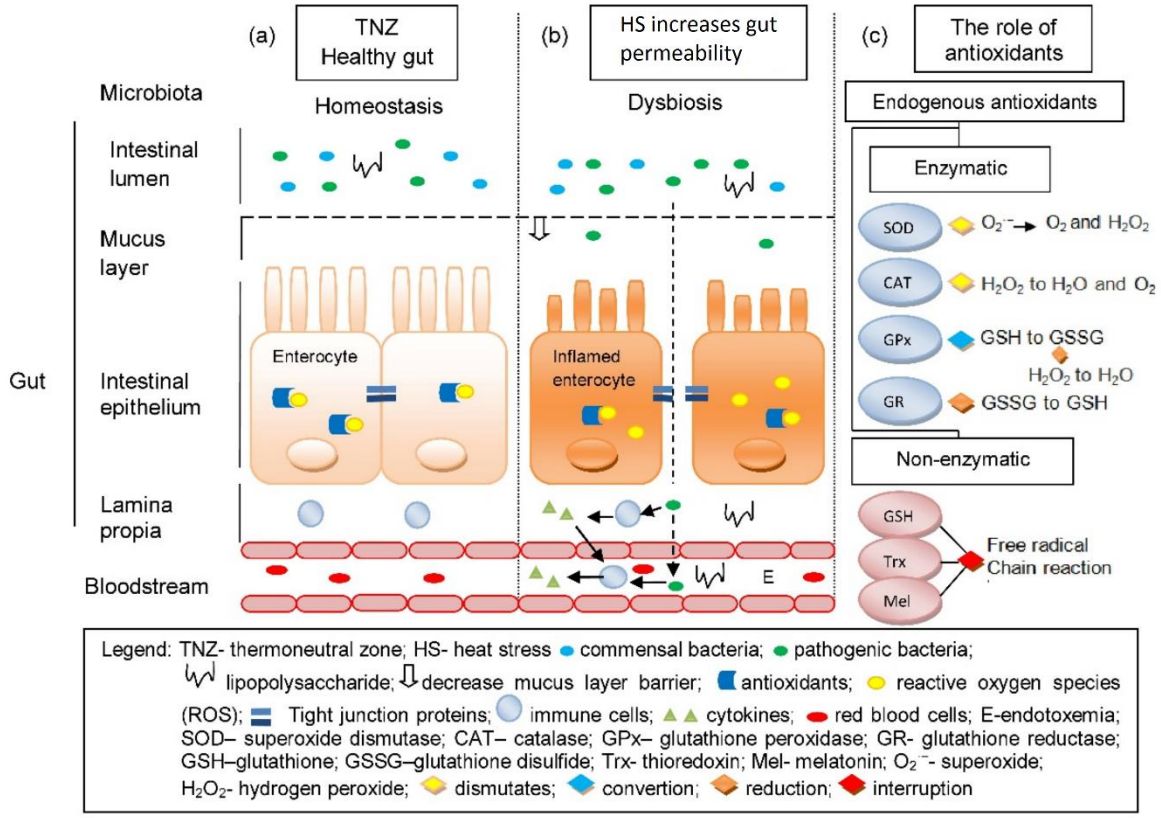
Heat stress (HS) effects on the intestinal integrity and function of pigs

Parameter	HS Effects	HS intensity	RH [a], %	HS Length	References
Intestinal mucus layer	Reduction of goblet cells in jejunum and ileum, which leads to decreased production of mucin	33.6 °C, 35°C,38.51°C	30–40	3,12hand3days	(1,2,3)[b,c,d]
Tight junction	Altered expression and localization and reduced expression of TJ proteins (ZO-1 and occludin), TER reduction in the jejunum and ileum, and manifestation of endotoxemia	35°C, 38°C	35–43	12,24hand7 days	(3,4,6)[e,f,d]
Enteric immune system	Inhibition of cellular apoptosis and gut permeability	35°C, 39°C	43	24hand10days	(3,6) [g,h]
Antioxidant system	Decreased glutathione concentration and imbalance of ROS and endogenous antioxidants in the jejunum and ileum	30°C,35°C	35–60	7and21days	(5,7) [i,f]

[a] RH – relative humidity; [b] (average live weight 19.5kg); [c] (average live weight 79.0kg); [d] (average live weight 70.2kg); [e] (average live weight 46.0kg); [f](average live weight 48.0kg); [g] (85–95 days old miniature pigs); [h] (average live weight 46.0kg); [i] (average live weight 79.0kg). 1 –ABUAJAMIEH et al., 2018; 2 – KPODO et al., 2020; 3 – YI et al., 2020; 4 – PEARCE et al., 2013*b*; 5 – PEARCE et al., 2013*a*; 6 – PEARCE et al., 2012; 7 – CUI and GU, 2015.

Figure 1.

Intestinal integrity of pigs under thermal comfort and HS condition and the role of antioxidants in ROS elimination



Source: ORTEGA and SZABÓ, 2021

2.1.4. Effect of HS on nutrient digestibility and retention

Higher temperature is hazardous for growing to finish animals. Temperatures above the upper critical level reduce animal performance by altering the body's homeostasis, energy, and nutrition, negatively affecting immune function and product quality. Pigs are one of the most vulnerable livestock species to the adverse effects of HS, and many essential attributes of their productivity are negatively affected by this stressor (QUNIOU et al., 2000; COLLIN et al., 2001*c*; BABINSZKY et al., 2011). Studies have reported that pigs' exposure to HS has increased N-excretion and decreased protein and mineral retention (FERGUSON and GOUS, 2002; LIAO and VEUM, 1994; PATIENCE et al., 2005). Other studies further support the adverse effects of HS on nutrient digestibility and retention. RENAUDEAU et al. (2013) reported that nitrogen retention rates decreased when growing pigs were subjected to 3 weeks of HS. Furthermore, metabolizable energy (ME) intake and retained energy (RE)

decreased between pigs under 24 °C for seven days and pigs under 32 °C for three consecutive days (−38.3% and −56.1%, respectively). Similar results were also reported by COLLIN et al. (2001*a*), wherein pigs reared under 33 °C have lower nitrogen retention than pigs under thermal comfort (23 °C).

Furthermore, KIEFER et al. (2012); reported that barrows exposed to 31 °C have poor digestibility of protein, phosphorus, calcium, zinc, iron, and magnesium. Moreover, these pigs had high percentage of nutrients excreted in their feces as compared to pigs under thermal comfort (23 °C). Such compromise in the digestibility of these nutrients could be attributed by HS detrimental impact on the pigs' intestinal morphology affecting its functionality (YI et al., 2020). This increase in nutrient excretion might lead to low retention of the said nutrients. Moreover, pigs exposed to HS increased water intake to attenuate its effect, consequently leading to nutrient loss through excretion. With the high possibility of intestinal hypoxia induced by HS, the animals' digestive and absorptive function can be jeopardized and exacerbate the situation (PEARCE et al., 2012; COTRELL et al., 2015).

2.1.5. Effect of HS on nutrient metabolism and electrolyte balance

Heat stress causes metabolic changes in pigs, including altered insulin profiles, decreased lipid mobilization, and compromised intestinal integrity (ZHAO et al., 2018). Alteration of insulin levels can lead to poor health in the animal. Proper insulin homeostasis is critical for adapting to and surviving under HS as it reduces body protein deposition while increasing body fat gain (SANZ-FERNANDEZ et al., 2015*a*). This protein synthesis suppression occurs at the transcriptional level. Acute HS has been shown to alter protein synthesis and ribosomal gene transcription and decrease protein deposition (JACOB, 1995). LE BELLEGO et al. (2002) reported that the decrease in body protein deposition of growing pigs under high temperatures (30 °C) was due to reduced feed intake's indirect and direct effects. When pigs were exposed to high temperatures (33 °C), there was a reduced content of pig carcass protein (KERR et al., 2003). VOET et al. (2016) said that when pigs are exposed to short-term HS, there is a reduction of protein synthesis and retention and an increase in protein degradation. Furthermore, it can also decrease plasma levels of aspartic acid, serine, tyrosine, and cysteine (essential in the biosynthesis of proteins) in fattening pigs. While long-term HS decreases blood levels of amino acids and protein breakdown while blocking protein synthesis (especially sulfur and branched-chain amino acids). Aspartic acid, glutamic acid, and phenylalanine plasma levels are also rising concurrently. Additionally, it inhibits

protein catabolism by triggering the gluconeogenesis pathway, which raises glucose levels to produce more energy and decreases protein usage (TEMIM et al., 2000).

Typically, metabolic adjustments favor muscle growth over adipose tissue accretion during times of low feed intake. However, during times of HS, when nutrient intake is restricted, there is an increase in lipid retention and a decrease in skeletal muscle mass observed in multiple species (sheep, cattle, pigs, and poultry). It is contradictory that HS promotes food intake reduction and growth suppression but boosts carcass fat accretion and lowers carcass nitrogen content (JOHNSON et al., 2013). Adipose formation outpaces the synthesis of lean muscle mass in the composition of gain in animals raised under HS. Long-term HS also reduced the body's lipid metabolism and fat distribution; the body's tendency was for fat to go from its outer layer to its inner layer to better dissipate heat (LEDIVIDICH, 1998; SHWARTZ et al., 2009). WU et al. (2015) revealed that high temperatures (33 °C) tended to diminish the intramuscular fat of the longissimus muscle in finishing pigs. It also impeded the ability to synthesize fatty acids by reducing the enzyme acetyl coenzyme A carboxylase (ACC) activity. Under HS, lipolytic enzyme activity is decreased while lipoprotein lipase activity is elevated in adipose tissue, suggesting that hyperthermic animals have a larger capacity to store intestinal and hepatic triglycerides (SANDERS et al., 2009). A readily available energy source, carbohydrates can be broken down into a number of metabolic intermediates, which can then be employed in synthesis processes or generate adenosine triphosphate (ATP). Three main signaling mechanisms, including glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation, control the production of ATP. When animals are exposed to mild HS, the muscle glycogen phosphorylase and pyruvate dehydrogenase activation is evident. Chronic HS decreased the plasma level of glucose, increased the amount of glucose that entered tissues from the circulation to provide energy, and decreased the amount of fat that was providing energy. Rebalancing the energy in heat-stressed pigs is necessary because when feed intake and blood glucose levels fall, the production declines and the glucose no longer satisfies demand (BAUMGARD and RHOADS, 2013; SLIMEN et al., 2016; CUI et al., 2019).

Electrolytes are required for basic life functions such as cell electrical neutrality and generating and transmitting action potentials in nerves and muscles. Along with magnesium, calcium, phosphate, and bicarbonates, the major electrolytes are sodium, potassium, and chloride. One of the essential electrolytes in the extracellular fluid is sodium, an osmotically active cation. It is in charge of maintaining extracellular fluid volume and regulating cell membrane potential. Sodium is exchanged along with potassium across cell membranes as

part of active transport. Potassium functions primarily as an intracellular ion. The sodium-potassium adenosine triphosphatase pump is the primary regulator of sodium and potassium homeostasis, pumping sodium out in exchange for potassium, which moves into the cells. The regulation of sodium and filtration of potassium happens occurs in the kidneys. Chloride is an anion that is mainly found in extracellular fluid. The kidneys are the primary regulators of serum chloride levels. The majority of the chloride filtered by the glomerulus is reabsorbed by both the proximal and distal tubules (mostly the proximal tubule) via active and passive transport. These electrolytes can become unbalanced, resulting in either high or low levels. High or low electrolyte levels disrupt normal bodily functions and can lead to potentially fatal complications (MORRISON, 1990; PALMER and SCHNERMANN, 2015; SHRIMANKER and BHATTARAI, 2021). Several animals have been observed with an imbalance of electrolytes due to HS exposure. Exposure to high ambient temperature in broilers has led to their low blood electrolyte balance, subsequently affecting their growth performance (ADYEMO et al., 2018). In the case of pigs, their exposure to high ambient temperature could influence the loss of electrolytes and imbalance through their physiological process of thermoregulation, which involves increased respiration (panting) and behaviour response such as increased water intake, which leads to excessive urination. Moreover, as observed in pigs and broilers, HS can cause respiratory alkalosis and renal failure, exacerbating the situation (COTRELL et al., 2020; TANG et al., 2018).

2.1.6. HS effect on carcass quality

Several studies have shown that the carcass quality of pigs was negatively affected by HS. Pigs' exposure to high AT could cause detrimental effects on pork quality parameters (YANG et al., 2014). Pigs exposed to HS at 30 °C decreased the pH value and increased the crude fat, drip loss percentage, and toughness (Shear Force) of their meat (YANG et al., 2014; MUN et al., 2022). HSs' influence on the pigs' meat pH is critical as low meat pH can increase protein denaturation, resulting in decreased water holding capacity, lighter color, and poor eating quality (KIM et al., 2016; JANKOWIAK et al., 2021). Such depression in performance can also be attributed to OS induced by HS. The balance between the ROS and the endogenous antioxidant is disturbed through HS. HS causes the excessive generation of ROS and the reduction of endogenous antioxidants, which causes the accumulation of dysfunctional protein, lipid peroxidation products, and impaired mitochondrial DNA (SLIMEN et al., 2014, CUI et al., 2019). These ROS can induce protein modification upon direct reac-

tion with proteins leading to protein oxidation which can lead to higher drip loss and toughening of the meat (HUFFLONERGAN et al., 2010; TRAORE et al., 2012). Moreover, OS can reduce collagen synthesis, decreasing collagen solubility and greater meat toughness (ARCHILE-CONTRERAS and PURSLOW, 2011). Pigs raised under HS conditions had more fat tissue and less muscle mass (ROSS et al., 2015). LE BELLEGO et al. (2002) stated that pigs' prolonged exposure to HS directly negatively affects protein deposition and affects the partitioning of energy gain between protein and fat deposition. Based on the findings of their investigation, pigs raised at HS condition (30 °C) and weighing 24 to 65 kg acquired fatter carcasses that were associated with reduced protein compared to pigs kept at thermal comfort (23 °C). Their findings show that high temperatures directly affect the maximum protein deposition rate. In this perspective, higher lipid deposition indirectly results from protein deposition limitation. Because the energetic efficiency of lipid deposition is greater than that of protein deposition, it can be perceived as a metabolic response to reduce internal heat production in hot environmental conditions (CAMPOS et al., 2017).

Hormonal changes are triggered by HS, which influences the metabolism of nutrients. AGGARWAL and UPADHYAY (2013) stated that the two most important responses of the animals to HS are the activation of the hypothalamic-pituitary-adrenal axis and the consequent increase in plasma glucocorticoid concentrations. The short- and long-term environmental heat affects endocrine glands and releases hormones, namely, thyroxine, cortisol, growth hormone, and catecholamine. SABER et al. (2009) stated that when animals are exposed to high AT, the thyroid activity is depressed, causing a relatively lower concentration of thyroid hormones. Triiodothyronine (T3) and thyroxine (T4), two thyroid hormones with tyrosine as their primary building block, are essential for development, differentiation, and metabolism. They significantly influence metabolic rate, oxygen consumption, and protein synthesis and are crucial for the healthiest operation of nearly all tissues (DEV et al., 2016). CHAKRABORTY et al. (2017) reported reduced T3 and T4 concentrations in pigs raised in summer (hot environmental conditions). The said reduction of thyroid hormones is believed to be the animals' adaptive response to HS. It might attempt to reduce metabolic rate and heat production under HS conditions.

Increased secretion of thyroid hormones increases body metabolism; thus, it also increases heat production. The reduced secretion of thyroid hormones during HS might cause deterioration of pork quality by increasing fat and lesser lean levels. CHRISTON (1988) reported that pigs in tropical environmental temperatures (22-32 °C) markedly affect their

metabolism due to the decreased concentration of thyroid hormones responsible for regulating metabolism. Additionally, pigs exposed to the aforementioned AT showed changes in their metabolic status, including elevated levels of plasma-free fatty acids, triglycerides, cholesterol, adipose tissue, and lipoprotein lipase activity. This is further supported by the results of the study of PEARCE et al. (2013*c*) and QU and AJUWON (2018), wherein heat-stressed pigs tended to have high levels of circulating triglycerides and cholesterol, leading to fatter deposition. Besides, HS influences the stimulation of catecholamine secretion that can cause rapid decomposition of muscle glycogen, leading to large amounts of lactic acid production. Consequently, this leads to decreased muscle pH and the formation of pale soft exudative (PSE) meat (SANDERCOCK et al., 2001), further deteriorating the meat quality.

2.2. HS-induced oxidative stress in pigs

Cellular level alterations and damages are evident upon the animal's exposure to high ambient temperatures. Numerous intracellular molecular structures are stabilized by various relatively weak interactions, which are easily disrupted by changes in the microenvironment (RHOADS et al., 2013). Normal cellular metabolism by living organisms and environmental factors produces ROS, which function in physiological cell processes at low and moderate concentrations. However, at high concentrations, they can damage and change the functionality of cell structures such as proteins, lipids, carbohydrates, and nucleic acids (BIRBEN et al., 2012). SLIMEN et al. (2014), stated that HS is an environmental factor that stimulates ROS production. A balance between ROS and antioxidants exists under normal physiological settings, but OS can result from this imbalance if the antioxidant defense system can no longer counteract the high ROS generation. ROS can attack amino acid residues, causing oxidation of side chains and cleavage of polypeptide bonds, impacting the non-covalent interactions that hold the protein together properly, resulting in protein destabilization and unfolding (DAVIES, 2016). Cell components are negatively impacted by extreme temperature and its induced stressors (OS) and can cause proteins to unfold and aggregate (TYDMERS et al., 2010; RHOADS et al., 2013). The animal's body is equipped with a system that counters ROS, and it is called the antioxidant defense system. The system is divided into two categories: (1) preventative antioxidants, which include enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and DNA repair enzymes, as well as some metal ion sequestrators (albumin); and (2) Scavenging antioxidants (2) Chain-breaking antioxidants) such as vitamin C retinol-vitamin A, uric acid, vitamin E, reduced glutathione, and polyphenols (ZHANG et al., 2020).

However, HS has a detrimental effect on the antioxidant system furthering oxidative damage and is summarized in *Table 3*. HS induces OS in pigs' intestines, causing a damaging effect on their integrity and function (CUI and GU, 2015). Nevertheless, the antioxidant system is vital in eliminating the ROS responsible for OS (*Figure 1*).

Table 3.

Effect of heat stress (HS) on the antioxidant enzymes

Antioxidant Enzyme	**Effects of HS**	**References**
SOD	Decrease SOD mRNA levels, cytoplasmic SOD protein, and enzyme activity, leading to the increase in ROS generation	EL-ORABI et al., 2011
CAT, SOD, GPx	Finishing pigs exposed to 30℃ for three weeks had decreased antioxidant enzyme activity, resulting in OS.	CUI et al., 2019

2.3. Heat shock proteins in response to heat stress

HS promotes a rapid heat shock response by activating heat shock proteins (HSPs). This is especially noticeable in pigs' intestinal tracts within 2 to 4 hours of HS due to intestinal hypoxia (PEARCE et al., 2014). FLANAGAN et al. (1995); ROSS et al. (2015) further stressed that the small intestine is one of the first tissues that regulate HSPs during high thermal load. Their names have been derived because the expression of essential members of this large family of proteins is up-regulated by several non-infectious stressors, such as elevated temperature. The main HSPs are categorized based on size and function, and their molecular masses range from 15 to 110 kilo Daltons (kDa). They can be found in the nucleus, endoplasmic reticulum, mitochondria, and cytoplasm (GALLUCCI, 2016). Since HSPs are found in both prokaryotic and eukaryotic cells, it is likely that they are crucial to the basic functions of both types of cells. Selected HSPs, also referred to as molecular chaperones, are essential for folding or unfolding proteins, assembling multiprotein complexes, transporting or sorting proteins into the proper subcellular compartments, controlling and signaling the cell cycle, and defending cells from stress or apoptosis (LI and SRIVASTAVA, 2004). Heat,

hypoxia, OS, and nutritional stress can up-regulate these proteins. Their activity is widespread within several hours of the onset of severe stress and can last for a few days (HOROWITZ, 2002; GABLER and PEARCE, 2015).

The most well-studied and understood HSPs in mammals are those with molecular weights of 60, 70, 90, and 110 kDa. They are expressed at euthermic body temperature (37 °C) and under stress conditions (heat shock) and have distinct locations and functional characteristics. Temperature sensitivity and great conservation are both characteristics of the HSP70 family. The ATP-binding proteins known as the HSP70s (HSP72, HSP73, HSP75, and HSP78) show a 60–80% base identity in eukaryotic cells. Despite having similar protein sequences, members of the HSP70 group produce different proteins in response to different stimuli (KREGEL, 2002). HSP70-72 kDa is a significant member of the inducible HSP family that helps in protein folding, ubiquitination, renaturing proteins, and protecting against cellular stress (PETROF et al., 2004; GABLER and PEARCE, 2015). KREGEL (2002) mentioned that HSPs are essential for normal cellular function and survival after stress. Stress-induced accumulation of HSP 70 can influence the ability of a cell or organism to become resistant to HS after a prior sub-lethal exposure (thermo-tolerance). Based on the findings of the study done by NAKAMURA et al. (1991), when guinea pigs are exposed to HS (43 °C) for a brief period (one hour), the cells synthesize 72-kDa protein and increase the synthesis of 74- and 90-kDa proteins, which were detected using gel electrophoresis after methionine labeling of the cells. According to an immunoblot study, the 72- and 74-kDa proteins belonged to the HSP 70 family.

Furthermore, heat treatment significantly reduced damage in ethanol-treated animals; this reduction was inhibited by cycloheximide, a protein synthesis inhibitor, and was associated with the inhibition of HSP synthesis. They proposed that a key component of the intracellular gastric protective mechanism is the creation of HSPs. HSP70 has previously been shown to play significant in vivo and in vitro cytoprotective roles in gastric mucosa. HSP is produced by gastric mucosal cells, which boosts mucosal defense against potential shocks and rapidly causes tolerance to stressful situations (ROKUTAN, 2000). Its induction under HS conditions can positively impact the animals' performance. When pigs were exposed to HS (35 °C), an increase of HSP70 was observed (QU et al., 2016). Pig ileum and colon HSP70 protein expression were likewise induced with HS, according to PEARCE et al. (2014). HSP70 is significantly elevated 2 to 6 hours after heat exposure, specifically in thermal biology. This strengthens their protective role in stressful situations. The critical cellular activities of translation, membrane translocation, presenting degradation substrates,

assembling and disassembling macromolecular complexes or aggregates, gene activation, and apoptosis all entail the involvement of HSP70s, which usually occurs when animals are under HS (SHARMA and MASISON, 2009). Up-regulation and the importance of HSPs are shown in *Table 4*.

Table 4.

Up-regulation of heat shock proteins and their importance against stressors

Stressors	Proteins	Function	References
Heat stress (37 °C)	HSP 70	Promote cell thermo-tolerance	KREGEL, 2002
Cellular stress	HSP 72 and HSP 74	Protein folding and cell protection	PETROF et al., 2004; GABLER and PEARCE, 2015.
Heat stress (35 °C)	HSP 70	Gastric mucosal defense its anti-inflammatory properties suppressed inflammatory cytokine release or action in pigs.	Qu et al., 2016
Cellular stress and diseases	HSPs 40,60,70 and 90	Antigen presenter and immune modulator. By stimulating antigen-presenting cells of the immune system (macrophage and dendritic cells).	LI and SRIVASTAVA, 2004; ZININGA et al., 2018

2.4. Nutritional mitigation strategies against heat stress

Management practices such as environmental modifications (construction of modern facilities) and genetic improvement can mitigate the effect of HS. However, these technologies can be expensive, which urges the conduct of research on the precision nutrition of pigs, which involves the use of highly digestible feedstuffs and feed supplements like vitamins E, and C and trace minerals to alleviate the effects of HS. Nutritional therapies are a realistic, flexible, and economic strategy to reduce the harmful effects of HS and boost animal output (RHOADS et al., 2013; MAYORGA et al., 2018*a*). The performance of pigs under HS could be positively impacted by several dietary management techniques, which include but are not limited to:1) development of diets with a low thermic effect (obtained by boosting dietary

fat and decreasing the amount of crude fiber, crude protein, and precise amino acid supplementation), 2) Supplementation of dietary antioxidants to address the HS induced OS (LE BELLEGO et al., 2001; BABINSZKY et al., 2011; WOLP et al., 2012; PATIENCE et al., 2015; BABINSZKY et al., 2019).

2.4.1. The use of dietary antioxidants

Oxidation is a chemical reaction that can generate free radicals, causing chain reactions that can harm organisms' cells. Oxidation occurs when animals are under several stressors, such as HS. Dietary antioxidants are chemicals that lessen oxidative damage, including that brought on by free radicals, and they can counter oxidation's damaging effects, which animals often experience under HS. Well-known dietary antioxidants include enzymes and other substances, such as vitamins C and E, and minerals selenium (Se) and zinc (Zn) (SHIEL, 2017; MOCCHEGIANI and MALAVOLTA, 2018). Vitamin C's antioxidant properties defend cellular structures from the damaging effects of free radicals. In contrast, vitamin E, a lipid-soluble antioxidant, can counteract free radicals brought on by HS, thereby acting as a shield of the body's structures against oxidative damage (TRABER and STEVENS, 2011; SINBAD et al., 2019). Besides its function as an antioxidant, vitamin C is also responsible for synthesizing serotonin (a hormone essential in the functioning of the endocrine, nervous, immune, and digestive systems) (CARR and FREI, 1999; SINBAD et al., 2019). Micro-minerals such as Se and Zn have diverse antioxidant functions. Se, an essential trace mineral, is important for numerous physiological processes, particularly the immune system, metabolism of thyroid hormones, and antioxidant defense system. Moreover, it evinces the animals' antioxidant activity and anti-inflammatory and antibacterial effects (HOSNEDLOVA et al., 2017) essential responses to HS. Se incorporates into proteins in the form of selenoproteins (antioxidant enzymes) to prevent cell damage and OS by catalyzing the reduction of ROS such as hydrogen peroxide (H_2O_2) into water (KIELCZYKOWSKA et al., 2018; GONZÁLEZ DE VEGA et al., 2018; ZHANG et al., 2020). Zn, a vital trace mineral for all living organisms, prevents oxidative damage. It acts as powerful electrophilic scavenger and cytoprotective agents, delaying the oxidative process by promoting the development of metallothionein, which regulates zinc-related cell homeostasis (JAROSZ et al., 2017). Additionally, the antimicrobial properties of Zn have favorable benefits on pigs' intestinal health (LIU et al., 2018*a*). Several studies also demonstrated that supplementing these minerals improved the immune status, total antioxidant capacity, carcass yield and quality and performance of various livestock and poultry animals, including broilers

(SHAKERI et al., 2020), lambs (CHAUHAN et al., 2020), rabbits (HASSAN et al., 2021), and pigs (YOON et al., 2020).

These dietary antioxidants (vitamins C and E) and micro-minerals (selenium and zinc) are known to mitigate some of the adverse effects of heat stress in pigs. The substances' ability to avert cell damage and improve intestinal integrity and renal function can benefit animal performance under HS (LIU et al., 2016; DENNIS et al., 2017; FERNANDEZ et al., 2014). Supplementation of the said vitamins and micro-minerals can also enhance the acid-base balance and the metabolic and physiological functions of several species of livestock (SIVAKUMAR et al., 2010; LIU et al., 2018*b*). Also, vitamins E and C can suppress pro-inflammatory cytokines and regulate inflammatory response (LAURIDSEN et al., 2021; LEWIS et al., 2019). Moreover, alleviation of HS adverse effects on pigs' intestinal integrity and function (LIU et al., 2016; PEARCE et al., 2015*a*) and mitigated the HS adverse effects on the plasma metabolites of heat-stressed pigs (HS-induced increase in plasma urea nitrogen, decrease in plasma insulin, and decrease in blood potassium and chloride concentrations) were observed upon supplementation of some of the mentioned dietary antioxidants (LIU et al., 2018*c*; PEARCE et al., 2015*a*).

PEARCE et al. (2015*a*) observed that pigs exposed to acute HS (37 °C for 12 hours) and supplemented with dietary organic zinc (ZnAA) had a significant reduction in rectal temperature as compared to pigs under HS given with the basal or control diet. Elevation of blood metabolites essential for metabolism (glucose and insulin) and improvement in intestinal morphology was also observed in the said study, which greatly influenced the integrity and function of the intestine, leading to a decrease in blood endotoxin, better digestive and absorptive function. LIU et al. (2016) reported that supplementation of Se (1.0 ppm) and vitamin E (200 IU kg^{-1}) to pigs under HS conditions (35 °C) increased the TER and GPx activity (responsible for the protection of the organism from oxidative damage). This initiates intestinal integrity and mitigates the effects of HS associated with reducing OS. The effectiveness of dietary Se can also be enriched with probiotics. The study of GAN et al. (2014) revealed that weanlings supplemented with Selenium-enriched probiotic (0.46 Se/kg) have an improved growth performance with an increased blood GPx activity and tissue thioredoxin reductase 1 mRNA expression, which is essential in OS response. Moreover, increased SOD activity (an enzyme that helps break down potentially harmful oxygen molecules in cells and prevent damage to tissues) was observed along with increased glutathione content and proliferation of immune cells (T lymphocytes). Also, piglets exposed to 42 days HS (average temperature 42 °C) fed diet supplemented with selenium-enriched probiotics

(0.46 mg/kg Se in the diet) had significantly better performance. Their final body weight, ADG, feed conversion ratio, and ADFI is higher than pigs under a control diet that contains only 0.16 mg/kg. They also observed increased concentrations of T3 hormone in pigs supplemented with Se-enriched probiotics, which is typically decreased in pigs under HS (LV et al., 2015). *Table 5* summarizes the mitigation capability of the mentioned dietary antioxidants.

Table 5.

Effects of dietary antioxidants on the intestinal integrity and antioxidant system of heat-stressed pigs

Parameter	Antioxidant	Con [a] mg/kg	Supp [b] mg/kg	Effects	References
Intestinal barrier integrity	SeY [c] ZnAA + ZnSO4 [d] ZnAA + ZnSO4 [d]	0 0 + 120 0 + 120	250 200 + 120 60 + 60	Improved intestinal TJ, high ileum TER, reduction of blood endotoxin, and improved intestinal histology and morphology	LIU et al., 2020 [g] PEARCE et al., 2015*b* [h] SANZ FERNANDEZ et al., 2014 [i]
Antioxidant system	SeY c Se and VE [e] SeP	0 0.5 and 100 0.16	250 1 and 200 0.46	Elevation of antioxidant enzymes (catalase and glutathione peroxidase) and enhanced mucosal antioxidant capacity	LIU et al., 2020 [g] LIU et al., 2016 [j] LV et al., 2015 [k]

[A] Con—levels in the control diet; [b] Supp—supplemented in the experimental diet; [c] SeY—selenium-enriched yeast; [d] ZnAA+$ZnSO_4$—zinc amino acid complex and zinc sulfate; [e] Se—selenium and VE—vitamin E; [f] SeP—selenium enriched probiotic; [g] (average live weight 7.30kg); [h] (average live weight 64.0kg); [I] (average live weight 43.0kg); [j] (average live weight 20.0kg); [k] (average live weight 7.9kg).

2.5. Conclusions

Based on the literature, high ambient temperature is the leading cause for causing heat stress in pigs. However, it is not the sole culprit, as the relative humidity also contribute to such stressor. Being homeotherms, pigs under heat stress perform a thermoregulatory process which could lead to physiological strains, particularly on their metabolism, electrolyte balance, gastrointestinal tract, immune response, and antioxidant defense system. Heat stress causes metabolic changes in pigs, including altered insulin profiles and decreased lipid mobilization, and the increase in water intake and excessive urination by pigs under such stressor could lead to electrolyte imbalance affecting their growth and carcass quality. Heat stress increases gut permeability as it could negatively affect the expression of tight junction proteins essential in gut barrier integrity. Such detrimental effects could promote the translocation of harmful pathogens from the lumen into circulation, causing endotoxemia. Heat stress could also promote the expression of pro-inflammatory cytokines, which diverts nutrients away from growth to fuel the fight against the inflammatory response. Aside from the compromised integrity of the gut, heat stress also causes intestinal damage, as observed in the histology and morphology of the small intestine. Reducing villus height, crypt depth, and villus height to crypt depth ratio are some reported consequences of heat stress. Since the villi are essential for nutrient absorption, this function can be compromised under heat stress and could lead to inadequate nutrient digestion and absorption of exposed pigs. Moreover, HS can induce intestinal hypoxia and oxidative stress exacerbating the situation. Nevertheless, supplementation of dietary antioxidants such as Vitamins C and E and micro-minerals Se and Zn could mitigate some of the adverse effects of heat stress since these dietary antioxidants can neutralize the reactive oxygen species that are over-expressed in heat-stressed pigs. Therefore, the combination of these dietary antioxidants at elevated levels could benefit the nutrient digestibility, metabolism, electrolyte balance and immune response of heat-stressed pigs, hence the conduct of our research.

3. MATERIALS AND METHODS

3.1. Animals, diets, and management

All experimental procedures were reviewed and approved by the Hungarian authorities and registered and supervised by the University of Debrecen Animal Care Committee (Debrecen, Hungary – 9/2019/DEMÁB). A total of thirty-six DanBred hybrid barrows (65.1 ± 2.81kg) were assigned to the combination of two environmental conditions (*Table 6*) and three dietary treatments at the University of Debrecen, Institute for Agricultural Research and Educational Farm, Animal Husbandry Experimental Station (Kismacs, Hungary). All pigs were allowed a seven-day adaptation period to their pens (3 pigs per pen with a total of 12 pens), fed a*d libitum* (with basal feed) in a thermo-neutral (TN) environment (average 19.5 ± 1.5 °C). Afterward, the temperature of the thermo-neutral room, which housed nine pigs (three pens), was maintained at 19.5 ± 0.9 °C, RH- 85.9 ± 7.3% with a temperature-humidity index (THI) of 66.4 throughout the experiment. Meanwhile, the temperature of the HS room was gradually raised to 30 °C during seven days (heat increment period, days 8-14, HI), and the main period of the experiment commenced, which lasted 14 days (15 to 28 days of the trial) with an average temperature of 28.9 ± 0.9 °C and RH of 60.4 ± 4.3%, with a THI of 78.3. Room temperatures and humidity were recorded using digital thermal and humidity meters (TESTO 174H (Testo SE & Co. KGaA, Lenzkirch, Germany)) data logger with an accuracy of ± 0.5 °C , and ± 3 % RH. One data logger was used for every three pen (one in TN and three in high AT room). This equipment recorded the temperature and humidity in every minute 24h which data were retrieved daily and the averages were calculated. The mean temperature changes are shown in *Figure 2*.

A corn-soybean meal diet (basal feed - C) was formulated for 75 to 100 kg pigs having 155 g mean protein deposition per day (*Table 7* and *Table 8*) in accordance to the National Research Council (NRC, 2012) recommendation. Two additional dietary treatments (elevated diet 1 or single dose supplementation (T1) and elevated diet 2 or double dose supplementation (T2)) were formulated by providing vitamins C and E and micro-minerals Se and Zn (elevated levels), as shown in *Table 9*. The control diet contained levels of vitamins C and E and micro-minerals Zn and Se in accordance to the NRC recommendation (*Table 9*). Nutrient recommendation tables do not recommend vitamin C supplementation for growing and finishing pigs, but in DSM Optimum Vitamin Nutrition (OVN) guidelines (a standard for maximized performance) for breeders the recommendation is set at 315 mg/kg of maximum supplementation. We chose to add 300 mg/kg for T2 and 150

mg/kg for T1. Regarding vitamin E the OVN guidelines recommend 64–105 mg/kg, therefore we decided for a 30 mg/kg two-step increase of the basal diet concentration, resulting in 41 mg/kg in T1 and 71 mg/kg in T2. In case of Zn, the maximum allowed supplementation in the EU is 150 mg/kg, therefore which was chosen as the maximum value in T2, whereas concentration in T1 was 100 mg/kg, and in basal feed was 50 mg/kg. Due to toxicity problems complete pig feeds should not contain more than 0.5 mg/kg Se in total. Therefore, usually not more than 0.2–0.35 mg/kg is added to the feed. We decided to increase the NRC supplementation of the basal diet (0.16 mg/kg) for 0.05 mg/kg for two times, resulting in 0.21 mg/kg (T1) and 0.26 mg/kg (T2). The analyzed nutrient content of the diets is shown in *Table 10*.

The pigs were distributed among four treatment groups, which consisted of a combination of environmental and dietary treatments: 1) TC: TN+ C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and basal diet, 2) HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + basal diet; 3) HT1: HS + T1 (elevated diet 1: single dose supplementation, vitamin C and E and Se and Zn content (*Table 9*), and 4) HT2: HS + T2 (elevated diet 2: double dose supplementation, vitamin C and E and Se and Zn content). The experimental period, sampling, and measurement are summarized in *Table 11*.

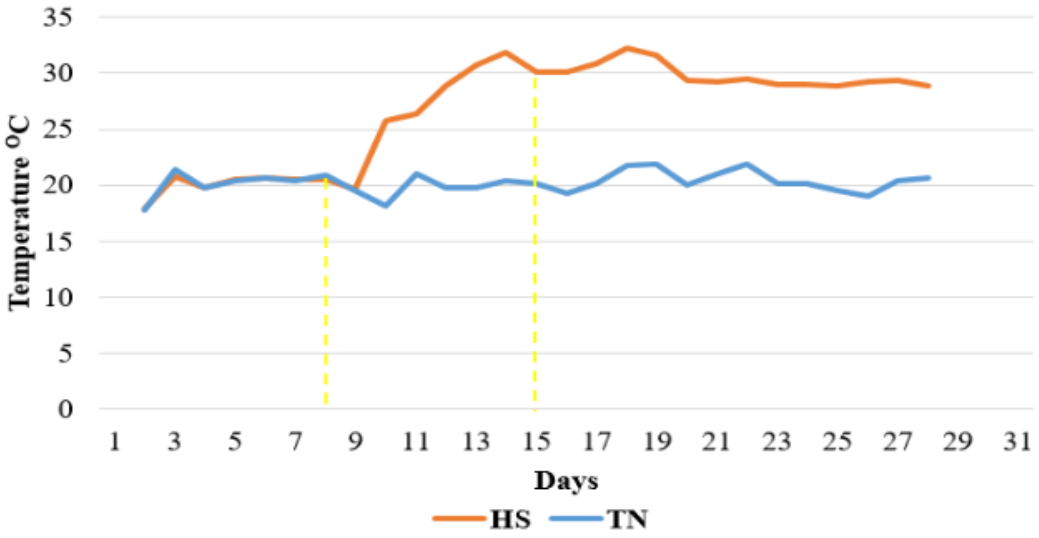
Table 6.

Allocation of thermal and dietary treatments

Temperature	**Heat stress**									**Thermo-neutral zone**		
Pen number	1	2	3	4	5	6	7	8	9	10	11	12
Number of animals	3	3	3	3	3	3	3	3	3	3	3	3
Treatment	HC	HT1	HT2	HC	HT1	HT2	HC	HT1	HT2	TC	TC	TC

Figure 2.

The temperature of heat stress (HS) and thermo-neutral (TN) rooms throughout the study



Activities

Day 1- 7	Adaptation period
Day 8 - 14	Gradual temperature increase in HS room and introduction to test diets
Day 15 - 28	Experimental period

Table 7.

Composition and calculated nutrient content of basal feed [a]

Ingredients	Inclusion rate (%)	Energy and Nutrients	Calculated value
Corn	78.68	Digestible energy, MJ/kg	14.24
Soybean meal	16.33	Crude protein, %	12.81
Sunflower oil	2.11	SID [b] Lys, %	0.78
Limestone	0.92	SID Met+Cys, %	0.45
MCP	0.80	SID Thr, %	0.49
L-Lysine	0.30	SID Trp, %	0.14
DL-Methionine	0.01	Ca, %	0.59
L-Tryptophan	0.03	digestible P, %	0.23
L-Threonine	0.06	Na, %	0.10
Salt	0.26		
Vit. and mineral premix	0.50		

[a] NRC (2012) recommendation for 75-100 kg live weight pigs having 155 g mean protein deposition per day, [b] standardized ileal digestible

Table 8.

*Nutrient content of the mineral and vitamin premix**

Nutrient	Unit	Level
Zn	mg/kg	9999
Cu	mg/kg	1454
Fe	mg/kg	7281
Mn	mg/kg	9999
I	mg/kg	136
Se	mg/kg	32
Vitamin A	IU/kg	410000
Vitamin D-3	IU/kg	82000
Vitamin E	mg/kg	2205
Vitamin K-3	mg/kg	82
Vitamin B-1	mg/kg	62
Vitamin B-2	mg/kg	205
Ca-d-pantothenate	mg/kg	492
Vitamin B-6	mg/kg	164
Vitamin B-12	mg/kg	1
Biotin	mg/kg	5
Niacin	mg/kg	1026
Folate	mg/kg	25
Choline chloride	mg/kg	60000

* At or above NRC (2012)

Table 9.

Dietary treatments (supplementation mg/kg)

Nutrient	**Basal feed**[1]	**Elevated 1**[2] **Single dose**	**Elevated 2**[3] **Double dose**
Vitamin C[4]	0	150	300
Vitamin E[5]	11	41	71
Zn[6]	50	100	150
Se[7]	0.16	0.21	0.26

[1]NRC (2012), supplemented to HC and TC treatment groups
[2]Supplemented to HT1 treatment group
[3]Supplemented to HT2 treatment group
[4]L-ascorbic acid (ROVIMIX® Stay-C, DSM, Heerlen, Netherlands)
[5]α-Tocopheryl acetate (ROVIMIX® E50, DSM, Heerlen, Netherlands)
[6]Zinc chelate of amino acid hydrate (Vevomin Zn, DSM, Heerlen, Netherlands)
[7]L-selenomethionine (Excential Se, ORFFA, Werkendam, Netherlands)

Table 10.

Nutrient analysis of the experimental feed (Dry matter basis)

Energy and Nutrients	**Basal feed**	**Elevated 1** **Single dose**	**Elevated 2** **Double dose**
Dry matter %)	91.83	91.82	92.04
Crude protein (%)	13.30	13.15	12.95
Crude fat (%)	5.85	5.63	5.54
Crude fiber (%)	2.80	3.34	3.51
Gross energy (MJ/kg)	18.85	18.92	18.86
Crude ash (%)	4.44	4.36	4.60
Acid insoluble ash (%)	0.0077	0.0041	0.0059
Ca (g/kg)	5.72	5.77	6.00
P (g/kg)	5.32	5.41	5.83
Na (g/kg)	1.36	1.58	1.66
Se (mg/kg)	0.3	0.5	0.7
Zn (mg/kg)	65.6	136.1	147.8
C-vitamin (mg/kg)	260	690	790
Organic matter (%)	95.56	95.64	95.40
N-free extract (%)	73.61	73.52	73.40

Table 11.

Experimental periods, samplings, and measurements

Days in experiment	Period	Live weight	Blood sampling	Digestibility test	Slaughter, ileal digesta and tissue sampling	Feed and feed intake	Room temperature and humidity	Skin and rectal temperatures
1	Adaptation	X				X	X	
2							X	
3							X	
4							X	
5							X	
6							X	
7							X	
8	Temperature rise in the heat stress room	X	X			X	X	X
9							X	
10							X	
11							X	
12							X	
13							X	
14							X	
15	Experimental period	X	X	Animal 1 adaptation		X	X	X
16							X	
17				Animal 1 collection			X	
18							X	
19							X	
20							X	
21							X	
22		X		Animal 2 adaptation		X	X	
23							X	
24				Animal 2 collection			X	
25							X	
26							X	
27							X	
28							X	
29		X				X	X	
30			X		X		X	X
31					X		X	

3.2. Measurements of temperature indices and production parameters

3.2.1. Body temperature measurement

Skin and rectal temperature were measured with an infrared (TESTO 830-T1, Testo SE & Co. KGaA, Lenzkirch, Germany, with an accuracy of ± 1.5 °C) and digital thermometer, respectively, on the days of blood sampling (but before on resting animals). The former was measured during day time on the pig's body parts: ear base, middle of ear, and back (center) with an approximate distance of 10 cm.

3.2.2. Growth performance

On arrival of the animals, all groups received the basal feed. Each pen had a designated plastic container, of which tare-weight was measured and written on the container. About 70 kg of feed was weighed in for each pen for one week. Semi ad-libitum feeding was followed, animals were supervised during the day, and if the feed level in the trough decreased it was filled to about the 50% of its capacity. After the one-week adaptation period, the feed residues (both from the trough and the container) were measured, and the feeds were replaced according to the allocated treatment. Every week on Monday, the feed residue (both from the trough and the container) was measured, and the container was refilled and weighed. Body weights (BW) were obtained weekly from the start of the adaptation period to the end of the experiment. Average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR) were calculated by week.

3.2.3. Slaughter and Pork Quality Measurement

At the end of the trial, six pigs from each treatment were slaughtered (three in one day from each treatment) after electrical stunning. About 500 g of *longissimus lumborum* muscle was removed between the pig's 12^{th} rib and 5^{th} lumbar vertebrae for meat quality measurements, as REZAR et al. (2017) described.

3.2.4. Physical meat quality assessment

The meat pH was measured at 45 minutes and 24 hours after slaughter in the loin meat by Testo AG Germany 205 pH value gauge (immersed in a buffer solution before measurement). The meat color was measured using Konica Minolta CR-410 Chroma Meter (Konica Minolta Corp., Japan) 24 hours after slaughter with a 21-minute blooming time. The Chroma Meter was calibrated with the use of a white calibration plate before the analysis,

setting the Y, x, and y illuminant coordinates (Y=93.7, x=0.3144, y=0.3204). Regarding meat color, L* (the degree of lightness, on a scale between 0 (black) and 100 (white), a* (is red-green), and b* (stands for the yellow-blue color characteristic) values represent a color space defined by CIE. In the CIELAB system, by using the measured a*, b*, and L* features (a* = red, b* = yellow, L* = paleness).

For drip loss (%) determination, meat pieces of 50 ± 5 g and 1 cm thick were cut from each meat part. Pieces were packed in an inflated nylon bag, then hung up in the fridge at 4 °C for 48 hours and weighed again. For freeze loss (%) determination, from the frozen meat samples (-20 °C), meat parts were cut into 100 ± 5 g and 1 cm thick pieces, stored at 4 °C for 24 hours, and weighed. The same samples used for thawing loss were cooked. For the evaluation of cooking loss, pieces were packed in nylon bags and cooked for half an hour until reaching the 75 °C core temperature, then weighed. After cooking, meat pieces were chilled at 4 °C overnight, then sliced into cuboids and measured. For the firmness, shear force measurement (N), a Warner-Bratzler shear machine (TA.XT + Texture Analyzer 6.1.18.0 version (Texture exponential, Stable Microsystems Ltd., Vienna Court, Lammas Road, Godalming, Surrey GU7 1YL, United Kingdom)) was applied on cooked samples, with a shear blade set using 25 kg load cell, with 1.5 mm/s test speed from 40 mm distance. Once the trigger force is attained, the blade proceeds to shear through the sample. The maximum force denotes the point at which the sample completely fills the triangular cavity of the blade and cuts through the sample surface. After this point, shearing continues throughout the sample until the blade passes through the base plate slot. The blade then returns to its starting position. Curves were evaluated to get shear force data.

3.2.5. Chemical analysis of the meat samples

Total nitrogen was analyzed through Kjeldahl-Method, and protein content was calculated using the factor of 6.25. Fat was measured according to MSZ ISO 1443:2002 standard. The procedure of BRASSÓ et al. (2021) for the mineral and vitamin C analysis was followed where in ground meat samples (1.000 g) were loaded into digester tubes. To all samples, ten ml of distilled concentrated HNO_3 was added and heated at 60 °C for 30 minutes, then 3 ml 30% (v/v) H_2O_2 (Scharlab, Magyarország Kft., Debrecen, Hungary) was added, and the samples were digested further at 120 °C for 90 minutes. After the digestion, all samples were washed into 50 ml volumetric flasks with distilled water, homogenized, and filtered (MN 640 W paper; Macherey-Nagel). The ICP-OES technique was applied to

the iCAP 7000 spectrophotometer (Thermo Scientific). For the calibration, a multielement standard solution was applied.

3.3. Nutrient Digestibility, Metabolism, and Immune response of fattening Pigs

3.3.1. Digestibility trial: Sample collection, measurement, and analysis

Six pigs in each treatment group (three for week one and three for week two) were randomly selected for the digestibility trial. The evaluation was performed for two weeks during the main experimental period, with weekly changes of pigs in the digestibility cages. Both weeks consisted of two days of adaptation to the cage and five days of collection. Feces, urine, and feed residue were collected, measured and sampled (about 10%) daily from each digestibility cage. For nitrogen fixation, initially 10 ml of 50% sulphuric acid was added to the urine collecting canister. Every morning the pH of the urine was checked by a quick litmus test, for those that had a pH value of above 3, the amount of sulphuric acid was increased to 25 ml. The collected samples were then pooled by cage, frozen at - 20 °C and sampled for analysis. Faeces, urine (not analyzed for dry matter and crude ash), and feed samples were analyzed for dry matter (ISO 6496), crude ash (ISO 5984), crude protein (CP) by the Kjeldahl method (ISO 5983-2), crude fat (Cfat) using petroleum ether extraction (ISO 6942), crude fiber (CF) with boiling samples alternating sulphuric acid and potassium hydroxide (ISO 6865). Calcium (Ca), phosphorus (P), sodium (Na), and zinc (Zn) analysis were carried out after 1.0000g samples were digested in a block digester (LABOR MIM, Budapest, Hungary) with 10 mL cc. Nitric acid at 60 °C for 30 min and 3 mL of 30% hydrogen peroxide alt. (Sigma-Aldrich, Saint Louis, MI, USA) at 90 min at 120 °C. For selenium (Se) analysis, a 0.5000 g sample was measured into high-pressure digestion bombs with 5 mL cc. Nitric acid and 3 mL of 30% hydrogen peroxide (Sigma-Aldrich, Saint Louis, MI, USA). The digestion was processed in a microwave digester (ETHOS Plus, Milestone) applying the digestion program suggested by the manufacturer (Application Note 076: 3 mins at 85 °C; 9 mins at 145°C; 4 mins at 200°C; 14 mins at 200°C). All digested samples were filled to 50 mL with distilled water and filtered through MN640W (155 mm; Macherey-Nagel) filter paper. The analysis was carried out with the ICP-OES technique (iCAP 7000, Thermo Scientific Kandell, Germany). The multi-element standard solution was applied from mono-element standards (for Ca, Na, P, and Zn from VWR, Leuven, Belgium, and for Se from Thermo Scientific, Kandell, Germany). The following wavelengths were tested and applied in the concentration measurement: Ca-393.366 nm; Na-589.592nm; P-177.495nm; Zn-202.548nm; Se-196.090nm.

3.3.2. Collection of ileal digesta and analysis

After the trial, all pigs were immobilized by using an electrical stunner and exsanguinated. The abdomen was opened and the small intestine was removed with care. The small intestine was closed at the beginning and at the end of the ileum. Digesta from the ileum (1 meter of ileum before the cecum) was collected and stored at -20 °C for chemical analyses and ileal digestibility calculation. Diets and ileal digesta were analyzed for dry matter (ISO 6496), crude ash (CA) (ISO 5984), crude protein (CP) by the Kjeldahl method (ISO 5983-2), crude fat (Cfat) using petroleum ether extraction (ISO 6942), and crude fiber (CF) with boiling samples alternating sulphuric acid and potassium hydroxide (ISO 6865). Calcium (Ca), phosphorus (P), sodium (Na), and zinc (Zn) analyses were carried out after 1.0000g samples were digested in a block digester (LABOR MIM, Budapest, Hungary) with 10 mL cc. Nitric acid at 60 °C for 30 min and 3 mL of 30% hydrogen peroxide alt. (Sigma-Aldrich, Saint Louis, MI, USA) at 90 min at 120 °C. For selenium (Se) analysis, a 0.5000 g sample was measured into high-pressure digestion bombs with 5 mL cc. Nitric acid and 3 mL of 30 % hydrogen peroxide (Sigma-Aldrich, Saint Louis, MI, USA). The digestion was processed in a microwave digester (ETHOS Plus, Milestone) applying the digestion program suggested by the manufacturer (Application Note 076: 3 mins at 85 °C; 9 mins at 145 °C; 4 mins at 200 °C; 14 mins at 200 °C). All digested samples were filled to 50 mL with distilled water and filtered through MN640W (155 mm; Macherey-Nagel) filter paper. The analysis was carried out with the ICP-OES technique (iCAP 7000, Thermo Scientific Kandell, Germany). The ileal digestibility of nutrients and minerals was calculated using acid-insoluble ash (AIA) as an internal marker using the following formula:

Ileal digestibility (%) = $(1-(A_{diet} / B_{digesta}) * (XB_{digesta} / XA_{diet}))*100$

Where:

A and B are marker concentrations (g/kg dry matter)

XA and XB are the concentrations of the test nutrient (g/kg dry matter)

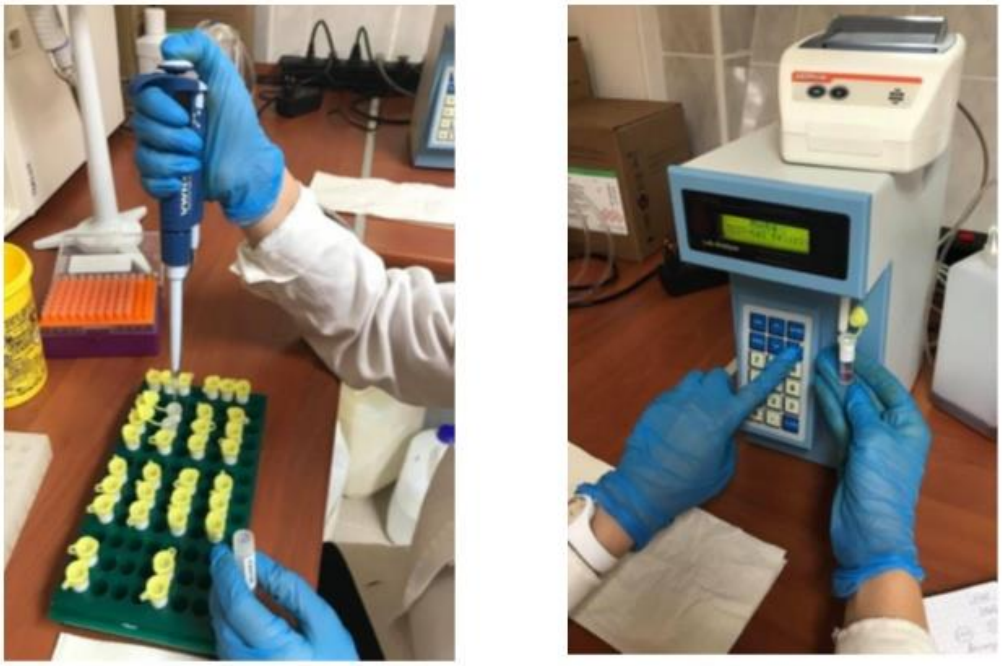
3.3.3. Plasma biochemical parameter: blood collection and analysis

On the first and last days of the experimental period (the 15^{th} and 30^{th} days of the trial, 7 days (Period 1) and 21 days (Period 2) of exposure, respectively), blood samples were collected from the external jugular vein of the pigs (*Picture 1*) into EDTA tubes. The collected blood samples were then centrifuged at 4 °C for 15 min at 3000x g after being clotted

for 20 min (XIN et al., 2018). The separated plasma samples were then stored at −80 °C for later analysis. Plasma concentrations of significant electrolytes (sodium (Na^+), potassium (K^+), and chloride (Cl^-)) were used as markers for electrolyte balance with reference to previous studies (HEIDARI et al., 2018; OLIVEIRA et al., 2017; PATIENCE et al., 2005; HASONA and ELASBALI, 2016), and as mentioned by SHRIMANKER and BHATTARAI (2021). The analysis of the plasma samples was performed in triplicate. Na^+, K^+, and Cl^- plasma levels were analyzed through the photometric method with a Lab-Analyse (Orvostechnika Ltd., Budapest, Hungary) half-automatic analyzer. There were two measurements; first measurement was the endpoint (for Cl^- measurement), wherein a 5 µl sample was pipetted into its respective reagent. Cl^- samples (mixed with reagents) were incubated for 5 minutes. One clear reagent was used as a blank before measuring the incubated samples. The second measurement was the two-point (for Na^+, and K^+ measurement), whereas 20, and 10 µl samples, respectively, were pipetted into their respective reagents, mixed, and measured directly (except for Na, which was incubated for 5 minutes). In the said measurement, distilled water was also used as a blank. The units used on the parameters measured were mmol/L (Na^+, K^+, and Cl^-).

Plasma concentrations of glucose, uric acid, urea, and creatinine were analyzed through the photometric method using Lab-Analyse (Orvostechnika Ltd., Budapest, Hungary) half-automatic analyzer (*Picture 2*). The measurement was performed in triplicate and at room temperature. It was made sure that the samples and reagents were at room temperature before and during the measurement. Three different types of measurement were performed depending on the parameter measured and the reagent used (varied among the parameters measured). The first measurement is the endpoint (for glucose and uric acid measurement), wherein 5 µl (glucose) and 12 µl (uric acid) sample was pipetted into their respective reagents. Glucose and uric acid were incubated for 10 minutes. One clear reagent for each parameter was used as a blank before the measurement of the incubated samples. The second measuring is the Kinetic in which distilled water was used as a blank. In this method, a 10 µl sample was pipetted into the urea reagent and was mixed and measured directly. The third measurement is the two-point (for creatinine measurement), whereas 50 µl sample was pipetted into the creatinine reagent, mixed, and measured directly. In the said measurement, distilled water was also used as a blank. The units used on the parameters measured were mmol/L (glucose and urea) and µmol/L (uric acid and creatinine).

Picture 1. **Collection of blood samples from the pig**



Picture 2. **Measurement of samples using the Lab-Analyse spectrophotometer**

3.3.4. Cytokines and heat shock proteins expression

Sample collection

At the end of the trial, whole blood was collected from the external jugular vein of the pigs. White blood cells were separated from the whole blood (following the method of SIPOS et al. 2004) to measure interleukins (*IL-1β, IL-10*). On the first and second days after the experiment, six pigs from each treatment were slaughtered (three in one day from each treatment) after electrical stunning, and tissue samples of jejunum were collected. Jejunum samples were cleaned in PBS and snap-frozen in liquid nitrogen to measure heat shock proteins (*HSP 70* and *90*) and tumor necrosis factor-alpha (*TNF-α*).

RNA isolation and reverse transcription

Addition of 500 µl TRI Reagent™ (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to white blood cell samples was done before RNA isolation which was carried out with Direct-zol™ RNA MiniPrep (Zymo Research, Orange, CA, USA) following the manufacturers' protocol and with the inclusion of DNase treatment. Total RNA from jejunum was extracted using peq GOLD Total RNA kit (C-line) (VWR International, LLC., Radnor, Pennsylvania, USA) following the manufacturers' protocol. RNA integrity was checked with 1% agarose gel electrophoresis. RNA concentration and purity were determined by HTX Synergy Microplate Reader (Agilent BioTek, Agilent Technologies INC., Santa Clara, USA). RNA was reverse-transcribed into cDNA with qScript® cDNA SuperMix (Quantabio, Beverly, USA) in a 20 µL final volume containing 5x cDNA supermix (including MMLV-type reverse transcriptase, MgCl2, dNTPs, oligo (d)T primer, random primers, recombinant RNase inhibitor protein, stabilizers), 600 ng RNA template and distilled water. The conditions consisted of reverse transcription at 25 °C for 5 minutes, 42 °C for 30 minutes, and 85 °C for 5 minutes.

qPCR analysis of cytokines and heat shock proteins

Intron-spanning forward and reverse primers for pig *ACTB, HMBS, HPRT, HSP70, HSP90, IL-1β, IL-10,* and *TNF-α* were designed by Oligo 7 software and checked for target identity using National Center for Biotechnology Information (NCBI) Primer Blast (YE et al., 2012). *ACTB*, *HMBS*, and 18S ribosomal RNA (*HPRT*) housekeeping genes were analyzed by three algorithms (ΔCt, BestKeeper, NormFinder). In white blood cells, *HPRT*, in jejunum *HMBS* were considered as the most stable reference genes for normalization. Quantitative PCR was performed by AriaMx Real-Time PCR System (Agilent Technologies

INC., Santa Clara, USA), and reactions were run in triplicates using 96-well plates (4titude, Surrey, UK). Each reaction included a three ng cDNA template, 2× Xceed qPCR SG Hi-ROX Mix (Institute of Applied Biotechnologies, Prague, Czech Republic), 200 nM of each primer, and distilled water in 10 μL final volume. No template controls were included for each primer. Real-time PCR conditions were the following: initial denaturation at 95 °C for 2 min, 40 cycles of denaturation at 95 °C for 5 s, and annealing/extension at 60 °C for 30 s.

Ct values and melting temperatures were collected with Aria Mx 1.7 software. Results were determined by the Livak method (LIVAK and SCHMITTGEN, 2001) by normalizing the expression of the target gene to a housekeeping gene. Results were determined as fold changes in the expression of the target gene in the experimental groups compared with the thermo-neutral control (TC).

3.4. Statistical analysis

Data were analyzed with variance analyses with GraphPad Prism 8.4.3 software (GraphPad Software Incorporated, San Diego, USA). One-way analysis of variance (ANOVA) evaluated the growth, pork quality, digestibility, and retention of nutrients and minerals (faecal and ileal), cytokines, and HSPs. A two-way ANOVA was used to determine the effects of HS duration and vitamin and micro-mineral supplementation on mineral digestibility, electrolyte concentration, and plasma biochemical parameters. The data were expressed as a mean, with a means separation by Tukey's multiple comparison test. Differences among the treatments were considered significant when $P < 0.05$.

4. RESULTS AND DISCUSSION

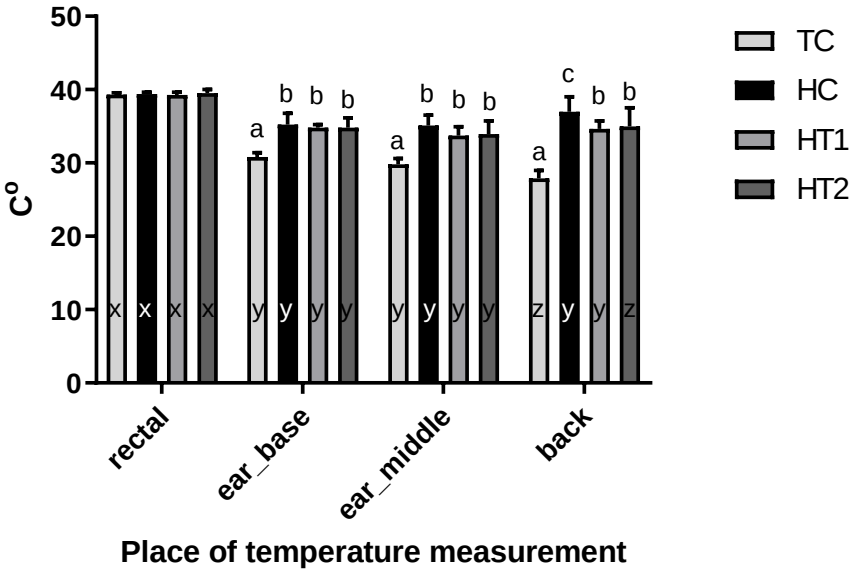
4.1. Temperature and Production performance of pigs

4.1.1. Rectal and skin temperature of pigs

Long-term exposure to HS (29 °C) and high vitamin and micro-mineral supplementation did not significantly affect ($P > 0.05$) the rectal temperature of pigs (*Figure 3*). However, a significant difference ($P< 0.05$) was observed in their skin temperature (measured from the ear base, middle of the ear, and the back). All treatment groups exposed to HS had higher temperature measurements on the ear base and middle than in the TC group. Regarding the temperature measured on the pigs' back, HT1 and HT2 groups had a significantly better ($P < 0.05$) temperature measurement than HC. Nevertheless, pigs in TC had much better temperature measurements than those treatment groups exposed to HS.

Figure 3.

Impact of heat stress and dietary antioxidant supplementation on the rectal and skin temperature of fattening pigs after two weeks of heat exposure (n=9/treatment)



Values are means, with their standard deviation represented by vertical bars; [a,b,c] Means with the same letters within a measurement place between treatments are not significantly different ($P > 0.05$); [x,y,z] Means with the same letters within a treatment between measurement places are not significantly different ($P > 0.05$). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content).

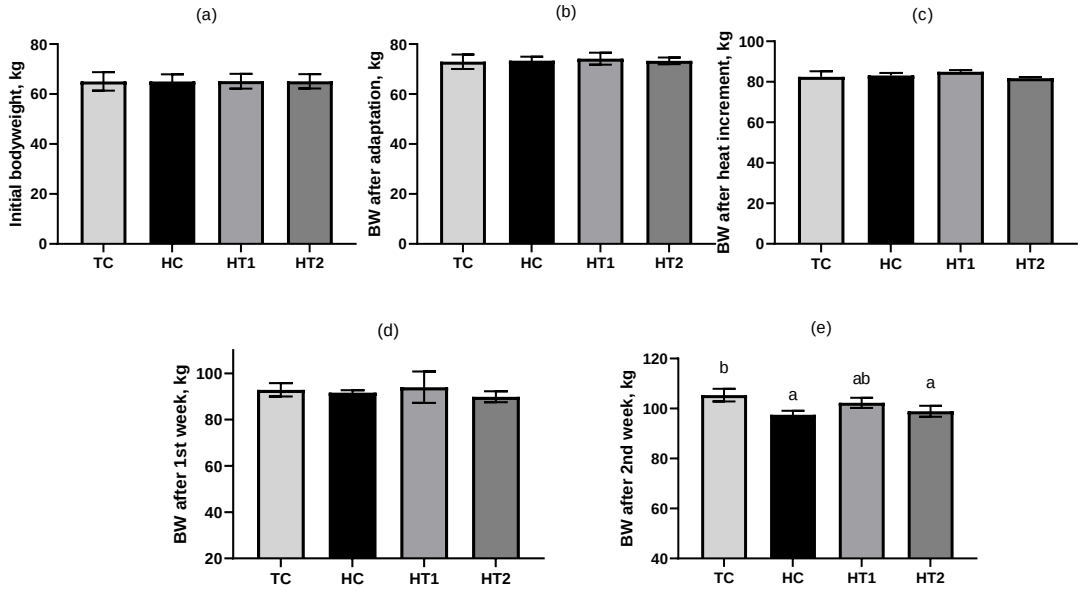
Pigs' exposure to heat stress can cause an increase in body heat, as proven by elevated rectal and skin temperature (COTRELL et al., 2020; QU et al., 2016; XIA et al., 2021; XIN et al., 2018). However, the results of our study about the rectal temperature of pigs suggest otherwise. HS and vitamin and micro-mineral supplementation did not significantly affect the rectal temperature of pigs. The similar rectal temperature observed between pigs in HC and TC groups might be due to the animals' acclimation to the said stressor. It was reported that pigs' can respond with gradual rectal temperature improvement upon exposure to chronic HS (7 days) (WALTZ et al., 2014). Nevertheless, HS caused a significant increase ($P < 0.05$) in the pigs' skin temperature (measured from the base and middle of the ear and at the pigs' back). Such increase was alleviated by the supplementation of vitamin and micro-minerals (HT1 and HT2), which measurements are significantly lower ($P < 0.05$) than in the HC group of pigs (measured at the pigs' back). Reduction of skin temperature in heat-stressed pigs upon supplementation of dietary antioxidants (zinc amino acid complex) was also reported by MAYORGA et al. (2018*b*), suggesting the antioxidants' capability to lower the body temperature of pigs exposed to HS.

4.1.2. Growth performance

After two weeks of exposure to HS, pigs in HC and HT2 groups were significantly lighter ($P < 0.05$) compared to those in the TC group. Interestingly, pigs in HT1 had comparable weights to TC (*Figure 4*). Various duration of HS and supplementation of elevated levels of vitamins (C and E) and micro-minerals (Zn and Se) did not significantly affect the rest of the growth performance parameters (*Table 12*).

Figure 4.

Initial body weight (a), body weight after adaptation period (b), body weight after heat increment (c), body weight after 1st week of experiment (d), and body weight after 2nd week of experiment (e) (n=9/treatment)



Values are means, with their standard deviation represented by vertical bars; [a,b] means with a common letter are not significantly different (P > 0.05). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content).

Table 12.

Growth performance of thermo-neutral and heat-stressed pigs fed basal diet and diets containing elevated levels of vitamins C and E and micro-minerals Zn and Se (n=9/treatment)

Parameter	Treatment				SEM[a]	P value
	TC	HC	HT1	HT2		
Average daily gain, g/d						
HI[b]	1375	1536	1626	1383	59.10	0.4031
HS[c], 1st week	1850	1598	1721	1483	86.18	0.5311
HS, 2nd week	1700	1119	1421	1357	100.89	0.2537
HS, 2 weeks [d]	1775	1358	1571	1420	65.38	0.0814
Average daily feed intake, g/d						
HI	3423	3498	3557	3265	45.44	0.0970
HS, 1st week	3806	3544	3665	3495	86.43	0.6500
HS, 2nd week	3915	3898	3501	4227	114.57	0.1573
HS, 2 weeks	4017	3730	3781	3498	106.83	0.1018
Feed conversion ratio						
HI	2.51	2.30	2.20	2.41	0.08	0.5896
HS, 1st week	2.06	2.23	2.21	2.42	0.09	0.7117
HS, 2nd week	2.50	3.53	2.95	2.61	0.17	0.1369
HS, 2 weeks	2.26	2.73	2.41	2.51	0.07	0.0955

TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content). [a] standard error of the mean; [b] heat increment; [c] heat stress; [d] average of 1st and 2nd week.

Several research pieces reported that high ambient temperature-induced detrimental effects on growing to finish pigs (KELLNER et al., 2016; CERVANTES et al., 2016; DA FONSECA DE OLIVEIRA et al., 2019). Slow growth, decreased feed efficiency, and carcass lean are consequences experienced by pigs under such stressors, which are significant contributors to swine economic losses (GONZALEZ-RIVAS et al., 2020). Mitigation of such adverse effects through vitamins and micro-minerals has been reviewed and documented (COTRELL et al., 2015); however, information regarding the mitigation capacity of their combinations is limited. In our study, 14 days of HS caused a significant decrease (P <

0.05) in the body weight (BW) of pigs fed with basal feed or control diet (HC) and higher vitamin and micro-mineral diets (HT2). Interestingly, pigs fed an elevated 1 (T1) diet (HT1) had a comparable body weight with those in TN conditions (TC). A decline in BW is commonly observed in several animals as a response to HS, which can be attributed to the lower feed and nutrient intake of such animals exposed to the said stressor (XIN et al., 2018; GOO et al., 2019). However, the reduction in the BW observed in HT2 pigs was unexpected. This observation might be due to the decreased feed consumption as supported by numerically lower feed intake by pigs in HT2 regardless of the supplementation. A similar observation was reported by SANZ FERNANDEZ et al. (2013), where pigs exposed to HS-fed diets containing inorganic and organic zinc supplemented at a level of 120, 100+120, and 120+200 mg/kg, respectively, had comparable performance in terms of feed intake and BW. Contrastingly, ROMU-VALDEZ et al. (2019), and LV et al. (2015), reported that high levels of antioxidants (organic zinc 360 mg/kg, and selenium-enriched probiotics 0.46 mg/kg, respectively) in the diet positively influence the production performance of pigs under HS, as supported by improvements in BW, FI, ADG, and FCR. Statistically, comparable performance was exhibited by pigs in all treatment groups in terms of ADG, FI, and FCR under all periods of observation in our experiment. However, pigs under HS and fed basal diet (HC) had the least observed values of all the parameters studied numerically. At the same time, supplementation of vitamins and micro-minerals at an elevated 1 level (vitamins C -150 mg/kg, E – 41 mg/kg, and minerals Se – 0.21 mg/kg and zinc – 100 mg/kg) in the diet indicates slight improvement. Such a level of supplementation might be effective enough as there are also studies that reported improvements in the growth of pigs under HS-fed diets containing a slight increase in dietary antioxidant (Zn – 75 mg/kg) supplementation (MANI et al., 2019).

4.1.3. Meat quality

Chemical and physical analysis of meat samples obtained from thermo-neutral and heat stressed-pigs fed their respective dietary treatments (basal, T1, and T2 diets) have similar results among all experimental treatments ($P > 0.05$) (*Table 13*). HS and vitamin C, E, and micro-minerals Zn and Se supplementation did not significantly affect the meat quality parameters of pigs ($P > 0.05$).

Table 13.

Meat quality of thermo-neutral and heat-stressed pigs fed basal diet and diets containing elevated levels of vitamins C and E and micro-minerals Zn and Se (n=6/treatment)

	Treatment					
Parameter	TC	HC	HT1	HT2	SEM[a]	P value
Moisture, %	67.45	67.87	67.68	68.17	0.29	0.8636
Protein, %[f]	22.38	22.12	22.85	22.25	0.22	0.7095
Fat, %[f]	8.62	8.46	7.93	8.04	0.13	0.1937
Vitamin C, mg/100g[b]	9.26	9.60	8.31	8.40	0.64	0.8807
Zn, mg/kg [b]	11.70	12.48	11.74	12.30	0.33	0.8075
Se, mg/kg [b]	0.3172	0.3910	0.3385	0.3342	0.02	0.6864
pH 45 minutes	6.46	6.38	6.44	6.44	0.04	0.9118
pH 24 hours	5.47	5.53	5.53	5.22	0.01	0.3297
L* (lightness)	49.84	51.09	50.87	51.52	0.38	0.4764
a* (redness)	15.09	15.95	16.47	15.88	0.22	0.1824
b* (yellowness)	3.60	4.22	4.17	4.62	0.15	0.1126
Drip loss, %	2.54	2.68	2.96	2.41	0.15	0.6453
Freeze loss, %	11.25	9.58	11.51	12.48	0.40	0.0764
Cook loss, %	23.60	25.10	24.86	24.31	0.42	0.6370
Firmness, N	55.48	53.58	62.83	58.98	1.83	0.3089
Shear force, N	499.50	528.70	587.80	554.30	16.64	0.2933

TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content). [a] standard error of the mean; [b] in dry matter basis.

Chronic HS can gravely influence the chemical composition and physical character of meat, which have been observed in meat obtained and evaluated from various species of meat-type animals such as broilers, beef cattle, goats, sheep, and pigs (GREGORY et al., 2010; WEGLARZ, 2010; ZHANG et al., 2012; CRUZEN et al., 2015; GONZALES-RIVAS et al., 2020). Pig productivity is vulnerable to HS, and the stressor highly affects pork quality attributes. Decrease in lean tissue, increase in carcass fatness and impaired pork quality such as pale soft exudative (PSE) meat, manifested by increased lightness and yellowness values, and decreased redness value of meat are common adverse effects of HS on pigs' productive

and meat quality performance (SANZ FERNANDEZ et al., 2015; KELLNER et al., 2016; LIU et al., 2021). Nevertheless, as observed in different poultry and livestock animals (broiler, lamb, and pig), vitamins and micro-mineral supplementation through the diet can be an effective tool to mitigate the HS detrimental effect on meat quality (SHAKERI et al., 2019; SILVA et al., 2019; CHAUHAN et al., 2020; LIU et al., 2021). Interestingly, the 14-day chronic HS exposure of Danbred hybrid pigs in our study did not cause any significant changes in the quality of their meat. And high vitamins and micro-mineral supplementation in combination also did not significantly influence the meat quality of pigs exposed to the said stressor. Although our result is in contrast to the previous observations of YANG et al. (2014); SHI et al. (2016); MA et al. (2019); LIU et al. (2021*a*), it is in agreement with what was observed by LEHOTAYOVÁ et al. (2012) in pigs exposed to constant HS throughout the growing and finishing period. They concluded that several meat quality parameters, such as shear force, drip loss, and meat color, were not significantly affected by HS. The pigs' resilience to HS adverse effects, as observed in the quality of their meat evaluated in this study, might be due to their ability to tolerate such stressors over time (CAMPOS et al., 2017). Several studies reported that a longer duration of HS exposure could result in gradual performance improvement in pigs. Such observation may result from the pigs' adaptive changes, such as a decrease in heat production during the acclimation stage upon chronic exposure to HS (RENAUDEAU et al., 2008; RENAUDEAU et al., 2010, RENAUDEAU et al., 2013).

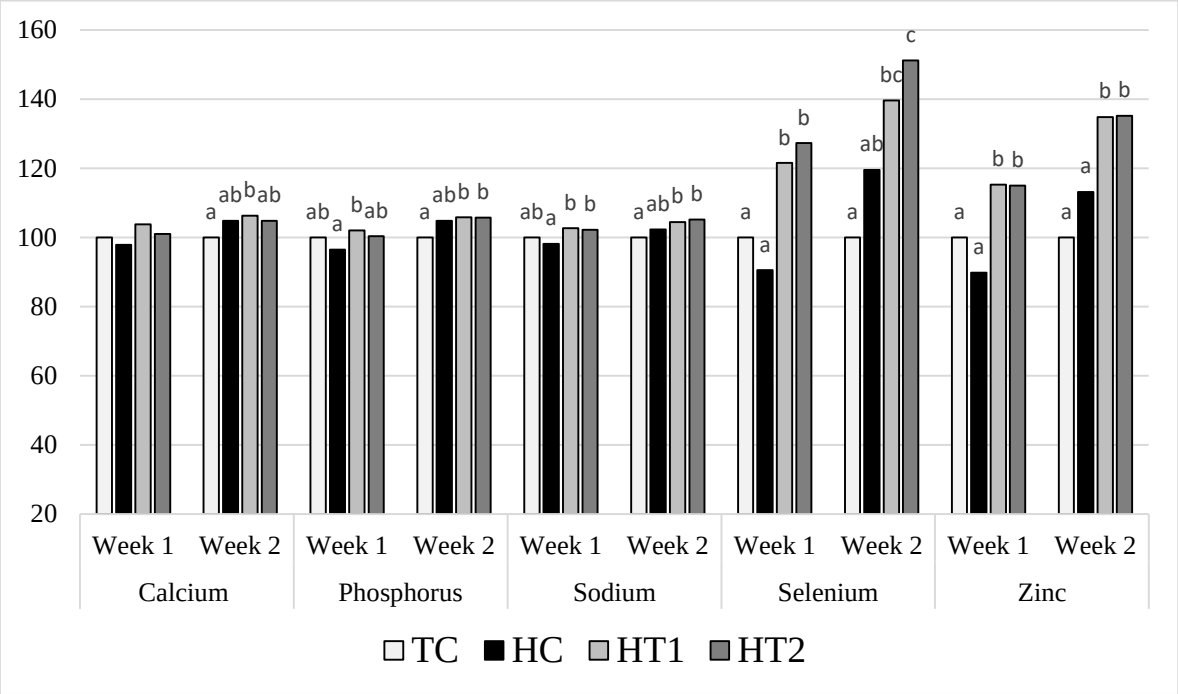
4.2. Mineral digestibility and electrolyte balance of pigs

4.2.1. Mineral digestibility

There was a significant HS duration (period) effect observed in the case of sodium and zinc ($P < 0.05$) (*Figure 5*). The environmental and dietary treatments significantly affected the faecal digestibility of all minerals ($P < 0.05$). As observed in both periods, pigs in the TC and HC groups had a similar mineral digestibility ($P > 0.05$), indicating that the genotype used in the trial has some resilience to HS. Single dose supplementation of some vitamins and trace minerals (T1) resulted in increased digestibility ($P < 0.05$) in the second week of the heat stress period compared to the TC treatment. double dose supplementation of vitamin C, vitamin E, Zn, and Se (T2) did not improve the digestibility of the minerals tested ($P > 0.05$).

Figure 5.

Effects of heat stress and vitamin and micro-mineral supplementation on the faecal digestibility (%) of some minerals in fattening pigs relative to TC group (n=3/treatment/week)



[a,b,c] means in a row with the same superscripts do not differ (P > 0.05); TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content).

The pigs' exposure to different durations of HS had varying responses with a high possibility of thermal acclimation at a more extended period of exposure (RENAUDEAU et al., 2008; RENAUDEAU et al., 2010). HS is noted to have a deleterious impact on pigs' intestinal integrity and digestive function, and it was reported that HS could negatively influence the nutrient and mineral retention capability (GABLER and PEARCE, 2015; YU et al., 2010; SANTOS et al., 2018). Our results partly agree with PATIENCE et al. (2005) and KIM et al. (2020), wherein no significant changes in the mineral digestibility of pigs reared under HS conditions were observed. Our results might be due to the pigs' ability for thermoregulation, which provides an avenue for their acclimation to high ambient temperature (CAMPOS et al., 2020). As previously reported, pigs show improved tolerance to heat with the duration of exposure, resulting in a positive production performance (RENAUDEAU et al., 2008; RENAUDEAU et al., 2010). Interestingly, a significant increase in Ca, P, Na, Se,

and Zn digestibility was observed in pigs that were fed elevated levels of vitamins and micro-minerals (HT1 vs. TC) during week 2. Our findings agree with the results reported by XIE et al. (2019). The increased levels of Se and Zn in the HT1 and HT2 diets might influence their digestibility. The significant increase in Ca, P, and Na digestibility does not apply in this situation, as the contents of these minerals were similar in all diets. Such results might be attributed to vitamins and micro-minerals capability to improve the integrity of the animals' gastrointestinal (GIT) tract through their antioxidant effect.

Vitamins E and C and micro-minerals Se and Zn are notable dietary antioxidants. Vitamin E works as a chain-breaking antioxidant that prevents the propagation of free radicals in membrane and plasma lipoproteins, while vitamin C has the capability of protecting cell membranes, DNA, cell proteins, and lipids against reactive oxygen species (ROS) during OS and is also essential in the regeneration of other antioxidants, such as alpha-tocopherol (vitamin E) and glutathione (REBOUL et al., 2017; TRABER et al., 2011; PADAYATTY and LEVINE., 2016; SHENKIN et al., 2006). Se, which is absorbed in the duodenum and cecum by active transport through a sodium pump, acts as a dietary antioxidant by forming selenoproteins and regulating endogenous enzymes' activity. In contrast, Zn, which is absorbed by the small intestine by transcellular transport processes, activates antioxidant peptides and enzymes by inducing metallothionein expression, which is vital in protecting cells against ROS (MEHDI et al., 2013; KIELCZYKOWSKA et al., 2018; MAARES et al., 2020; JAROSZ et al., 2017). Several research reported that supplementation of these vitamins and micro-minerals improved the heat-stressed pigs' intestinal integrity and function by enhancing the intestinal epithelial function, alleviating HS-induced OS, and improving intestinal health (LIU et al., 2016; PEARCE et al., 2015*a*; MAYORGA et al., 2018*b*; MANI et al., 2019). Along with the impact of these substances on GIT's integrity and functionality, they influence the utilization of other vitamins essential for absorbing minerals. Vitamin C and Zn are cofactors of vitamin D, which promotes the absorption of calcium and phosphorus (phosphate) in the intestine via active transport and diffusion. Phosphate is transported into the epithelial cell by co-transport with sodium (sodium phosphate cotransporter), which is enhanced by said vitamin (VIVO PATHOLOGY; WASSERMAN et al., 1981; CRAIG et al., 2001; CHRISTAKOS et al., 2011).

4.2.2. Markers for electrolyte balance

More prolonged chronic heat stress elevates the plasma Na^+ level ($P< 0.05$) (*Table 14*). The plasma levels of K^+ were similar ($P> 0.05$) despite the thermal and dietary treatments. However, a significant reduction of plasma Cl^- ($P < 0.05$) was observed in pigs due to heat stress (HC group). The supplementation of vitamins and micro-minerals was wholly or partly able to mitigate this adverse effect.

Table 14.

Effects of heat stress and vitamin and micro-mineral supplementation on the plasma concentration (mmol/l) of major electrolytes as markers of electrolyte balance in pigs (n=9/treatment/period)

	Treatment					***P* values**	
Electrolytes	TC	HC	HT1	HT2	SEM	Period	Treatment
Sodium						0.0315	0.2798
Day 7	204.3	194.0	205.7	213.1	2.95		
Day 21	219.7	210.7	210.8	213.6	3.12		
Potassium						0.1540	0.3365
Day 7	8.9	7.2	7.9	8.9	0.36		
Day 21	9.6	9.0	8.7	8.5	0.34		
Chloride						0.2098	0.0013
Day 7	100.3 ^{b}	88.5 ^{a}	96.2 ^{ab}	100.9 ^{b}	1.60		
Day 21	104.7 ^{b}	93.3 ^{a}	97.7 ^{ab}	101.0^{ab}	1.65		

^{a,b}means in a row with the same superscripts do not differ $p > 0.05$. TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH-60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content).

Previous observations regarding the concentrations of significant electrolytes (Na^+, K^+, and Cl^-) under HS suggested no changes; therefore, no losses of electrolytes were determined (PATIENCE et al., 2005). However, our results suggest otherwise, although such losses did not significantly affect all the studied parameters. Acute and chronic HS reduces metabolic rate and causes metabolism disorders, acute phase response, and respiratory alkalosis that might affect the electrolyte balance of pigs (COTTRELL et al., 2020;

FAUSNACHT et al., 2021; XIONG et al., 2020; CUI et al., 2019). The concentration of plasma Cl^- obtained from pigs under HS + fed basal diet (HC) was significantly reduced ($P< 0.01$) and is below the reference range in pigs (94-106 mmol/l) (KANEKO et al., 2008), regardless of the duration of exposure, which is in contrast to the results observed by PEARCE et al. (2014) in pigs exposed to acute HS (2–6 h). Moreover, it was also reported that one to three-day HS exposure led to significant changes in plasma Cl^- concentration in pigs. However, after 7 days and 28 days, similar electrolyte concentrations were observed between pigs under HS and thermal comfort (PEARCE et al., 2013*c*; MENDOZA et al., 2017). Such discrepancies might be due to the pigs' blood pH changes. Progressive alkalinity of the pigs' blood pH was reported in response to HS. Such a condition can cause metabolic alkalosis, one of the major causes of the reduction in Cl^- concentration in the blood (COTTRELL et al., 2020; CUI et al., 2019). Another possibility is HS-induced hepatic cellular apoptosis, which can affect the regulation of blood Cl^- by the liver (CUI et al., 2016). Supplementation of dietary antioxidants (vitamins and micro-minerals) has shown significant improvement in some of the blood biochemical parameters of pigs under various stressors (LIU et al., 2016; XIE et al., 2019; PEARCE et al., 2015*a*; YOON et al., 2020). In our study, the concentration of Cl^- was significantly improved ($P< 0.01$) in HT2 pigs after 7 days of exposure (period 1), and slight improvements were also observed after 21 days (period 2). This observation might be due to the capability of vitamins and minerals to improve the regulation of the pigs' blood pH under HS and mitigate its adverse effect on the pigs' liver. High-level vitamin E and selenium supplementation reportedly improved blood pH in pigs reared in high ambient temperatures (LIU et al., 2016; LIU et al., 2018). Moreover, LIU et al. (2021b) observed that supplementation of organic selenium beyond nutrient requirements (0.4 and 0.6 mg/kg in the diet) alleviated the adverse effects of chronic HS in pig liver.

4.3. Nutrient digestibility, metabolism, and immune response of fattening pigs

4.3.1. Dry matter and nutrients, macro and micro-minerals digestibility and retention

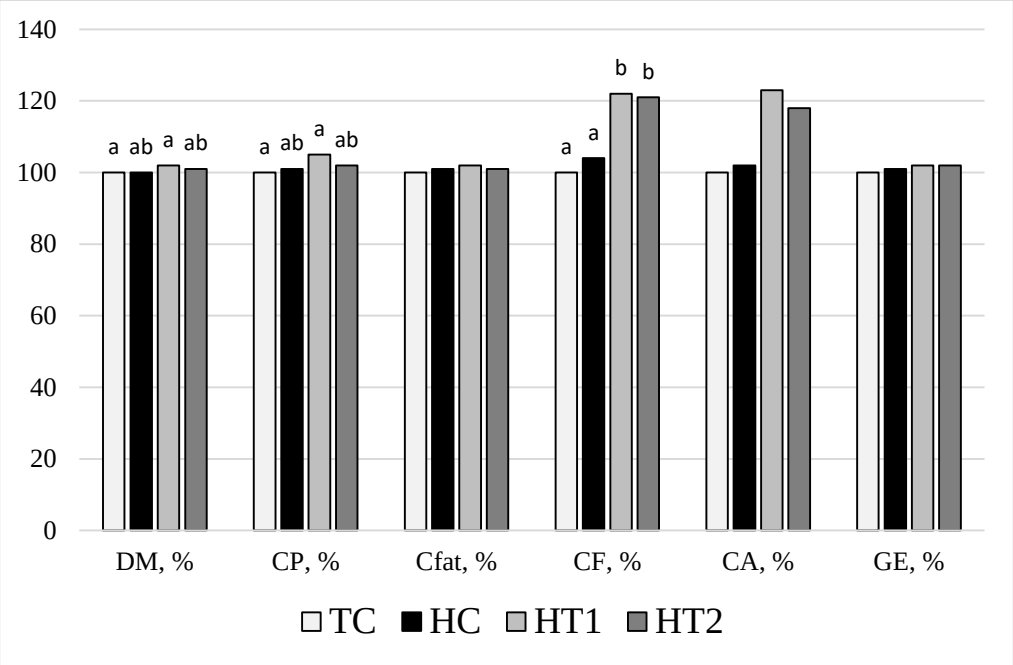
HS did not significantly affect ($P > 0.05$) pigs' dry matter and nutrient digestibility. However, vitamin and micro-mineral supplementation improve pigs' DM and CP digestibility under HS. Pigs in HT1 had a significantly higher DM and CP digestibility ($P < 0.05$) as compared to pigs in TC groups (*Figure 6*). Moreover, the crude fiber digestibility of heat-stressed pigs supplemented with vitamins and micro-minerals (HT1 and HT2) was significantly higher ($P < 0.05$) than those in the TC and HC group. Nevertheless, no significant differences were observed in the groups' digestibility of crude fat, energy, and crude ash ($P > 0.05$).

Interestingly, pigs in TC and HC groups have similar mineral digestibility ($P > 0.05$). P and Na digestibility were not affected by HS and the supplementation of vitamins and micro-minerals at elevated levels. However, significantly higher digestibility of Ca, Zn, and Se ($P < 0.05$) was observed in pigs fed elevated levels of vitamins and micro-minerals (HT1 and HT2) than in pigs fed a control diet (HC and TC), despite being exposed to HS (*Figure 7*).

CP retention of pigs was not significantly affected ($P > 0.05$) by HS and vitamin and micro-mineral supplementation (*Figure 8*). However, significant differences ($P < 0.05$) were observed in the Ca, Na, Zn, and Se retention. Wherein pigs fed elevated levels of vitamins and micro-minerals (HT1 and HT2) showed significantly better performance. Among the various minerals, only P was not significantly affected.

Figure 6.

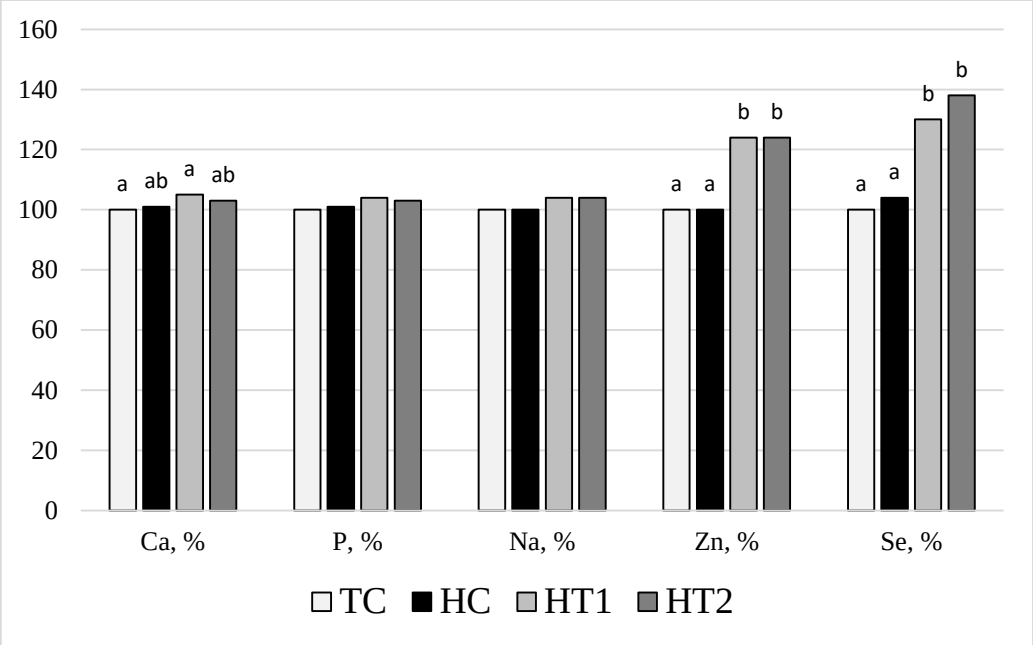
The impact of long-term heat exposure and high vitamin and micro-mineral supplementation on the faecal digestibility of nutrients in fattening pigs relative to TC group (n=6/treatment)



[a,b] means in a row with the same superscripts do not differ (P > 0.05). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content).

Figure 7.

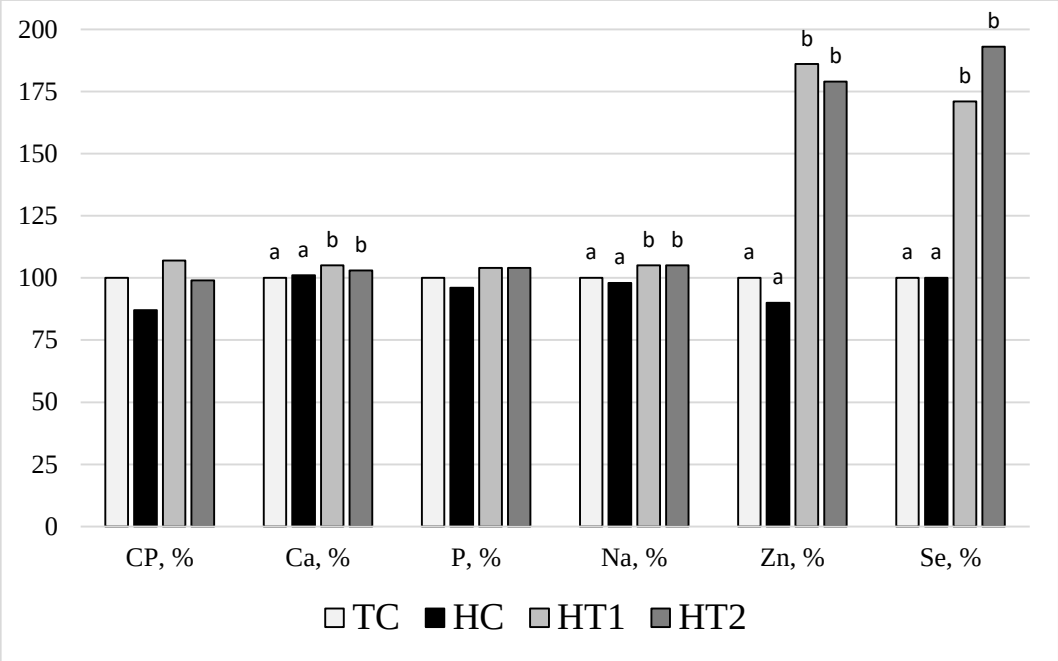
The impact of long-term heat exposure and high vitamin and micro-mineral supplementation on the faecal digestibility of minerals in fattening pigs relative to TC group (n=6/treatment)



[a,b] means in a row with the same superscripts do not differ (P > 0.05). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content).

Figure 8.

The impact of long-term heat exposure and high vitamin and micro-mineral supplementation on the faecal retention of CP and minerals in fattening pigs relative to TC group (n=6/treatment)



[a,b] means in a row with the same superscripts do not differ (P > 0.05). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content).

The nutrient and mineral digestibility and retention of pigs were not significantly affected (P > 0.05) by HS. Our observation indicates that HS conditions in this study have no significant impact on the gastrointestinal digestibility function of the pigs. Our findings contradict the observation of BRESTENSKY et al. (2012) and HAO et al. (2014), which reported that the digestibility of nitrogen, gross energy, DM, CP, and crude ash were reduced in heat-stressed pigs. However, it agrees with the report of Kim et al. (2020*a*), wherein pigs reared under HS (33 °C) had comparable nutrient and mineral digestibility to pigs under thermal comfort (25 °C). Moreover, RENAUDEAU et al. (2008) observed a significant increase in DM digestibility of pigs over a long period of HS despite having a relatively lower feed intake. Although, it was reported that HS negatively affects the nutrient digestibility of pigs by impairing intestinal integrity and function (PEARCE et al., 2015*b*). Most of these cases were observed after acute HS (24 hours), where digestive capacity alteration and post-

absorptive metabolism are prominent (PEARCE et al., 2012). The comparable values observed in HC and TC groups might be due to the adaptability of pigs to HS when acclimatized. From a physiological standpoint, pigs' negative response to HS is more prominent during the first 2 to 3 days (LIU et al., 2009; PEARCE et al., 2014; YU et al., 2010) and might decrease as animals become acclimated over time. RENAUDEAU et al. (2010) reported that pigs under constant HS for 20 days had elevated rectal temperature in the first 24 hours of exposure, but it was gradually reduced with successive days of exposure. This indicates that pigs can become partially acclimated within weeks of exposure to HS (MORALES et al., 2016*a*).

MORALES et al. (2016*b*) reported that pigs under HS had lower CP digestibility than pigs in thermal comfort. In contrast, RENAUDEAU et al. (2008) and CAMPOS et al. (2014) reported that pigs' exposure to HS (32 °C) showed an increase in N digestibility, despite having significantly lower average daily gain, and its digestibility is greater than in pigs under thermal comfort. Although there were contradicting results from previous studies, our observation agrees with what was reported by LIAO and VEUM (1994), that faecal excretion and digestibility of proteins remained unchanged in pigs under constant high temperatures as opposed to pigs under TN conditions. Previous studies revealed that pigs' exposure to HS (30-33 °C) could lead to low retention of N (BRESTENSKY et al., 2012; RENAUDEAU et al., 2013) and protein (COLLIN et al., 2001*d*). However, results in our study revealed that both HC and TC groups have similar retention of protein and minerals. Our finding is similar to LE DIVIDICH et al. (1980) and PATIENCE et al. (2005), which observed no difference in protein and mineral retention between pigs housed at 20 and 28 °C, and diurnal HS (20-38 °C within 24 hours), respectively. Therefore, either too high or low temperatures affect protein metabolism (CLOSE and MOUNT, 1978; FERGUSON and GOUS, 2002; LIAO and VEUM, 1994). Similarly, no significant differences were observed in the mineral retention between HC and TC groups ($P > 0.05$). Therefore, the experimental HS had no significant impact on nutrient digestibility and retention in the pigs in this trial. Aside from the pigs' capability of acclimation to HS, the pigs in our study did not respond with severe tissue damage as *TNF-α* expression of HC and TC pigs were comparable. A similar observation was reported by WEN et al. (2019) in pigs under long-term exposure to HS. While an increase in the expression of *TNF-α* has been associated with damaged intestinal barrier functions (ABUAJAMIEH et al., 2018), our observation indicates that there is only a low incidence of intestinal barrier damage, supporting our results.

LIU et al. (2016) demonstrated that the combination of selenium and vitamin E at high levels reduced oxidative stress and intestinal leakiness. Zn supplementation improved pigs' intestinal integrity, leading to better digestibility of nutrients under HS (PEARCE et al., 2015*a*). It was hypothesized that supplementing pigs under HS with dietary antioxidants (vitamins E and C, Se and Zn) would mitigate against HS-induced OS. Our findings confirmed this hypothesis as our results indicate that despite being subjected to HS conditions, pigs fed T1 and T2 diets (HT1 and HT2) have better mean performance in terms of DM, nutrients, and mineral digestibility and retention. The effects of supplementation with dietary antioxidants (vitamin and micro-minerals) can be observed in DM and CP digestibility between HT1 and TC groups. DM and CP digestibility were significantly higher in HT1 than in TC ($P < 0.05$). Moreover, CF digestibility was significantly higher in HT1 and HT2 than in HC and TC groups. The digestibility and retention of minerals were also influenced by dietary antioxidants' supplementation and were observed in Ca, Zn, and Se (digestibility) and Ca, Na, Zn, and Se (retention). Dietary antioxidant supplementation at T1 and T2 levels significantly improved ($P < 0.05$) the digestibility and retention of these minerals, which can be supported by the fact that dietary antioxidants (vitamins C and E and minerals Se and Zn) can alleviate HS-induced damage in the intestinal epithelial cells (TANG et al., 2019) and can improve their intestinal barrier integrity and function (LIU et al., 2016; PEARCE et al., 2015*a*; SANZ FERNANDEZ et al., 2014).

4.3.2. Metabolite concentration in plasma

The glucose, uric acid, and urea plasma concentration were not significantly affected by HS and vitamin and micro-mineral (vitamin E, C, Se, and Zn) supplementation. Nevertheless, the plasma concentration of creatinine at day 7 of exposure was significantly ($P < 0.05$) increased in pigs housed in HS and fed with a T2 diet (HT2) than in pigs in the TC group. Furthermore, there was a significant HS duration (period) effect observed in the case of urea and creatinine ($P < 0.05$) (*Table 15*).

Table 15.

Impact of different duration of heat exposure and vitamin and micro-mineral supplementation on the plasma biochemical parameters of fattening pigs (n=9/treatment/period)

	Treatment					***P* values**	
Parameters	TC	HC	HT1	HT2	SEM	Period	Treatment
Glucose, mmol/l						0.5309	0.2627
Day 7	6.26	4.76	5.71	5.38	0.27		
Day 21	5.49	5.05	5.03	5.67	0.21		
Uric acid, µmoL/L						0.7454	0.4888
Day 7	36.15	25.12	33.95	33.30	2.09		
Day 21	36.22	34.79	27.79	33.77	2.29		
Urea, mmol/l						0.0061	0.3318
Day 7	4.83	4.36	4.91	4.81	0.17		
Day 21	5.62	5.14	5.38	6.36	0.27		
Creatinine, µmoL/L						0.0087	0.0017
Day 7	123.57^{a}	133.33^{ab}	138.22^{ab}	158.16^{b}	4.08		
Day 21	136.20	160.34	151.10	165.58	4.43		

^{a,b} means in a row with the same superscripts do not differ (P > 0.05). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated 2 diet: double dose supplementation of vitamin C and E and Se and Zn content).

HS experienced by pigs upon exposure to high ambient temperature can influence their metabolism as it can cause alteration in the plasma concentration of metabolic compounds (SANZ FERNANDEZ et al., 2015*b*). In our study, HS and supplementation of vitamins (C and E) and micro-minerals (Se and Zn) above the recommended levels did not significantly affect the plasma concentration of glucose, uric acid, and urea of pigs. In previous related studies, PEARCE et al. (2013*c*); CUI et al. (2019) observed a high reduction of plasma glucose concentration after HS exposure of 7 and 21 days. While supplementation of ZnAA complex + zinc sulfate (60 + 60 mg/kg of diet) and high levels of selenium (1.0 ppm), respectively, had positively improved the plasma glucose concentration in finishing pigs (LIU et al., 2018*b*; PEARCE et al., 2015*a*). In our study, we observed similarly; however, the

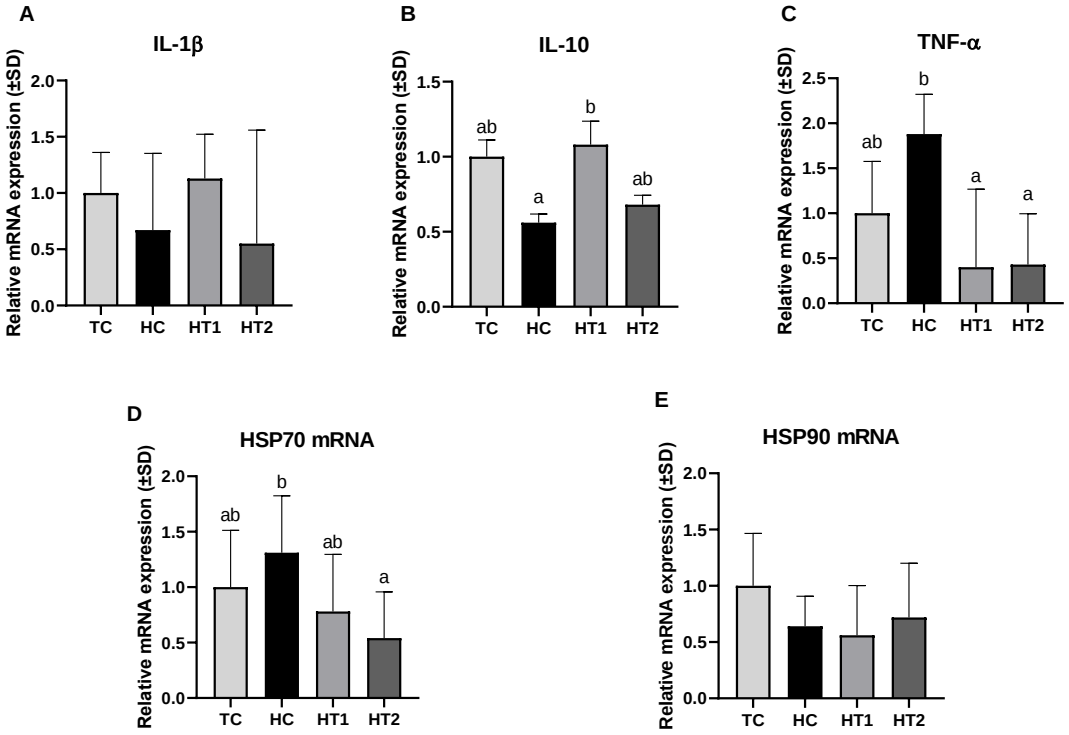
reduction and increase in the plasma glucose levels of pigs upon HS challenge and supplementation of dietary antioxidants, respectively, is not enough to reach a statistical level. Similar to our observation, the plasma concentration of urea (slight decrease in heat-stressed pigs) was also reported by KIM et al. (2020*a*), SANTOS et al. (2018), and SHI et al. (2016). Although contradicting the reports of MAYORGA et al. (2018*b*), PEARCE et al. (2015*a*), and XIONG et al. (2020), where the said plasma concentrations were elevated upon HS challenge, our observed decrease was not at the level of significance. However, the plasma concentration of creatinine was significantly affected ($P < 0.05$) by HS and supplementation of dietary antioxidants at T2 levels observed at day 7 of exposure. HT2 pigs had higher plasma creatinine concentrations than TC pigs. This observation is interesting as it was previously reported that HS influences the synthesis of creatinine and increases its plasma concentration in pigs (PEARCE et al., 2013*c*; KIM et al., 2020*a*). Moreover, the plasma creatinine level in all groups was slightly elevated at day 21 of exposure. This might be associated with the age of the animals, as a similar observation was reported by MENDOZA et al. (2017) upon measuring the creatinine levels of pigs under thermal comfort and HS on days 3 and 28 of the experiment. Although the creatinine level was increased in the second period, all treatment groups had similar concentrations, which agreed with the result observed by OLIVEIRA et al. (2018) on finishing pigs.

4.3.3. Cytokine and heat shock protein expression

HS did not significantly affect ($P > 0.05$) the expression of cytokines and *HSP* in pigs (*Figure 9*). However, supplementation of vitamins and micro-minerals at the T1 level significantly improves ($P = 0.0488$) the expression of *IL-10* and significantly reduces the expression of *TNF-α* in HT1 ($P = 0.0345$) and HT2 ($P = 0.0434$) pigs compared to HC group. The mRNA expression of *HSP 70* was also significantly reduced (*P = 0.0487*) in HT2 pigs compared to TC pigs. Nevertheless, the expression of *IL-1β* and *HSP 90* in all groups was similar ($P > 0.05$).

Figure 9.

The expressions of IL-1β (A), IL-10 (B) in blood, TNF-α (C), HSP 70 (D), and HSP 90 (E) in the jejunum of pigs under thermo-neutral and heat stress conditions and supplemented with elevated levels of dietary antioxidants (n=6/treatment)



Values are means, with their standard deviation represented by vertical bars; [a,b] Means with the same letters do not differ ($P > 0.05$). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated 2 diet: double dose supplementation of vitamin C and E and Se and Zn content).

Chronic heat stress can have a detrimental impact on the animal immune system through cell-mediated and humoral immune responses (AL-ZGHOUL et al., 2019; BAGATH et al., 2019; HUO et al., 2019). Such exposure can result in severe immune disorders in pigs (CHAUHAN et al., 2021; HUO et al., 2019). These days, only a few studies focus on the immune responses of pigs, especially the cellular immune functions of the intestine (HUO et al., 2019). During cellular immune responses, cytokines act as an extracellular signal between cells (KAISER and STÄHELI, 2008). *IL-1β* and *TNF-α* are involved in pro-inflammatory responses (JOHNSON et al., 2020). *IL-1β* has a role in inflammatory reactions and activates T-cells and macrophages (CORWIN et al., 2000; KLASING et al., 1988;

LOTZ et al., 1988). In this study, the mRNA level of *IL-1β* was not changed among treatments. In contrast, LAN et al. (2019) reported increased *IL-1β* concentration in the liver of rats during cyclical heat stress (for 4 hours per 7 days), while chitosan oligosaccharides decreased the level of the pro-inflammatory cytokine, so the inflammation was alleviated. *TNF-α* is a cytokine that inhibits activated immune cells during inflammation and can be applied as an indirect immune index for detecting immune functions (KIM et al., 2004). It also activates the NF-κB pathway, which is vital for regulating inflammatory factors (GOSH et al., 1998; LI et al., 2013). Gene expression level of *TNF-α* was not altered in the HC group compared to the TC pigs. LIU et al. (2016) measured the same when gene expression levels of *TNF-α* along with *IL-8* have not increased in heat-stressed (8 hours for 2 days) pigs, and the authors discussed it might indicate that inflammation was not a significant factor. However, in our study, *TNF-α* mRNA expression level was higher in the HC group than in the HT1 and HT2 groups, suggesting that the applied treatments can decrease the level of *TNF-α* and so the inflammation during heat stress or high ambient temperature conditions. In contrast, the mRNA level of *TNF-α* was not altered when increased concentrations of Se and Vitamin-E were supplemented in heat-stressed pigs (LIU et al., 2016). *IL-10* is an anti-inflammatory cytokine that inhibits apoptosis (KAISER and STÄHELI, 2008; HAKIMI et al., 2014; KINZENBAW et al., 2013). Relative gene expression levels of *IL-10* were not altered among TC, HC, and HT2 groups. Nevertheless, the mRNA level of the mentioned anti-inflammatory cytokine could increase upon the T1 diet (HT1) supplementation, which is significantly higher than the HC group in this study. Similarly, the concentration of *IL-10* was higher in heat-stressed (for 4 hours and 7 days) rats when chitosan oligosaccharides were applied as a treatment, and inflammation could be inhibited (LAN et al., 2019).

Besides cytokines, HSPs' is also a remarkable indicator of alterations in immunity (WILLIAMS – IRELAND, 2008). HSPs' are a family of proteins expressed in response to heat and other stressors and are significant for cell survival during stress conditions. WATANABE et al. (2004) said that HSP could be classified into four families based on their molecular weight. Such classification includes *small HSP*, *HSP60, HSP70,* and *HSP90*. Among them, *HSP70* protects the cells from stress damage and inhibits cellular proteins' irreversible aggregation (GAN et al., 2013). *HSP90* is vital to normal cellular functions and helps the cellular adaptation to stress (ZHANG et al., 2011). HSPs' is part of the third of the three-level antioxidant defense system and have a role in repairing damaged macromolecular segments and restoring the structure of proteins with defective conformations (IRSHAD and

CHAUDHURI, 2002; HORVÁTH AND BABINSZKY, 2019). In this study, the gene expression level of *HSP70* was not changed among the TC, HC, and HT1 groups. However, the mRNA level of *HSP70* was elevated in the heat-stressed pigs fed the control diet (HC) compared to HT2. As a result of HT2 treatment, the mentioned heat shock protein gene expression level could decrease ($P = 0.0487$). GAN et al. (2013) defined the same, and different treatments (inorganic and selenium-enriched probiotics as organic selenium) could result in lower *HSP70* mRNA levels in the spleen, liver, and kidney of heat-stressed pigs (8 hours for 42 days). The authors discussed this lower expression of the mentioned HSP may be due to the increased tissue Se level by the inorganic form, but organic Se was suggested to be even more beneficial during heat stress conditions. YU et al. (2010) reported that the gene expression level of *HSP70* was significantly higher in pigs after heat exposure for 5 hours during 10 days and discussed as heat stress conditions rapidly enhance the denaturation and mis-aggregation of proteins, which triggers *HSPs* expression as a response (KELLER et al., 2008; SONNA et al., 2002; YOUNG et al., 2009). In contrast to our results, LIU et al. (2016) defined a higher gene expression level of *HSP70* in heat-stressed pigs; however, antioxidant supplementation (elevated Se and Vitamin-E) did not decrease the level of the mentioned heat shock protein. In this study, none of the treatments influenced the mRNA level of *HSP90*. In contrast, YU et al. (2010) defined upregulated gene expression levels of *HSP90* after heat exposure, and the authors explained that these changes in gene expression are a major part of the cellular mechanisms during heat stress, which can control protein translation and so cellular functions. MORALES et al. (2014) also reported increased mRNA expression of *HSP90* in pigs exposed to heat stress for 20 days compared to the thermo-neutral group. CERVANTES et al. (2016) defined the same, and the gene expression level of *HSP90* was higher in the duodenum and *longissimus dorsi* of 21-day-heat-stressed pigs compared to the thermo-neutral ones, and authors discussed that *HSP90* might be involved in their long term acclimation mechanism.

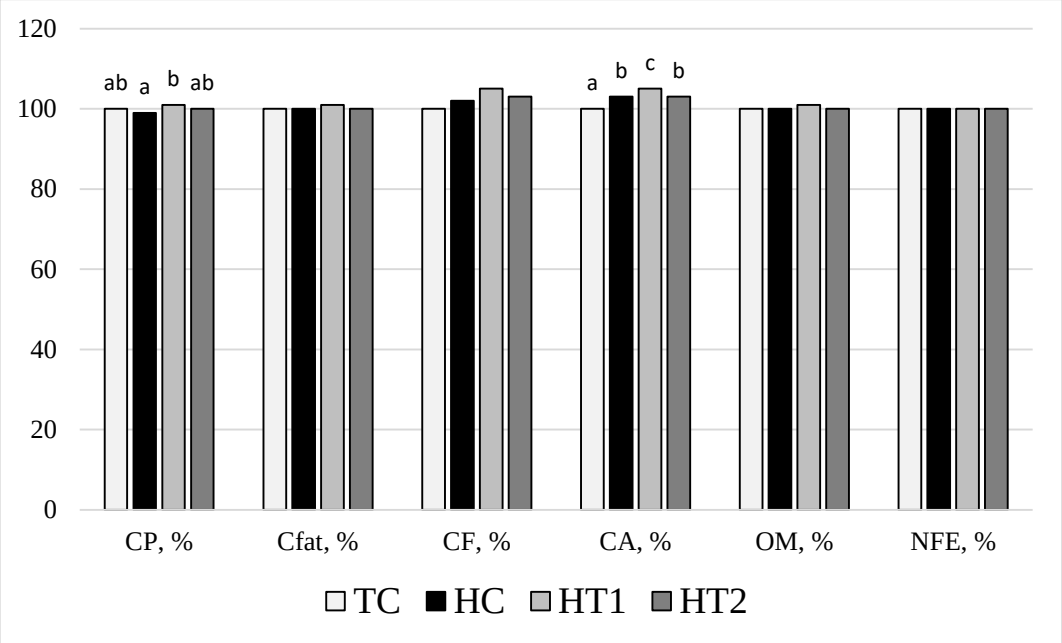
4.4. Ileal digestibility of nutrients and minerals

The ileal digestibility of CP, Cfat, CF, organic matter (OM), and nitrogen-free extract (NFE) was not affected by HS (*Figure 10*). Surprisingly, pigs in TC had significantly lower CA digestibility ($P<0.05$) compared to other treatment groups. Nevertheless, supplementation of dietary antioxidants at an elevated 1 level or T1 (HT1) in the diet significantly improves ($P<0.05$) the CA digestibility and CP digestibility despite the HS challenge.

Heat stress did not significantly affect (P> 0.05) the ileal digestibility of the minerals studied (*Figure 11*). However, high dietary antioxidant supplementation at an elevated 1 level in the diet given to HT1 pigs significantly increased (P< 0.05) the digestibility of Zn, Se, and Na compared to HC pigs. Further increase in the level of dietary antioxidant supplementation in the pigs' diet under HS (HT2) did not significantly (P> 0.05) influence their digestibility.

Figure 10.

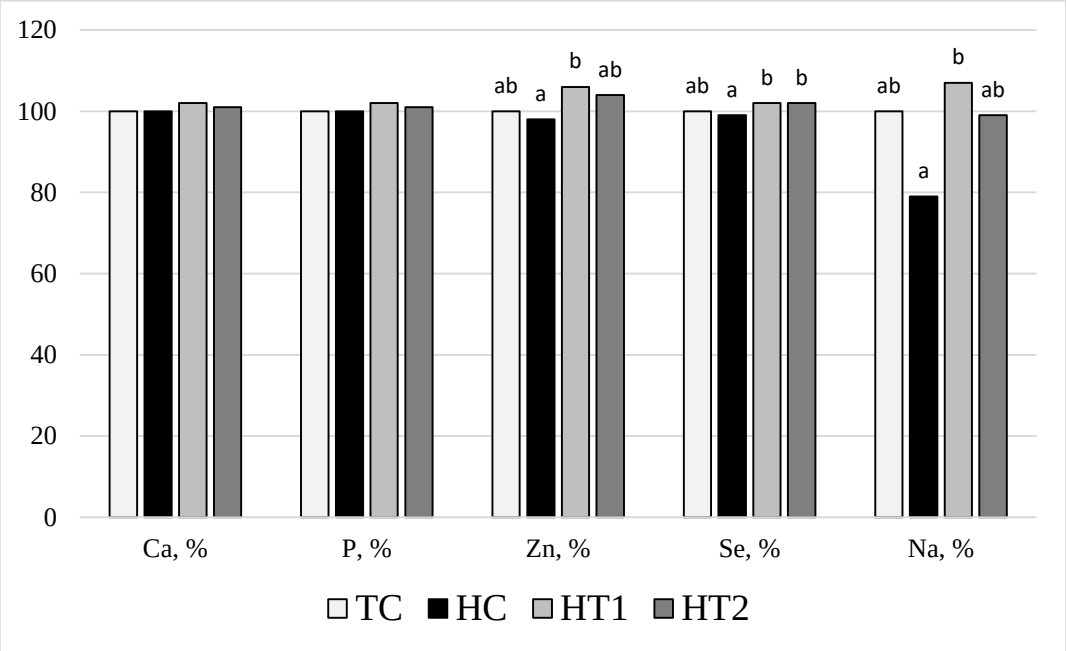
The effect of heat stress and high dietary antioxidant supplementation on the ileal digestibility of nutrients in fattening pigs relative to TC group (n=6/treatment)



Values are means, with their standard deviation represented by vertical bars; [a,b] Means with the same letters do not differ (P> 0.05). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated 2 diet: double dose supplementation of vitamin C and E and Se and Zn content).

Figure 11.

The effect of heat stress and high dietary antioxidant supplementation on the ileal digestibility of minerals in fattening pigs relative to TC group (n=6/treatment)



Values are means, with their standard deviation represented by vertical bars; [a,b] Means with the same letters do not differ (P> 0.05). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated 2 diet: double dose supplementation of vitamin C and E and Se and Zn content).

The susceptibility of fattening pigs to HS is a major concern, as the said stressor can negatively influence the integrity and functionality of the animals' gastrointestinal tract (GIT) (GABLER and PEARCE, 2015). Several studies have reported that HS causes a reduction in intestinal integrity and induces damage to the pigs' small intestine; thereby possibility of affecting its digestive and absorptive function is high (YU et al., 2010; PEARCE et al., 2013*b*; PEARCE et al., 2015*b*; CUI and GU, 2015; GABLER et al., 2018). In the study of HAO et al. (2014), exposure of pigs to HS (30 °C) caused a reduction in their digestibility of nutrients. However, in our study, HS did not significantly (P> 0.05) affect the digestibility of nutrients studied, except for the crude ash, of which digestibility from the HS group pigs (HC) was significantly higher (P< 0.05) than the TC pigs. Despite the insignificance, our observation of the CP digestibility in the TC group is slightly higher (P> 0.05) than the HC group of pigs. This is similar to the observation of MORALES et al. (2016*b*) in pigs exposed to HS for 7 days. The ileal digestibility of minerals studied was not significantly affected by

HS (P> 0.05). Although there were several reports about the impairment of intestinal integrity and function of heat-stressed pigs (PEARCE et al., 2014; PEARCE et al., 2015*b*), our observation indicates that these adverse effects were not experienced by the pigs used in this study. The comparable performance of TC and HC groups could possibly be due to the pigs' acclimation to HS conditions upon prolonged exposure (RENAUDEAU et al., 2008; RENAUDEAU et al., 2010). Moreover, WEN et al. (2019) reported that prolonged exposure of pigs to HS (33 °C for 21 days) did not induce tissue damage and systemic inflammation, which might also be the case experienced by the pigs in this study.

The supplementation of dietary antioxidants at T1 level in the pigs' diet (HT1) significantly increased (P< 0.05) the digestibility of CP and crude ash compared to the HS group fed basal diet (HC) and the rest of the treatment groups, respectively. The said level of supplementation also increased the ileal digestibility of Zn, Se, and Na significantly (P< 0.05) compared to the HC group. Although comparable, the digestibility of the other minerals (Ca and P) studied also shows a similar trend. The improvement in the digestibility of the aforementioned nutrients and minerals in pigs despite their exposure to HS condition could be attributed to the effectiveness of dietary antioxidants' in improving the integrity and functionality of the pigs' GIT (COTRELL et al., 2015; CELI et al., 2017). The dietary antioxidants (vitamins C and E and micro-minerals Se and Zn) used in this study have various roles in influencing the GIT of pigs. Vitamins C and E can promote the integrity of the gut barrier as they play a vital role in modulating the animals' immune function and GIT inflammation (MOUSAVI et al., 2019; LEWIS et al., 2019; LAURIDSEN et al., 2021). Se and Zn also promote pigs' intestinal barrier integrity under HS conditions. The effectiveness of these micro-minerals is associated with improved intestinal tight junction, high ileum transepithelial electrical resistance, and intestinal histology and morphology (SANZFERNANDEZ et al., 2014; PEARCE et al., 2015*b*; LIU et al., 2016). The said influence of the above vitamins and micro-minerals on gut health could also promote better nutrient and mineral digestibility (BROOM et al., 2021; LAURIDSEN et al., 2021; DIAO et al., 2021; ZHENG et al., 2022), of which we observed in our study involving pigs under HS challenge. The digestibility values observed in our study are high. This could be due to the concentration of the inert marker (acid insoluble ash, AIA) in our experimental diet. In the experimental diets that we formulated, the concentration of AIA was quite low: basal diet (0.077 g/kg), elevated diet 1 (0.041 g/kg), and elevated diet 2 (0.059 g/kg). It has been recommended that dietary AIA content should exceed 7.5 g/kg on a dry matter basis to get accurate measurements (THONNEY et al., 1985). Although it is also important to note that dietary AIA of 2 g/kg could also

give good results (PRAWIRODIGDO et al., 2020). The low AIA content in our experimental diet could be the cause of the high ileal nutrients and mineral digestibility values in our study. Since it has been mentioned that such values can be generated when the concentration of AIA in the diet is low, subsequently, low AIA concentration could also be recovered from the digesta and faeces, which could introduce an error to the digestibility calculation (JONES and DE SILVA, 1998; SALES and JANSSENS, 2003). Addition of exogenous sources of AIA and its addition to the diet could increase the AIA content of the feed. Several studies have reported the use of silica, celite, and acid-washed sand as supplemental AIA indigestible markers (CHENG et al., 1990; GODDARD and MCLEAN, 2001; BRESTENSKÝ et al., 2017; KIM et al., 2020*b*). The increase of the AIA marker in the diet through the addition of exogenous AIA markers could improve the precision of the measurement. Nevertheless, if such precision in our calculation was affected due to the inert marker, it seems that it influenced all treatment groups as reflected by their high values, thus the difference still stands. Therefore, we presented our data in a manner that the TC is the 100% and we compared the values of the rest of the treatment groups relative to the TC group.

5. CONCLUSIONS AND RECOMMENDATIONS

It is fascinating that the high genetic capacity genotype (DanBred pigs) used in this trial did not respond with impaired growth, meat quality, nutrient digestibility and retention, as well as detrimental changes in the concentration of plasma biochemical parameters expression of cytokines and HSPs. This occurred, despite the NRC recommendation to provide minimum vitamin and mineral levels in the diet. However, chronic HS at 7 and 21 days of exposure caused a significant reduction in the plasma concentration of chloride, indicating an electrolyte imbalance. Nevertheless, supplementation of elevated levels of dietary antioxidants (vitamin C and E, micro-minerals Zn and Se) corrected this issue, wherein at T2 (Vitamins C (300 mg/kg) and E (71 mg/kg) and micro-minerals (Zn (150 mg/kg) and Se (0.26 mg/kg)) level of supplementation (HT2), the pigs' plasma chloride significantly increased despite exposure to HS. Furthermore, supplementation at T1 (Vitamins C (150 mg/kg) and E (41 mg/kg) and micro-minerals (Zn (100 mg/kg) and Se (0.21 mg/kg)) level (HT1) improved the nutrient (DM, CP, CF) and mineral (Ca, Na, Zn, and Se) digestibility and retention performance of pigs exposed to high ambient temperature (29 °C). Despite the exposure to the HS challenge, pigs fed an antioxidant-fortified diet improved their chloride plasma concentration, correcting the imbalance. Moreover, the ileal digestibility of nutrients (CP and crude ash) and minerals (Zn, Se, and Na) improved. T1 and T2 diets also increased the expression of anti-inflammatory *IL-10* cytokine and could decrease the mRNA level of pro-inflammatory *TNF-α*, and the highest vitamin and mineral contents in the diet (T2) lowered the mRNA expression of *HSP70*.

Therefore, the genotype used did not exhibit the harmful effect of HS on the growth performance, meat quality, digestibility (faecal and ileal) and retention of nutrients and minerals, plasma biochemical parameters and immune response in the experiment. These findings indicate that a purposive genetic selection of pigs can influence their resilience against HS. Nevertheless, supplementation of dietary antioxidants at the T1 level (Vitamins C (150 mg/kg) and E (41 mg/kg) and micro-minerals (Zn (100 mg/kg) and Se (0.21 mg/kg)) can improve the digestibility and retention of nutrients and minerals despite HS challenge. Further increase in dietary antioxidants in the diet (T2) did not influence the digestibility of the nutrients and minerals studied. However, T2 (Vitamins C (300 mg/kg) and E (71 mg/kg) and micro-minerals (Zn (150 mg/kg) and Se (0.26 mg/kg)) level of supplementation could improve concentration of electrolytes. Both diets containing high levels of vitamins and micro-minerals supplementation as follows: vitamin C: 150 mg/kg, vitamin E: 41 mg/kg; Zn: 100

mg/kg; and Se: 0.21 mg/kg and vitamin C: 300 mg/kg, vitamin E: 71 mg/kg; Zn: 150 mg/kg; and Se: 0.26 mg/kg could alleviate inflammatory response and mitigate cell damage during pigs' exposure to HS conditions.

Having the curiosity about the various genotypes response to HS, the said findings can be further verified on other genotypes, particularly those that are reared in HS prone environment.

6. NEW SCIENTIFIC RESULTS

1. High-producing pig genotypes (DanBred) can be resilient to 14-day chronic heat stress exposure (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %).
2. Dietary antioxidant supplementation at elevated levels (vitamin C: 150 mg/kg; vitamin E: 41 mg/kg; Zn: 100 mg/kg; and Se: 0.21 mg/kg) in the diet can reduce the skin temperature of pigs raised in high ambient temperature. Such abatement can help the pigs in their thermoregulatory response and thus suffer less from chronic heat stress.
3. DanBred genotypes respond with a greater extent to vitamin and mineral supplementation under heat stress than to heat stress and fed with a basal diet in terms of metabolic and immune response as well as nutrient and mineral digestibility and retention. This calls attention to re-evaluate the vitamin and mineral requirements of these high-production potential genotypes.
4. Heat stress compromises plasma electrolyte balance (Na^+, K^+, and Cl^-) of DanBred pigs particularly reducing their plasma Cl^- concentration that can be improved through supplementation of dietary antioxidants if commercial fattening hog feed is supplemented with 300 mg/kg vitamin C, 0.71 mg/kg vitamin E, 150 mg/kg Zn and 0.26 mg/kg Se upon heat stress challenge. It is highly recommended during the 1st 7 days of heat stress.
5. Vitamin and micro-mineral supplementation of 150 mg/kg vitamin C, 30 mg/kg vitamin E, 50 mg/kg Zn and 0.05 mg/kg Se in a single and double dose too may alleviate inflammatory response and could mitigate cell damage induced by high ambient temperature (28.9 ± 0.9) exposure of pigs.

7. PRACTICAL USABILITY OF RESULTS

1. The resilience of the pigs to chronic heat stress (14 days at 28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) in this study can be used as a reference for further evaluation on how heat stress duration and intensity affect the physiological status and performance of modern growing to finish pigs.
2. The observed variability of the pigs' phenotypic response to chronic heat stress in rectal temperature (comparable among treatments) and skin temperature (higher in heat stress groups than in thermal comfort group), signifies the importance of skin temperature measurement in verifying the response of pigs under chronic heat stress.
3. To regulate the skin temperature of heat-stressed pigs, supplementation of vitamins C: 150 mg/kg, and E: 41 mg/kg and micro-minerals Zn: 100 mg/kg, and Se: 0.21 mg/kg diet level is recommended. Vitamins C: 300 mg/kg diet, and E: 71 mg/kg diet and micro-minerals Zn: 150 mg/kg diet, and Se: 0.26 mg/kg level of supplementation is recommended to improve the concentration of plasma chloride to correct the electrolyte imbalance experienced by pigs under heat stress conditions.
4. Based on the results, vitamins C: 150 mg/kg, and E: 41 mg/kg and micro-minerals Zn: 100 mg/kg, and Se: 0.21 mg/kg diet level of supplementation can improve the nutrient and mineral digestibility of heat-stressed challenged pigs and could also alleviate their inflammatory response; thus a premix with the inclusion of vitamins and micro-minerals levels stated above can be developed.

8. SUMMARY

Climate change is an infinite threat to livestock farming, particularly to swine. One of its direct effects is the increase in the ambient temperature (AT), especially during summer months, and induces heat stress (HS) in livestock animals. HS causes significant losses in swine production as these animals are less tolerant to high environmental temperatures due to their anatomy. Although it is a fact that pigs can acclimate to such exposure; still their production performance is jeopardized due to HS-induced physiological changes that cause disturbance in the normal functioning of the systems responsible for nutrient digestion, absorption, and metabolism, which in turn affects the product derived from the animal. Therefore, there is a constant need for effective, economic, and practical mitigation strategies to alleviate such ill effects of HS. As it is scientifically proven that abatement of such HS-induced physiological strains can be achieved through nutritional intervention, hence the use of combination and elevated levels of dietary antioxidants (vitamins C and E, and micro-minerals (Se and Zn).

In this study, the pigs' phenotypic and physiological responses to HS and with the supplementation of dietary antioxidants: vitamins (C and E), and minerals (Zn and Se) were observed. Growth performance, rectal and skin temperature, blood biochemical parameters (glucose, uric acid, urea, and creatinine), markers for electrolyte balance (plasma sodium, potassium, and chloride concentration) were measured from 36 finishing DanBred pigs, while meat quality evaluation, nutrient and mineral digestibility (faecal and ileal), retention, and expression of pro and anti-inflammatory cytokines were measured from 24 finishing pigs with an average weight of 65 ± 2.81kg at the start of the trial. The pigs were placed in digestibility cages in two phases (12 pigs in each phase) after the seven days adaptation period to the thermo-neutral conditions (20 °C) and fed basal diet and seven more days for the adaptation to the test diets and gradual exposure to HS (30 °C). The observation for the growth performance lasted for four weeks, electrolyte balance determination and plasma biochemical parameters (21 days), and 14 days for the digestibility trial. The weekly weighing was done to obtain the weight changes of pigs reared under thermo-neutral and HS environments fed their respective experimental diet. After the rearing period, six pigs from each treatment were slaughtered after electrical stunning. About 500 g of *longissimus lumborum* muscle was removed for meat quality measurements. Blood samples were collected on the 15^{th} and 30^{th} day of the experiment (for the plasma biochemical and electrolyte concentration determination trial). The 14-day digestibility trial was divided into two phases

consisting of different batches of pigs in each phase. The first two days in the digestibility cage served as the acclimatization period; the collection of faeces, urine, and feed residue was done for the remaining five experimental days for each pig that was in the digestibility cage. Pigs were exposed to four different treatments TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and basal diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content) (the variables were temperature and diet). The basal diet given to TC and HC groups was formulated as per NRC 2012 recommendations, and the diets given to HT1 and HT2 contain elevated levels of dietary antioxidants (vitamins C and E and minerals Zn and Se).

The pigs' rectal temperature was not significantly affected ($P > 0.05$) by HS and elevated levels of dietary antioxidants. However, the skin temperature reading from the ear base and the middle of the ear was significantly affected by HS ($P < 0.05$). The capability of antioxidants to reduce the pigs' skin temperature was also observed on the measurement from the pigs' back, where HT1 and HT2 pigs had significantly lower ($P <0.05$) skin temperature as compared to pigs in the HC group.

The pigs' growth performance and meat quality were not significantly affected ($P > 0.05$) by HS and elevated levels of dietary antioxidants. However, numerically, Despite HS exposure, pigs fed an antioxidant-fortified diet had slightly higher values in terms of the parameters studied than thermo-neutral pigs.

More prolonged HS exposure elevated the plasma concentration of sodium and regardless of its duration, pigs' plasma chloride concentration was significantly ($P < 0.05$) reduced by the said stressor, indicating an imbalance. High dietary antioxidant supplementation (T2) significantly improves ($P < 0.05$) the concentration of plasma chloride.

The analysis for the digestibility trial (faecal digestibility) was based on nutrient intake and faecal losses, while retention was based on nutrient intake, faecal, and urine losses. Surprisingly, HS did not affect pigs' nutrient digestibility and retention, as both HC and TC had comparable results in the parameters studied. Nevertheless, dry matter (DM) and crude protein (CP) digestibility were significantly higher in HT1 as compared to TC ($P <0.05$), and crude fiber (CF) digestibility was significantly lower in HC and TC groups ($P <0.05$) as compared to HT1 and HT2 groups. While the digestibility of crude fat (Cfat), energy, and crude ash (CA) was not significantly affected ($P > 0.05$). Regarding the digestibility of minerals, it was observed that calcium (Ca) was significantly higher in HT1 as compared to

TC, while zinc (Zn) and selenium (Se) digestibility was significantly higher in HT1 and HT2 as compared to HC and TC ($P < 0.05$). While phosphorus (P) and sodium (Na) were not significantly affected.

No significant difference was observed in the protein retention of pigs ($P > 0.05$); however, it was observed that the protein retention of pigs in the HC group was slightly lower as compared to those pigs fed an elevated diet and in thermo-neutral (TN) condition. Furthermore, retention of Ca, Na, Zn, and Se were significantly higher ($P < 0.05$) in HT1 and HT2 groups as compared to HC and TC groups. Among the minerals, only P was not significantly affected. This implies that the observed differences among the groups in digestibility and retention of nutrients were influenced by the supplementation of elevated levels of dietary antioxidants, hence its effectiveness in the improvement of the animals' performance exposed to high ambient temperature or HS conditions.

As observed, the plasma biochemical parameters of pigs (glucose, urea, and uric acid) were not significantly affected ($P > 0.05$) by HS and with the supplementation of elevated levels of dietary antioxidants. However, at day 7 of exposure of exposure, the plasma creatinine level was significantly lower in thermal comfort pigs (TC) fed a control diet compared to HT2. At day 21 of exposure, however, the plasma concentration levels were similar.

HS did not significantly affect ($P > 0.05$) the expression of cytokines and HSP in pigs. However, supplementation of vitamins and micro-minerals at T1 level ((Vitamins C (150 mg/kg diet) and E (41 mg/kg diet) and micro-minerals (Zn (100 mg/kg diet) and Se (0.21 mg/kg diet)) and T2 (Vitamins C (300 mg/kg diet) and E (71 mg/kg diet) and micro-minerals (Zn (150 mg/kg diet) and Se (0.26 mg/kg diet)) significantly improves ($P = 0.0488$) the expression of anti-inflammatory cytokine (*IL-10*) and significantly reduces the expression of pro-inflammatory cytokine *TNF-α* of HT1 ($P = 0.0345$) and HT2 ($P = 0.0434$) pigs compared to HC group. The mRNA expression of *HSP 70* was also significantly reduced ($P = 0.0487$) in pigs fed T2 (HT2 group) diet ((Vitamins C (300 mg/kg diet) and E (71 mg/kg diet) and micro-minerals (Zn (150 mg/kg diet) and Se (0.26 mg/kg diet)) compared to TC pigs. Nevertheless, the expression of *IL-1β* and *HSP 90* in all groups was similar ($P > 0.05$).

The ileal digestibility of nutrients was based on the sampled digesta obtained from the ileum right after slaughtering the pigs after electrical stunning. Prepared and analyzed through proximate analysis and calculated by the following equation:

Ileal digestibility (%) = (1-(A diet / B digesta) * (XB digesta / XA diet))*100

Where

A and B are marker concentrations (g/kg dry matter)

XA and XB are the concentrations of the test nutrient (g/kg dry matter)

CP, Cfat, CF, organic matter (OM), and nitrogen-free extract (NFE), and the minerals studied were not significantly affected ($P > 0.05$) by HS. Supplementation of dietary antioxidants at single dose (T1) in the diet significantly improved ($P < 0.05$) the CA and CP and some minerals (Zn, Se, and Na) digestibility of pigs despite the HS challenge. Further increase in the dietary antioxidant supplementation (T2) level in the pigs' diet did not influence their digestibility.

Therefore, the genotype used in the experiment did not exhibit the harmful effect of HS on growth performance, meat quality, digestibility (faecal and ileal), and retention of nutrients and minerals, plasma biochemical parameters, and immune response. These findings suggest that selective genetic selection of pigs can influence their resistance to HS. Nonetheless, dietary antioxidants at the T1 level (Vitamins C (150 mg/kg) and E (41 mg/kg) and micro-minerals (Zn (100 mg/kg) and Se (0.21 mg/kg)) can improve nutrient and mineral digestibility and retention despite HS challenge. An increase in dietary antioxidants (T2) had no effect on the digestibility of the nutrients and minerals studied. T2 (Vitamins C (300 mg/kg) and E (71 mg/kg) and micro-minerals (Zn (150 mg/kg) and Se (0.26 mg/kg) supplementation, on the other hand, could improve electrolyte concentration. Supplementation with T1 and T2 levels could reduce inflammatory responses and cell damage in pigs exposed to HS conditions.

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10. PUBLICATIONS

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List of publications related to the dissertation

Foreign language scientific articles in international journals (4)

1. **Ortega, A. D. S. V.**, Szabó, C.: Metabolism and endocrine alterations in growing and finishing pigs under different duration of heat stress - a review.
   Anim. Sci. Pap. Rep. 4, 1-19, 2023. ISSN: 0860-4037.
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2. **Ortega, A. D. S. V.**, Babinszky, L., Rózsáné Várszegi, Z., Ozsváth, X. E., Oriedo, O. H., Oláh, J., Szabó, C.: Effects of high vitamin and micro-mineral supplementation on growth performance and pork quality of finishing pigs under heat stress.
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4. **Ortega, A. D. S. V.**, Szabó, C.: Adverse Effects of Heat Stress on the Intestinal Integrity and Function of Pigs and the Mitigation Capacity of Dietary Antioxidants: A Review.
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Foreign language conference proceedings (1)

5. **Ortega, A. D. S. V.**, Xayalath, S., Lugata, J. K., Szabó, C.: Effects of heat stress-induced oxidative stress on the reproduction of sows and its alleviation by dietary antioxidants: a review.
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Foreign language abstracts (6)

6. **Ortega, A. D. S. V.**, Babinszky, L., Ozsváth, X. E., Oriedo, O. H., Oláh, J., Szabó, C.: Effects of heat stress and high dietary antioxidant supplementation on the ileal digestibility of nutrients and certain minerals in pigs.
In: 30th International Symposium Animal Science Days : Book of Abstracts. Ed.: Vladimir Brajković, University of Zagreb Faculty of Agriculture, Zagreb, 75, 2022. ISBN: 9789538276361

7. **Ortega, A. D. S. V.**, Xayalath, S., Lugata, J. K., Szabó, C.: Effects of heat stress-induced oxidative stress on the reproduction of sows and its alleviation by dietary antioxidants: a review.
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8. **Ortega, A. D. S. V.**, Szabó, C.: Effects of heat stress on the performance of primiparous and multiparous sows and their progeny: a review.
In: 19th Wellmann International Scientific Conference : Book of abstract. Ed.: Kiss Orsolya, University of Szeged Faculty of Agriculture, Hódmezővásárhely, 37, 2022. ISBN: 9789633068601

9. **Ortega, A. D. S. V.**, Babinszky, L., Ozsváth, X. E., Oriedo, O. H., Oláh, J., Szabó, C.: Effects of antioxidant supplementation on the growth performance of pigs exposed to heat stress.
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List of other publications

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12. Xayalath, S., Mujitaba, M. A., **Ortega, A. D. S. V.**, Rátky, J.: Opportunities and challenges for pig production in Vientiane Capital, Laos: a review.
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14. **Ortega, A. D. S. V.**, Mujitaba, M. A., Xayalath, S., Gutierrez, W., Soriano, A. C., Szabó, C.: Perspectives of the livestock sector in the Philippines: A review.
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15. Lugata, J. K., **Ortega, A. D. S. V.**, Szabó, C.: The Role of Methionine Supplementation on Oxidative Stress and Antioxidant Status of Poultry-A Review.
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18. **Ortega, A. D. S. V.**, Szabó, C.: Takarmány-kiegészítők okszerű használata a sertéstakarmányozásban.
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19. **Ortega, A. D. S. V.**, Szabó, C.: Fiatal sertések egészségvédelme takarmányozási szempontból.
Értékálló aranykorona. 21 (2), 19-22, 2021. ISSN: 1586-9652.

Total IF of journals (all publications): 10,837
Total IF of journals (publications related to the dissertation): 7,429

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

29 March, 2023

11. DECLARATIONS

DECLARATION

I hereby declare that I have prepared this dissertation in the framework of the Doctoral School of Animal Science, University of Debrecen, in order to obtain the doctoral (Ph.D.) degree in Animal Husbandry.

Debrecen, 2023.03.29.

……
Arth David Sol Valmoria Ortega
Ph.D. candidate

DECLARATION

I hereby declare that **Arth David Sol Valmoria Ortega** Ph.D. candidate has carried out his work under my/our supervision between 2019 and 2023 within the framework of the Doctoral School of Animal Science, University of Debrecen. The findings of the dissertation represent the candidate's own ideas and independent work. I recommend accepting the dissertation.

Debrecen, 2023. 03.29.

……
Dr. Csaba Szabó
supervisor