Theses of Doctoral (PhD) Dissertation

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

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1. INTRODUCTION AND AIM OF THE THESES

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*). 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 (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; 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). 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 (CUI and GU, 2015). 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 (TANG et al., 2018; COTTRELL et al., 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 and micro-minerals are potential nutritional tools to mitigate the HS adverse effects. These substances can neutralize the excess reactive oxygen species (ROS) produced during HS-induced OS and protect cells against the toxic effects of free radicals (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 dietary antioxidants 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.

Research aims

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

2.1. Animals, diets, and management

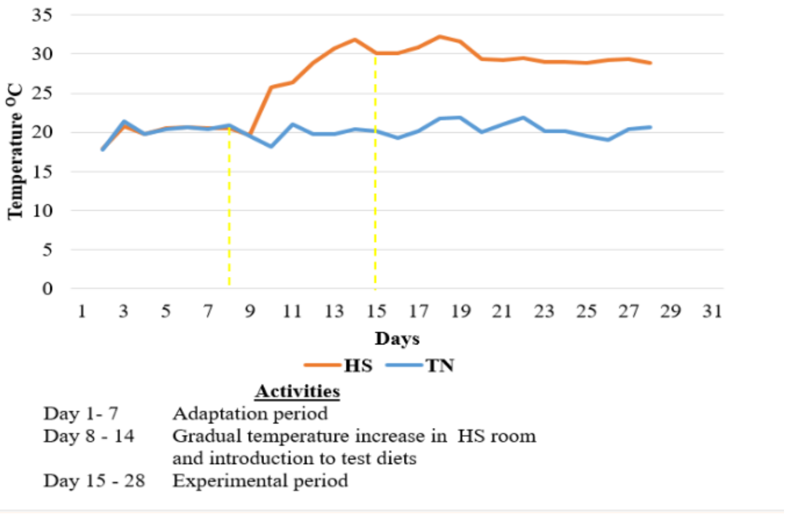
All experimental procedures were reviewed and approved by the Hungarian authorities and registered and supervised by the University of Debrecen Animal Care Committee (Debrecen, Hungary – 9/2019/DEMÁB). A total of thirty-six Danbred hybrid barrows (65.1 ± 2.81kg) were assigned to the combination of two environmental conditions (Table 1) 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 *ad 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% 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) (Figure 1).

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 2 and Table 3) 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 double dose supplementation (T2)) were formulated by providing vitamins C and E and micro-minerals Se and Zn (elevated levels), as shown in Table 4. 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 1: single dose supplementation of vitamin C and E and Se and Zn content (Table 4)), and 4) HT2: HS + T2 (elevated diet 2: double dose supplementation of vitamin C and E and Se and Zn content).

Table 1: 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 1: 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 2: 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
Plant 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 3: 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 4: Dietary treatments (supplementation mg/kg)

Nutrient	Basal feed*	Elevated 1	Elevated 2
Vitamin C	0	150	300
Vitamin E	11	41	71
Zn**	50	100	150
Se**	0,16	0,21	0,26

*NRC (2012); **organic source

2.2. Measurements of temperature indices and production parameters

Body temperature measurement. Skin and rectal temperature were measured with an infrared (TESTO 830-T1, Testo SE & Co. KGaA, Lenzkirch, Germany) and digital thermometer, respectively, on day 15th and 30th of the experiment. The former was measured on the pig's body parts: ear base, middle of ear, and back (center).

Growth performance. 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.

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 12th rib and 5th lumbar vertebrae. Chemical (moisture, protein, fat, vitamin and minerals) and physical (pH, meat color, drip, freeze and cook loss, and texture) assesment was done to the meat samples as described by REZAR et al. (2017).

2.3. Electrolyte balance and plasma biochemical parameters

Blood collection and analysis. Blood samples were collected from the external jugular vein of the pigs on the first and last day of the main experimental period (15th (7 days of exposure) and 30th (21 days of exposure) days of the trial) into EDTA tubes. The separated plasma samples were then stored at -80 °C, before analysis. Plasma concentrations of significant electrolytes (sodium (Na^+), potassium (K^+), and chloride (Cl^-)) were used as markers for electrolyte as mentioned by SHRIMANKER and BHATTARAI (2021). The analysis of the plasma samples was performed in triplicate in room temperature. Na^+, K^+, and Cl^- plasma levels, along with the plasma concentrations of glucose, uirc acid, urea, and creatinine were analyzed through the photometric method with a Lab-Analyse (Orvostechnika Ltd., Budapest, Hungary) half-automatic analyzer.

2.4. Digestibility trial

Faecal digestibility and retention. Random selection of six pigs in each treatment group was done and were evaluated for two weeks during the main experimental period, with weekly changes in the digestibility cages. Both weeks consisted of two days of adaptation to the cage and five days of collection. Faeces, urine, and feed residue were collected daily, pooled by cage, frozen at −20 °C, and sampled for analysis.

Ileal digestibility. 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 was collected and stored at −20 °C for chemical analyses and ileal digestibility calculation with the following equation.

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

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)

Chemical analysis. Feed and feces (fecal digestibility) and digesta (ileal digestibility) 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.

2.5. Cytokine and heat shock protein expression

Sample collection and analysis. 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*). Expression of the cytokines and heat shock proteins were determined through RNA isolation and reverse transcription, and qPCR analysis.

2.6. Statistical analysis

Data were analyzed with variance analyses with GraphPad Prism 8.4.3 software (GraphPad Software Incorporated, San Diego, USA). One-way ANOVA evaluated the growth, pork quality, digestibility, and retention of nutrients and minerals (faecal and ileal), cytokines, and HSPs. A two-way analysis of variance (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 $P < 0.05$.

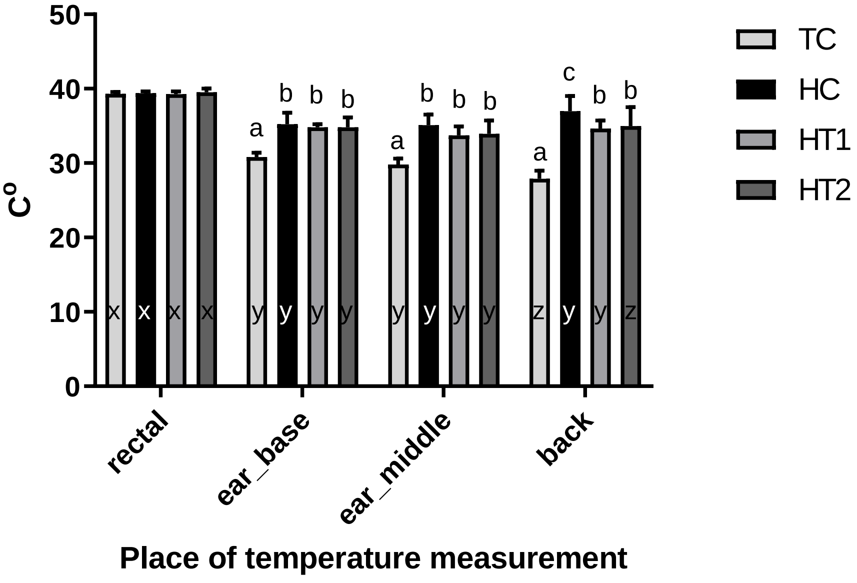
3. RESULTS AND DISCUSSION

3.1. Temperature and Production performance of pigs

Rectal and skin temperature

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 2). 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. 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. Nevertheless, pigs in TC had much better temperature measurements than those treatment groups exposed to HS.

Figure 2: Impact of heat stress and dietary antioxidant supplementation on the rectal and skin temperature of fatenning pigs after two weeks of exposure (n=9/treatment)

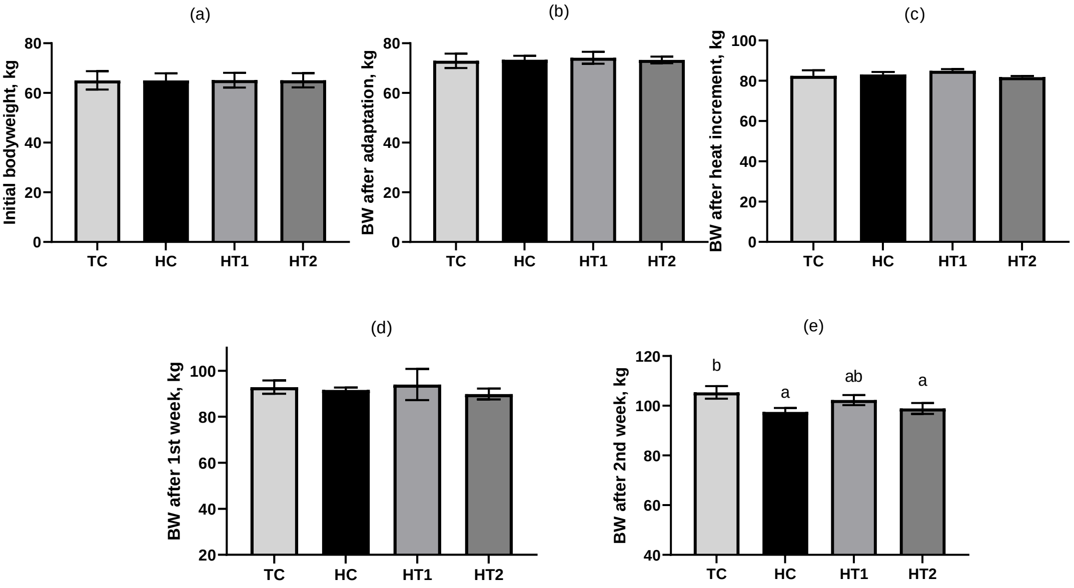


Values are means, with their standard deviation represented by vertical bars; a,b,c Means with the same letters within a measurement place between treatments are not significantly different ($P > 0.05$); x,y,z Means with the same letters within a treatment between measurement places are not significantly 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).

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. A decline in body weight (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. Interestingly, pigs in HT1 had comparable weights to TC (Figure 3). 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 5).

Figure 3: 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 5: 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)

	Treatment					
Parameter	TC	HC	HT1	HT2	SEM[a]	P value
			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.

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 6). 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). Although our result is in contrast to the previous observation of 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).

Table 6: 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

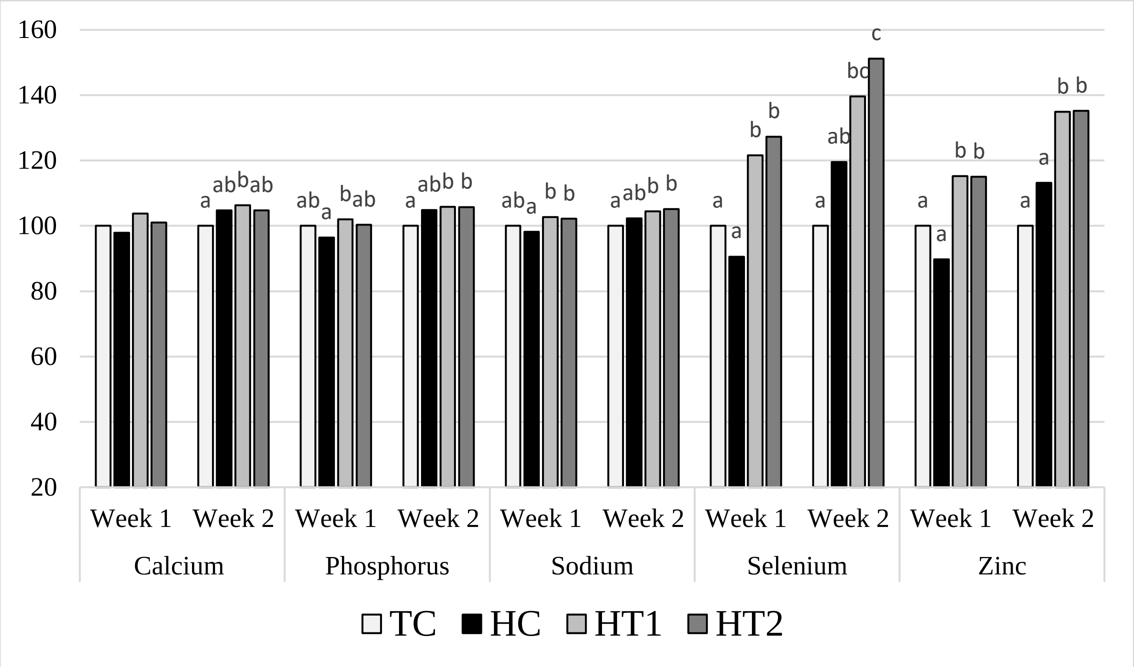
TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation of vitamin C and E and Se and Zn content), and HT2: HS + T2 (elevated 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.

3.2. Mineral digestibility and electrolyte balance pigs

Mineral digestibility

There was a significant HS duration (period) effect observed in the case of sodium and zinc ($P < 0.05$) (Figure 4). The environmental and dietary treatments significantly affected the fecal 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. This could be due to the pigs' ability for thermoregulation and tolerance to heat upon prolonged exposure (RENAUDEAU et al., 2008; RENAUDEAU et al., 2010; CAMPOS et al., 2017). Elevated levels of some vitamins and trace minerals (treatment HT1) resulted in increased digestibility ($P < 0.05$) in the second week of the heat stress period compared to the TC treatment. 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 (MAYORGA et al., 2018*b*). Further elevation of vitamin C, vitamin E, Zn, and Se (treatment HT2) did not improve the digestibility of the minerals tested (P > 0.05).

Figure 4: **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).

Markers for electrolyte balance

More prolonged chronic heat stress elevates the plasma Na^+ level (P < 0.05) (Table 7). 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 reduction might be due to the HS-induced hepatic cellular apoptosis, which can affect the regulation of blood Cl^- by the liver (CUI et al., 2016). The supplementation of vitamins and micro-minerals was wholly or partly able to mitigate this adverse effect. 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). Moreover, LIU et al. (2021*b*) 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.

Table 7: 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^{a}	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 $p > 0.05$. HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: elevated vitamin C and E and Se and Zn content), HT2: HS + T2 (further elevated vitamin C and E and Se and Zn content), and TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet.

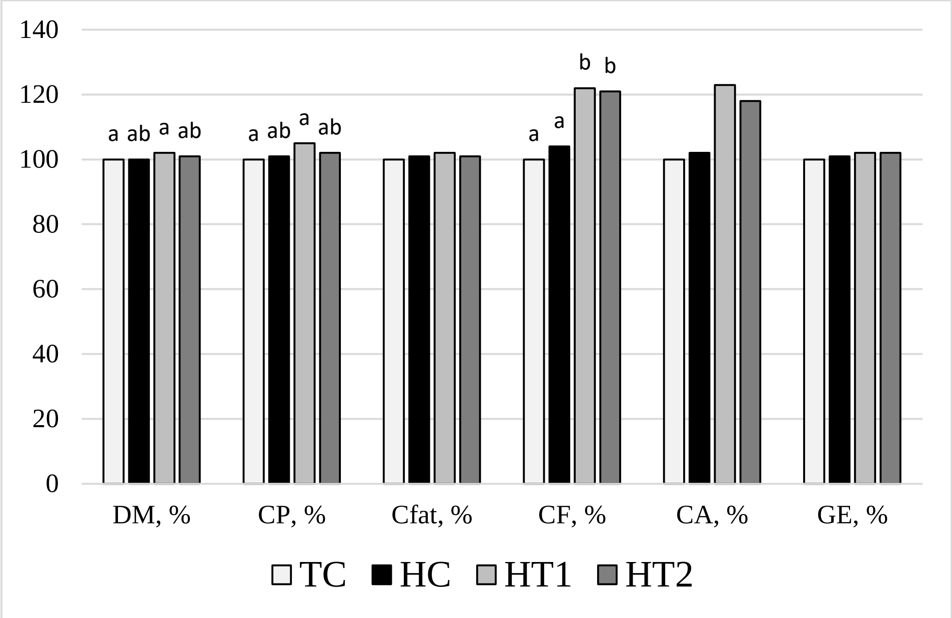
3.3. Nutrient digestibility, metabolism, and immune response of fattening pigs

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 5). 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 6).

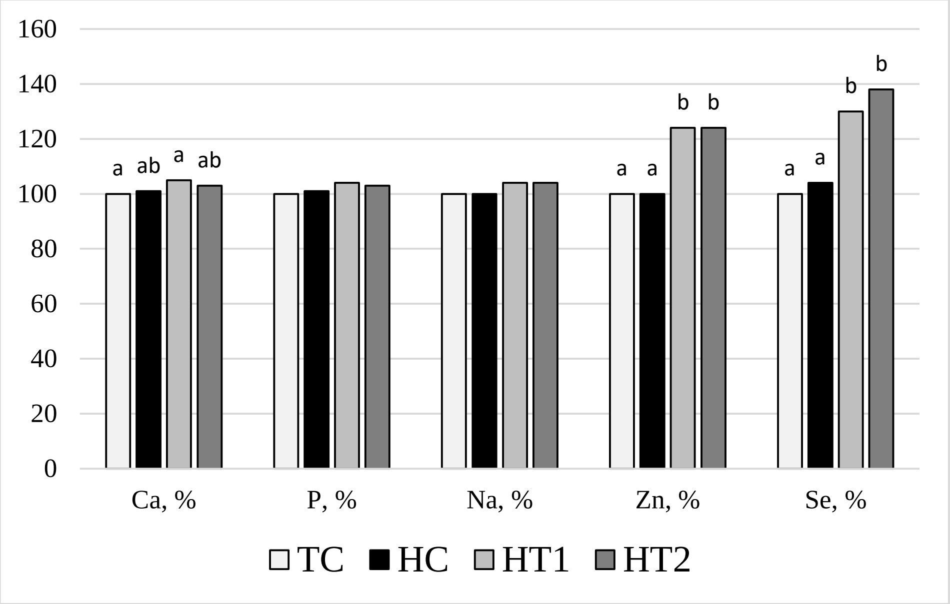
CP retention of pigs was not significantly affected ($P > 0.05$) by HS and vitamin and micro-mineral supplementation (Figure 7). However, significant differences ($P < 0.05$) were observed in the Ca, Na, Zn, and Se retention. Wherein pigs fed elevated levels of vitamins and micro-minerals (HT1 and HT2) showed significantly better performance. Among the various minerals, only P was not significantly affected. The similar nutrient and mineral digestibility observed between TC and HC groups in our study agreed with the results reported by KIM et al. (2020). 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). Previous studies revealed that pigs' exposure to HS (30-33 °C) could lead to low retention of N and protein (BRESTENSKY et al., 2012; RENAUDEAU et al., 2013) However, results in our study revealed that both HC and TC groups have similar retention of protein and minerals. Our finding is similar to PATIENCE et al. (2005), reared in diurnal HS (20-38 °C within 24 hours). The significantly better diegestibility of DM and CP in HT1 group compared to TC and the higher digestibility of CF in HT1 and HT2 groups than in HC and TC group can be attributed to the effectiveness of the dietary antioxidants supplementation in improving the digestive function of the intestine despite exposure to high ambient temperature. Moreover, 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).

Figure 5: The impact of long-term heat exposure and high vitamin and micro-mineral supplementation in the faecal digestibility of nutrients in fattening pigs relative to TC group (n=6/treatment)



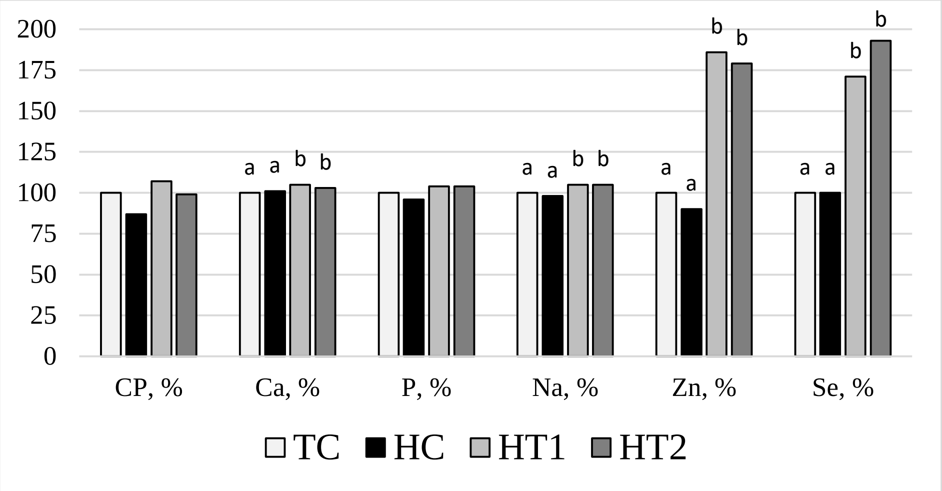
^{a,b} means in a row with the same superscripts do not differ (P > 0.05). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet, HT1: HS + T1 (elevated diet 1: single dose supplementation 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 6: The impact of long-term heat exposure and high vitamin and micro-mineral supplementation in the faecal digestibility of some 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 7: The impact of long-term heat exposure and high vitamin and micro-mineral supplementation in the faecal retention of CP and some 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).

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 8).

Table 8: Impact of different duration of heat exposure and vitamin and micro-mineral supplementation on the plasma biochemical parameters of fattening pigs (n=9/treatment)

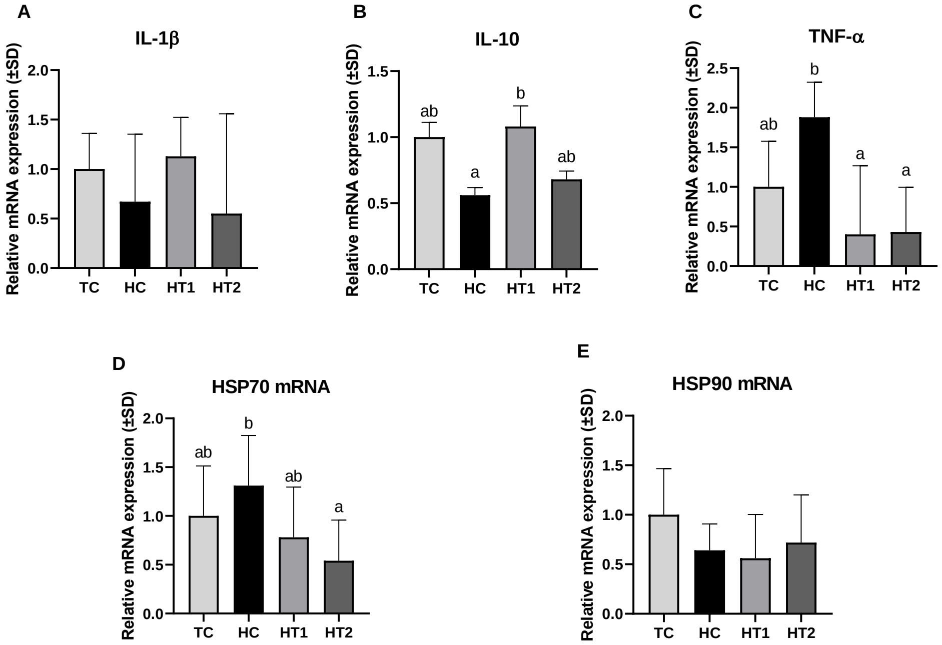
	Treatment					***P* values**	
Parameters	TC	HC	HT1	HT2	SEM	Period	Treatment
Glucose, mmol/l						0.5309	0.2627
Day 7	6.26	4.76	5.71	5.38	0.27		
Day 21	5.49	5.05	5.03	5.67	0.21		
Uric acid, µmoL/L						0.7454	0.4888
Day 7	36.15	25.12	33.95	33.30	2.09		
Day 21	36.22	34.79	27.79	33.77	2.29		
Urea, mmol/l						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).

Cytokine and heat shock protein expression

HS did not significantly affect ($P > 0.05$) the expression of cytokines and *HSP* in pigs (Figure 8). 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, suggesting that the applied treatments can decrease the level of *TNF-α* and so the inflammation during heat stress or high ambient temperature conditions. The mRNA expression of *HSP 70* was also significantly reduced ($P = 0.0487$) in HT2 pigs compared to HC pigs. 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. Nevertheless, the expression of *IL-1β* and *HSP 90* in all groups was similar ($P > 0.05$).

Figure 8: 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)

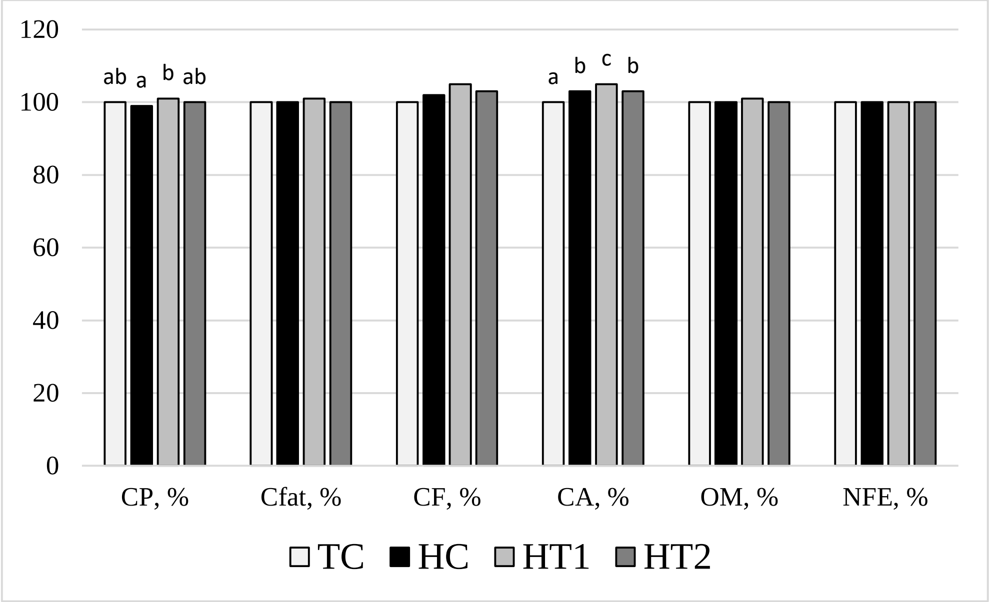


Values are means, with their standard deviation represented by vertical bars; ^{a,b} Means with the same letters do not differ ($P> 0.05$). TC: TN + C: thermo-neutral ambient temperature (19.5 ± 0.9 °C, RH- 85.9 ± 7.3%) and control diet, HC: HS + C: heat stress (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control 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).

3.4. Ileal digestibility of nutrients and minerals

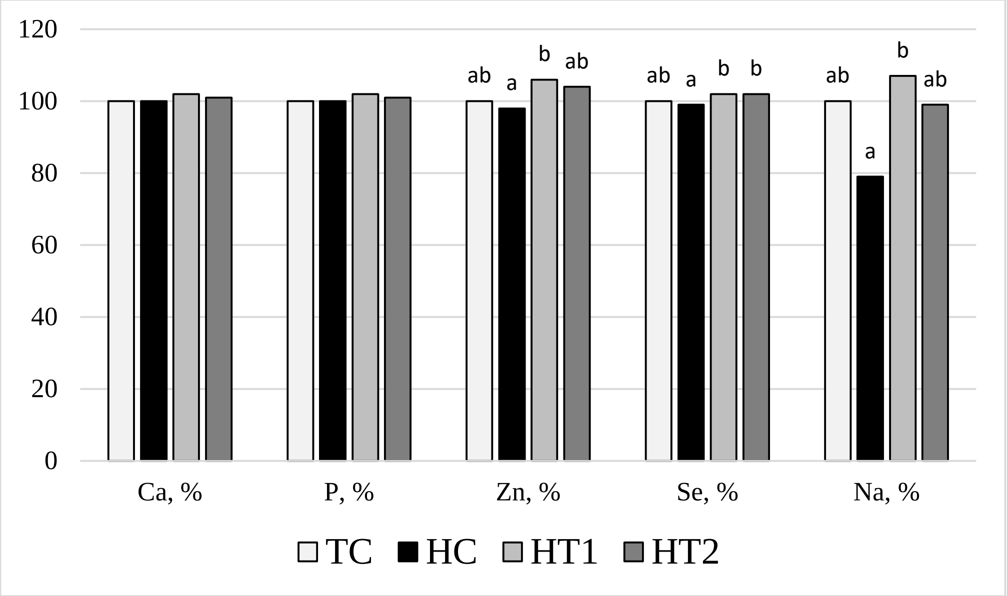
The ileal digestibility of CP, Cfat, CF, organic matter (OM), and nitrogen-free extract (NFE) was not affected by HS (Figure 9). Surprisingly, pigs in TC had significantly lower CA digestibility ($P < 0.05$) compared to other treatment groups. Nevertheless, supplementation of dietary antioxidants at an elevated 1 level (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 10). 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 9: 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 10: The effect of heat stress and high dietary antioxidant supplementation on the ileal digestibility of some 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).

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) 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). 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 (SANZ FERNANDEZ 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.

4. CONCLUSSION 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. ED1 and ED2 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 (fecal 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 single dose supplementation (T1) 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 level of supplementation could improve concentration of electrolytes. Both T1 and T2 levels of supplementation could alleviate inflammatory response and mitigate cell damage during pigs' exposure to HS conditions.

5. 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 fed 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.

6. 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.

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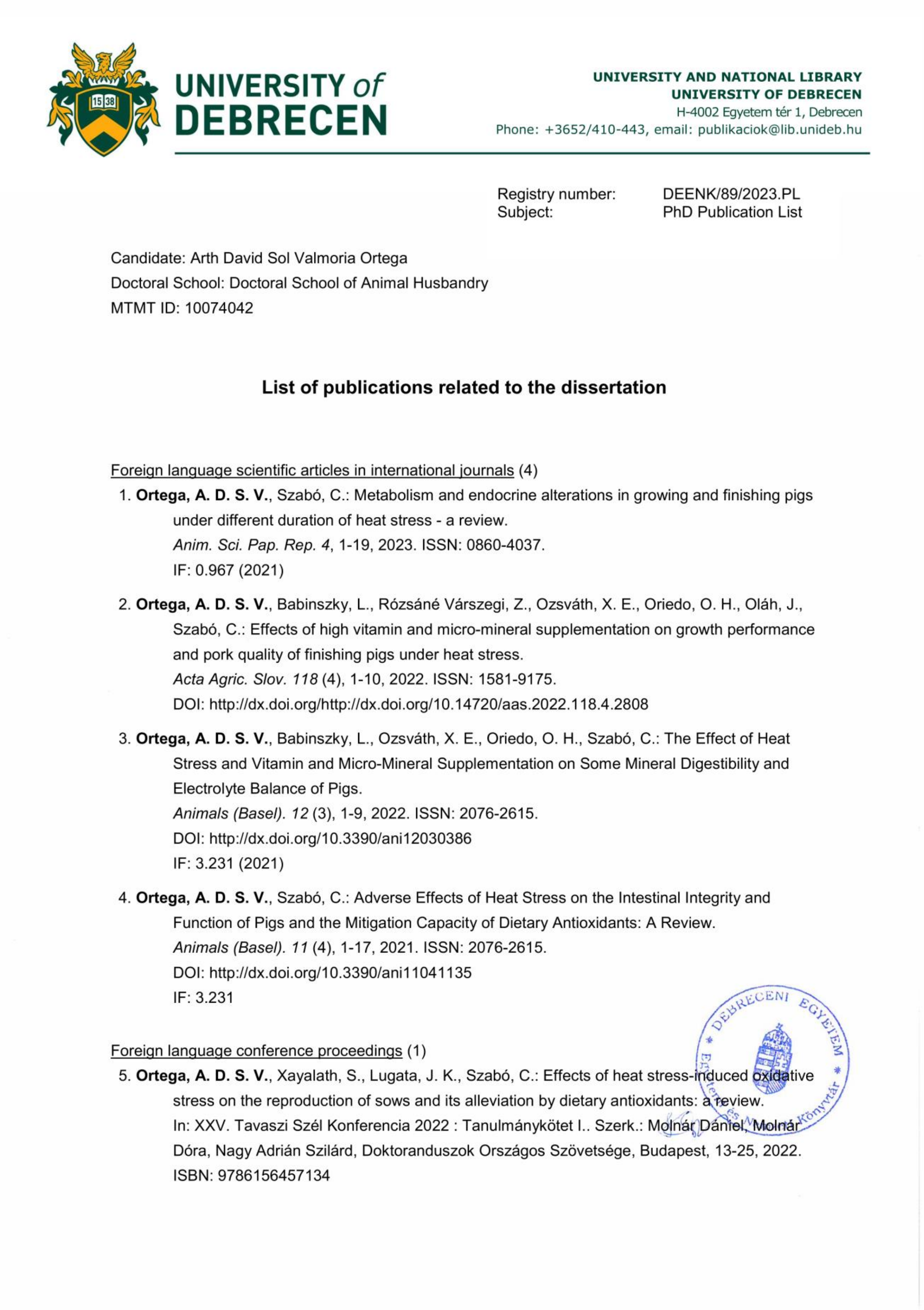
8. PUBLICATIONS



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Registry number: DEENK/89/2023.PL
Subject: PhD Publication List

Candidate: Arth David Sol Valmoria Ortega
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10074042

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.
   Acta Agric. Slov. 118 (4), 1-10, 2022. ISSN: 1581-9175.
   DOI: http://dx.doi.org/http://dx.doi.org/10.14720/aas.2022.118.4.2808
3. **Ortega, A. D. S. V.**, Babinszky, L., Ozsváth, X. E., Oriedo, O. H., Szabó, C.: The Effect of Heat Stress and Vitamin and Micro-Mineral Supplementation on Some Mineral Digestibility and Electrolyte Balance of Pigs.
   Animals (Basel). 12 (3), 1-9, 2022. ISSN: 2076-2615.
   DOI: http://dx.doi.org/10.3390/ani12030386
   IF: 3.231 (2021)
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.
   Animals (Basel). 11 (4), 1-17, 2021. ISSN: 2076-2615.
   DOI: http://dx.doi.org/10.3390/ani11041135
   IF: 3.231

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.
   In: XXV. Tavaszi Szél Konferencia 2022 : Absztraktkötet. Szerk.: Molnár Dániel, Molnár Dóra, Doktoranduszok Országos Szövetsége, Budapest, 72-73, 2022. ISBN: 9786158205481

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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    In: XXIV. Tavaszi Szél Konferencia 2021 : Absztraktkötet. Szerk.: Molnár Dániel, Molnár Dóra, Doktoranduszok Országos Szövetsége, Budapest, 89, 2021. ISBN: 9786155586996

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List of other publications

Foreign language scientific articles in Hungarian journals (3)

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.
    Rev. Agric. Rural Dev. 11 (1-2), 3-8, 2022. ISSN: 2063-4803.
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13. Xayalath, S., Mujitaba, M. A., **Ortega, A. D. S. V.**, Rátky, J.: A review on the trend of livestock breeds in Laos.
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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.
    Acta Agrar. Debr. 2021 (1), 175-188, 2021. ISSN: 2416-1640.
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Foreign language scientific articles in international journals (2)

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.
    Agriculture-Basel. 12 (10), 1-20, 2022. EISSN: 2077-0472.
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16. **Ortega, A. D. S. V.**: A glance at the 21st-century livestock industry and breeding.
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Informational/educational articles (3)

17. **Ortega, A. D. S. V.**, Szabó, C.: A genetika és a takarmányozás kapcsolata sertésekben.
    Értékálló aranykorona. 22 (3), 21-24, 2022. ISSN: 1586-9652.

18. **Ortega, A. D. S. V.**, Szabó, C.: Takarmány-kiegészítők okszerű használata a sertéstakarmányozásban.
    Értékálló aranykorona. 22 (10), 26-, 2022. ISSN: 1586-9652.

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
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