

Effects of high vitamin and micro-mineral supplementation on growth performance and pork quality of finishing pigs under heat stress

Arth David Sol Valmoria ORTEGA^{1,2,3,4}, László BABINSZKY¹, Zsófia RÓZSÁNÉ-VÁRSZEGI⁵, Xénia Erika OZSVÁTH^{2,5}, Ogonji Humphrey ORIEDO⁶, János OLÁH⁷, Csaba SZABÓ¹

Received August 08, 2022; accepted November 11, 2022.

Delo je prispelo 08. avgusta 2022, sprejeto 11. novembra 2022

Effects of high vitamin and micro-mineral supplementation on growth performance and pork quality of finishing pigs under heat stress

Abstract: The objective of this study was to determine the effects of heat stress (HS) on the production performance of fattening pigs and whether the supplementation of vitamins (C and E) and micro-minerals (Se and Zn) at increased concentrations can mitigate HS adverse effects. Thirty six Danbred hybrid barrows (65.1 ± 2.81 kg) were randomly distributed into four treatments 1) HS (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + control diet (HC), 2) HS + diet 1 (HT1), 3) HS + diet 2 (HT2), and 4) thermo-neutral conditions (19.5 ± 0.9 °C, RH- 85.9 ± 7.3 %) + control diet (TC). Bodyweight and feed intake were measured weekly for four weeks. After the experiment, six pigs from each treatment were slaughtered, and the *longissimus lumborum* muscle was sampled to evaluate meat quality. At week four, HS significantly affected pig body weight ($p < 0.05$). However, the other parameters were not significantly affected by HS, while slight improvements in these parameters were observed by supplementing vitamins and micro-minerals in the diet of the pigs despite exposure to HS. Therefore, the pigs used in the study showed resilience to adverse effects of HS on growth and meat quality parameters. The content of vitamins C and E and micro-minerals Se and Zn in the diet seems to play an important role in resilience to HS, therefore their requirement and supplementation should be carefully evaluated.

Key words: pigs; animal nutrition; heat stress; feed additives; vitamins; micro-minerals; growth performance; meat quality

Vpliv dodatka vitaminov in mikrorudninskih snovi v krmo za prašiče pitance na prirast in kakovost mesa v pogojih vročinskega stresa

Izvleček: Cilj raziskave je bil ugotoviti učinke vročinskega stresa (HS) na prirast prašičev pitancev in ali lahko z dodajanjem vitaminov (C in E) in mikrorudninskih snovi (Se in Zn) v povečanih koncentracijah ublažimo škodljive učinke HS. Šestintrideset kastratov Danbred križancev ($65,1 \pm 2,81$ kg) je bilo naključno razdeljenih v štiri skupine 1) HS ($28,9 \pm 0,9$ °C, RV- $60,4 \pm 4,3$ %) + kontrolna krmna mešanica (HC), 2) HS + krmna mešanica 1 (HT1), 3) HS + krmna mešanica 2 (HT2) in 4) kontrolni pogoji ($19,5 \pm 0,9$ °C, RV- $85,9 \pm 7,3$ %) + kontrolna krmna mešanica (TC). Telesno maso in zauživanje krme smo merili štiri tedne. Po koncu poskusa smo žrtvovali šest živali iz vsake skupine in vzorčili mišico *longissimus lumborum* za oceno kakovosti mesa. Ugotovili smo, da je v četrtem tednu HS negativno vplival na telesno maso prašičev ($p < 0,05$). HS ni statistično značilno vplival na druge parametre, pri čemer smo opazili trend izboljšanja teh parametrov ob dodajanju vitaminov in mikrorudninskih snovi v krmo prašičev, ki so bili izpostavljeni HS. Prašiči, uporabljeni v študiji, so pokazali odpornost na škodljive učinke HS na rast in parametre kakovosti mesa. Zdi se, da ima vsebnost vitaminov C in E ter mikrorudninskih snovi Se in Zn v prehrani prašičev pomembno vlogo pri odpornosti na HS, zato je potrebno skrbno oceniti potrebe po dodajanju omenjenih dodatkov v krmo prašičev.

Ključne besede: prašiči; prehrana živali; vročinski stres; krmni dodatki; vitamini; mikrominerali; rast; kakovost mesa

1 University of Debrecen, Faculty of Agriculture and Food Sciences and Environmental Management, Institute of Animal Science, Biotechnology and Nature, Department of Animal Nutrition and Physiology, Debrecen, Hungary

2 University of Debrecen, Doctoral School of Animal Science

3 Central Mindanao University, College of Agriculture, Department of Animal Science, University town, Musuan, Maramag, Bukidnon, 8710, Philippines

4 Corresponding author, e-mail: ortega.david@agr.unideb.hu

5 University of Debrecen, Faculty of Agriculture and Food Sciences and Environmental Management, Institute of Animal Science, Biotechnology and Nature, Department of Animal Science, Debrecen, Hungary

6 Department of Agriculture, Livestock and Food Security, Veterinary services section, Country Government of Makueni, 78-90300, Kenya

7 University of Debrecen, Institute for Agricultural Research and Educational Farm, Kismacs Experimental Station of Animal Husbandry, Debrecen, Hungary

1 INTRODUCTION

The ongoing rise in global temperature caused by climate change and the ensuing heat stress (HS) intensification is a severe threat to food security that is expected to persist throughout the 21st century (FAO, 2016; Wang & Zhang, 2019). Pigs are negatively affected by HS, and it was suggested that modern genotypes are more sensitive to its negative effects than traditional genotypes (Brown-Brandl et al., 2001; Brown-Brandl et al., 2004; Renaudeau et al., 2011). Pigs under HS focus more on reducing metabolic heat production by reducing feed intake by up to 23 % per day since they have a low capacity for thermoregulation; as a result, production performance and product quality are compromised (Renaudeau et al., 2011; Song et al., 2011; Yang et al., 2014a). Regardless of the duration of exposure to high ambient temperature (AT), pig production parameters such as average daily gain (ADG), feed conversion ratio (FCR), and pork quality parameters are negatively affected (Song et al., 2011; Pearce et al., 2013; Morales et al., 2014; Yang et al., 2014a). Pigs exposed to HS at 30 °C have decreased pH value and increased crude fat, drip loss percentage, and toughness (Shear Force) of their meat (Yang et al., 2014a; Mun et al., 2022). Influence of HS on pigs' meat pH is critical as low pH can increase protein denaturation, resulting in decreased water holding capacity, lighter color, and subsequently poor eating quality (Kim et al., 2016; Jankowiak et al., 2021).

Such performance depression can also be attributed to oxidative stress (OS) induced by HS. The balance between the reactive oxygen species (ROS) and the endogenous antioxidant is disturbed by HS. HS causes excessive generation of reactive oxygen species (ROS) and reduction of endogenous antioxidants, which causes the accumulation of dysfunctional proteins, lipid peroxidation products, and impaired mitochondrial DNA (Slimen et al., 2014; Cui et al., 2019). ROS can induce protein modifications upon direct reaction with proteins leading to protein oxidation, which leads to higher drip loss and toughening of the meat (Huff Lonergan et al., 2010; Traore et al., 2012). Moreover, OS can reduce collagen synthesis, leading to decreased collagen solubility and greater meat toughness (Archile-Contreras and Purslow, 2011). Therefore, there is a need for mitigation strategies to combat detrimental effects of HS. Nutritional intervention strategies using nutritional tools have shown progress in abating adverse effects of HS on pig performance (Rhoads et al., 2013; Babinszky et al., 2019). Exogenous antioxidants, such as vitamins C and E, and minerals selenium (Se) and zinc (Zn) can respond to such stressors (Traber & Stevens, 2011; Jarosz et al., 2017; González de Vega et al., 2018; Kielczykowska et al., 2018).

Individual use of vitamins and micro-minerals as supplements in heat-stressed pigs has been studied in the following concentrations: Se (0.5 ppm), vitamin E (100 IU/kg), Zn (60 mg/kg Zn sulfate + 60 mg/kg Zn amino acid complex) (Liu et al., 2016; Mayorga et al., 2018). However, their effectiveness against negative effects of HS on production performance remains unclear. Therefore, the present study aims to examine the impact of increased concentrations of combination of Zn, Se, vitamin E, and vitamin C on growth performance and meat quality of pigs under HS conditions.

2 MATERIALS AND METHODS

2.1 ANIMALS, DIET, AND MANAGEMENT

All experimental procedures were reviewed and approved by the University of Debrecen Animal Care Committee (Debrecen, Hungary – 9/2019/DEMÁB). At the University of Debrecen's Institute of Agricultural Research and Educational Farm's Animal Husbandry Experimental Station (Kismacs, Hungary), a total of 36 Danbred hybrid barrows (65.1 ± 2.81 kg) were allocated to one of the following environmental and dietary treatments (Tables 1, 2 and 3): 1) HS (28.9 ± 0.9 °C, RH- 60.4 ± 4.3 %) + basal diet (treatment code: HC), 2) HS + diet 1 (treatment code: HT1), 3) HS + diet 2 (treatment code: HT2), and 4) thermo-neutral environment (19.5 ± 0.9 °C, RH- 85.9 ± 7.3 %) + control diet (treatment code: TC). All pigs were allowed a 7 day adaptation period to their pens (3 pigs per pen with a total of 12 pens), fed *ad libitum* (with control feed) in a thermo-neutral (TN) environment (average 19.5 ± 1.5 °C). Afterwards, the temperature of the HS room was gradually raised to 30 °C (heat increment, HI), and pigs in this period started receiving different dietary treatments. A week after the heat increment, the main period of the experiment commenced, which lasted for two weeks. Three diets were formulated: the basal or control feed (B), formulated on a corn-soybean basis according to the (NRC, 2012) recommendation for 75–100 kg live weight pigs having 155 g mean protein deposition per day (Table 1), and with the nutrient content of the premixture added (Table 2). The control diet contained levels of vitamins C and E and micro-minerals Zn and Se in accordance to the NRC recommendation (Table 3). 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 maximised performance) for breeders the recommendation is set at 315 mg/kg of maximum supplementation. We choose to add 300 mg/kg for diet

Table 1: Composition and calculated nutrient content of basal feeda^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		
Vitamin and mineral premixture	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

2 and 150 mg/kg for diet 1. 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 diet 1 and 71 mg/kg in diet 2. In case of Zn, the maximum al-

Table 2: Nutrient content of the premixture (in 1 kg of premixture)*

Nutrient	Inclusion rate	Concentration
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)

lowed supplementation in the EU is 150 mg/kg, therefore that was chosen as the maximum value in diet 2, whereas concentration in diet 1 was 100 mg/kg, and in basal diet 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 (diet 1) and 0.26 mg/kg (diet 2).

2.2 PRODUCTION PARAMETERS

Each pen had a designated plastic container, of which tare-weight was measured and written on the container. Weekly feed intake was measured as feed disappearance and feed residues (both from the feeder and the container) were measured and recorded. 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 weekly, and all represented the mean value per pen.

Table 3: Dietary treatments (supplementation mg/kg)

Nutrient	Basal feed*	Diet 1	Diet 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

2.3 SLAUGHTER AND PORK QUALITY MEASUREMENT

At the end of the trial, six pigs from each treatment (24 pigs in total) were slaughtered. The slaughtering comprised of two days where three pigs from each treatment (12 pigs in total per day) were slaughtered in similar manner after electrical stunning. The pigs for slaughter were selected randomly. About 500 g of *longissimus lumborum* muscle was removed between the 12th rib and 5th lumbar vertebrae of the pig carcass for meat quality measurements, as described by Rezar et al. (2017).

2.3.1 Physical meat quality assessment

The meat pH was measured at 45 minutes and 24 hours after slaughter in the loin 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 Corporation, 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^* , L^* features (a^* = red, b^* = yellow, L^* = paleness).

For drip loss (%) determination, one cm thick meat pieces of 50 ± 5 g were cut. 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 of 100 ± 5 g and 1 cm thick pieces was cut, 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 and weighed. After cooking, meat pieces were chilled at 4 °C overnight then sliced in cuboids and measured. For the firmness, shear force measurement (N) was conducted on cooked samples, with shear blade set using 25 kg load cell, with 1.5 mm/s test speed from 40 mm distance, using 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)). 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 whole sample until the blade has passed through the base plate slot. The blade then returns to its starting position. Curves were evaluated to get shear force data.

2.3.2 Chemical analysis of the meat samples

Total nitrogen was analyzed using Kjeldahl-Method and protein content was calculated using the factor of 6.25. Fat was measured according to MSZ ISO 1443:2002 standard. For the elemental analysis (vitamin C and minerals) ground meat samples (1.000 g) were loaded into digester tubes. To all samples ten ml of distilled conc. HNO_3 was added and heated at 60 °C for 30 min, 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 mins. After the digestion, all samples were washed into 50 ml volumetric flasks with distilled water, homogenized, and filtered (MN 640 W paper; Macherey-Nagel). ICP-OES technique was applied on the iCAP 7000 spectrophotometer (Thermo Scientific). For the calibration, a multielement standard solution was applied.

2.4 STATISTICAL ANALYSIS

Data were analyzed using GraphPad Prism 7.05 software (GraphPad Software Incorporated, San Diego, USA). One-way ANOVA was used to evaluate the production and pork quality parameters. Data were expressed as mean, and group means differences were separated by the Tukey test. Differences among the treatments were considered significant when $p < 0.05$.

3 RESULTS

3.1 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 1e). 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 4).

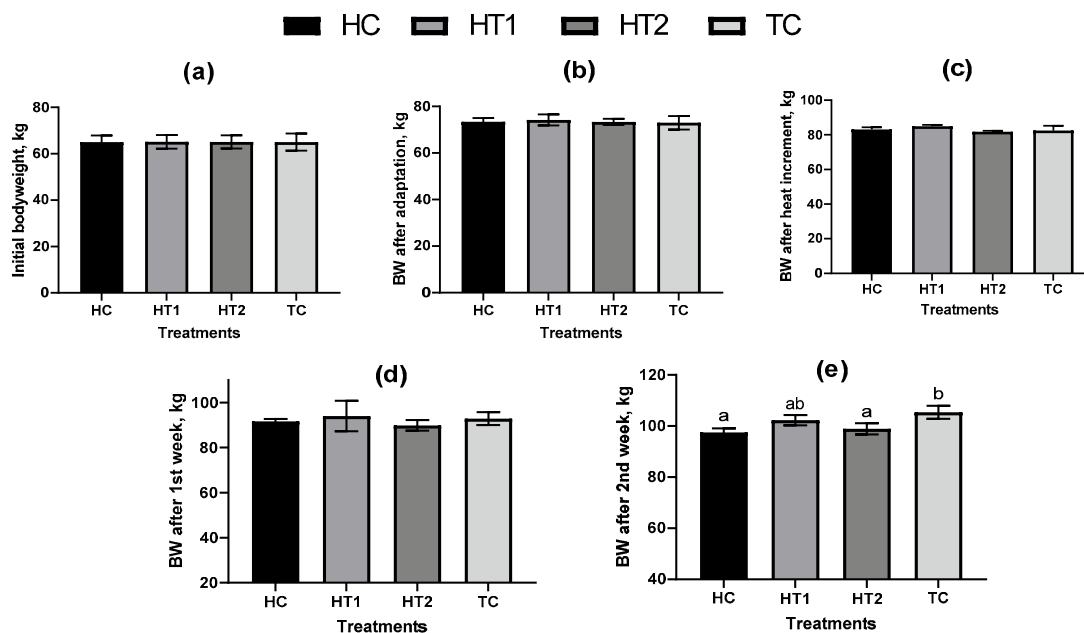


Figure 1: Initial body weight (a), body weight after adaptation period (b), body weight after heat increment (c), body weight after 1st week of the experiment (d), and body weight after 2nd week of the experiment (e). Values are means, with their standard deviation represented by vertical bars; ^{a,b} means with common letter are not significantly different ($p > 0.05$). HC = heat stress + basal diet; HT1 = heat stress + diet 1; HT2 = heat stress + diet 2; TC = thermal comfort + basal diet.

3.2 MEAT QUALITY

As shown in Table 5, chemical and physical analysis of meat samples obtained from pigs kept in TN and HC environment fed their respective dietary treatments (basal, diet 1, and diet 2) have similar results among all experimental treatments ($p > 0.05$). HS, vitamin C, E, and micro-minerals Zn and Se supplementation did not significantly affect the meat quality parameters of pigs ($p > 0.05$).

4 DISCUSSION

4.1 GROWTH PERFORMANCE

Several studies reported that high ambient temperature induced detrimental effects on growing parameters of 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 affected by pigs under such stressors, which are significant contributors to economic losses in pig production (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 signifi-

cant decrease ($p < 0.05$) in the body weight (BW) of pigs fed with basal feed (HC) and diets supplemented with higher concentrations of vitamins and micro-minerals (HT2). Interestingly, pigs fed diet 1 (HT1) had a comparable body weight with those in TN conditions (TC). A decline in BW is commonly observed in some animals as a response to HS, which can be attributed to the lower feed and nutrient intake of such animals exposed to the 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 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 have positive effects on 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 the treatment groups in terms of ADG, FI, and FCR under all periods of observation in our experiment. However, pigs under HS fed basal diet (HC) had

Table 4: Growth performance of pigs kept in thermo-neutral and heat-stress environment fed basal diet and diets containing increased concentrations of vitamins C and E and microminerals Zn and Se

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

^a heat stress + basal diet; ^b heat stress + diet 1; ^c heat stress + diet 2; ^d thermal comfort + basal diet; ^e standard error of mean; ^f heat increment; ^g heat stress

the lowest observed values of all the studied parameters. At the same time, supplementation of vitamins and micro-minerals at increased level in the diet 1 (vitamins C – 150 mg/kg, E – 41 mg/kg, and minerals Se – 0.21 mg/kg and Zn – 100 mg/kg) mitigated negative effects of HS. The proposed 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 only a slight increase in dietary antioxidant (Zn – 75 mg/kg) supplementation (Mani et al., 2019).

4.2 MEAT QUALITY

Chronic HS can influence the chemical composition and physical characteristics of meat, which has been observed in meat obtained and evaluated from various species of 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 affected by 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 meat (PSE), manifested by increased lightness and yellowness, and decreased redness 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). Moreover, HS induced OS can promote protein modifications due to the increase of ROS production. This can lead to protein oxidation that can decrease the water holding capacity (WHC) of the meat and causes its toughness (Huff Lonergan et al., 2010; Traore et al., 2012). As reported by Yang et al. (2014b), exposure of pigs to long term HS at 30 °C increased the drip loss and shearing force of longissimus muscle. These attributes are also correlated to HS effect on pork pH (significant decrease - 5.43, which is below the optimal pH range of 5.7–6.1) signifying its importance (Klont, 2005; Yang et al., 2014b; Jankowiak et al., 2021). Nevertheless, as observed in different poultry and livestock animals (broilers, lambs, and pigs), vitamins and micro-minerals supplementation to the diet can be an effective tool to mitigate detrimental effects of HS 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. Supplementation of vitamins and micro-minerals did not significantly influence the meat quality of pigs exposed to HS. Although our results are in contrast to the observations of Yang et al. (2014b) (pigs exposed to chronic HS (30 °C)); Shi et al. (2016) (pigs exposed to chronic HS (35 °C)); Ma et al. (2019) pigs exposed to chronic HS (35 °C)); and Liu et al. (2021) (pigs exposed to chronic HS (35 °C)). But it is in agreement with

Table 5: Meat quality of thermo-neutral and heat-stressed pigs fed basal diet and diets containing increased content of vitamins C and E and microminerals Zn and Se

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

^a heat stress + basal diet; ^b heat stress + diet 1; ^c heat stress + diet 2; ^d thermal comfort + basal diet; ^e standard error of mean; ^f in dry matter basis

the observations of Lehotayová et al. (2012) in pigs exposed to constant HS (30 °C) throughout the growing and finishing period. They concluded that several meat quality parameters, such as shear force, drip loss, and meat colour, were not significantly affected by HS. The resilience of pigs to HS, as observed by 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 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). Moreover, as concluded in the growth performance evaluation no decisive effect of either temperature or diet could be seen, it is therefore expected that the meat quality evaluation would have similar results.

5 CONCLUSIONS

The pigs used in the study were not severely affected by chronic HS. They responded with slightly lower growth performance (ADG, ADFI, and FCR) than pigs in TN environment and have comparable meat quality charac-

teristics. Supplementation of the diet with vitamins and micro-minerals (vitamin C (150 mg/kg) E (41 mg/kg), Zn (100 mg/kg) and Se (0.21 mg/kg)) contributed to the slight improvement in the growth performance of pigs under chronic HS; however, such supplementation did not significantly affect the meat quality of the pigs. Therefore, we can conclude that the high ambient temperature challenge of 14 days has no significant effect on the growth performance and meat quality of the pigs used in this study. Careful evaluation of vitamin and micro-mineral supplementation levels seems important in pigs reared under chronic HS conditions since the pigs in the study responded with improved growth performance in HT1 group, but not in HT2 group.

6 ACKNOWLEDGEMENTS

This study was supported by the EFOP 362-16-2017-00001 project, and the project was financed by the European Union and the European Social Fund. We also thank DSM Nutritional Products Hungary Ltd. (Újhartyán, Hungary) for providing the vitamin and mineral supplements. Ortega, ADSV appreciates the support of the Tempus Public Foundation, Stipendium Hungaricum Scholarship Programme.

7 REFERENCES

- Archile-Contreras, A. C., & Purslow, P. P. (2011). Oxidative stress may affect meat quality by interfering with collagen turnover by muscle fibroblasts. *Food Research International*, 44(2), 582–588. <https://doi.org/10.1016/j.foodres.2010.12.002>
- Babinszky, L., Horváth, M., Remenyik, J., Versteegen, M. W. A. (2019). The adverse effects of heat stress on the antioxidant status and performance of pigs and poultry and reducing these effects with nutritional tools (Chapter 8). In: W. H. Hendriks, M. W. A. Versteegen & L. Babinszky (Eds.), *Poultry and pig nutrition, Challenges of the 21st century* (pp. 187–208). Wageningen: Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-884-1_8
- Brown-Brandl, T. M., Eigenberg, R. A., Nienaber, J. A., & Kachman, S. D. (2001). Thermoregulatory profile of a newer genetic line of pigs. *Livestock production science*, 71(2–3), 253–260. [https://doi.org/10.1016/S0301-6226\(01\)00184-1](https://doi.org/10.1016/S0301-6226(01)00184-1)
- Brown-Brandl, T. M., Nienaber, J. A., Xin, H., & Gates, R. S. (2004). A literature review of swine heat production. *Transactions of the ASAE*, 47(1), 259. <https://doi.org/10.13031/2013.15867>
- Campos, P. H. R. F., Floc'h, L., Noblet, J., & Renaudeau, D. (2017). Physiological responses of growing pigs to high ambient temperature and/or inflammatory challenges. *Revista Brasileira de Zootecnia*, 46, 537–544. <https://doi.org/10.1590/s1806-92902017000600009>
- Cervantes, M., Cota, M., Arce, N., Castillo, G., Avelar, E., Espinoza, S., & Morales, A. (2016). Effect of heat stress on performance and expression of selected amino acid and glucose transporters, HSP90, leptin and ghrelin in growing pigs. *Journal of thermal biology*, 59, 69–76. <https://doi.org/10.1016/j.jtherbio.2016.04.014>
- Chauhan, S. S., Dunshea, F. R., Plozza, T. E., Hopkins, D. L., & Ponnampalam, E. N. (2020). The Impact of Antioxidant Supplementation and Heat Stress on Carcass Characteristics, Muscle Nutritional Profile and Functionality of Lamb Meat. *Animals: an open access journal from MDPI*, 10(8), 1286. <https://doi.org/10.3390/ani10081286>
- Cottrell, J. J., Liu, F., Hung, A. T., DiGiacomo, K., Chauhan, S. S., Leury, B. J., ... Dunshea, F. R. (2015). Nutritional strategies to alleviate heat stress in pigs. *Animal Production Science*, 55(12), 1391–1402. <https://doi.org/10.1071/AN15255>
- Cruzen, S. M., Boddicker, R. L., Graves, K. L., Johnson, T. P., Arklfeld, E. K., Baumgard, L. H., ... Lonergan, S. M. (2015). Carcass composition of market weight pigs subjected to heat stress in utero and during finishing. *Journal of animal science*, 93(5), 2587–2596. <https://doi.org/10.2527/jas.2014-8347>
- Cui, Y., Wang, C., Hao, Y., Gu, X., & Wang, H. (2019). Chronic Heat Stress Induces Acute Phase Responses and Serum Metabolome Changes in Finishing Pigs. *Animals: an open access journal from MDPI*, 9(7), 395. <https://doi.org/10.3390/ani9070395>
- da Fonseca de Oliveira, A. C., Vanelli, K., Sotomaior, C. S., Weber, S. H., & Costa, L. B. (2019). Impacts on performance of growing-finishing pigs under heat stress conditions: a meta-analysis. *Veterinary research communications*, 43(1), 37–43. <https://doi.org/10.1007/s11259-018-9741-1>
- FAO (2016). *Climate Change and Food Security: Risks and Responses* (Food and Agriculture Organization of the United Nations (FAO)). Retrieved from <http://www.fao.org/3/i5188e/i5188e.pdf>
- González de Vega, R., García, M., Fernández-Sánchez, M. L., González-Iglesias, H., & Sanz-Medel, A. (2018). Protective effect of selenium supplementation following oxidative stress mediated by glucose on retinal pigment epithelium. *Metallomics: integrated biometal science*, 10(1), 83–92. <https://doi.org/10.1039/C7MT00209B>
- Gonzalez-Rivas, P. A., Chauhan, S. S., Ha, M., Fegan, N., Dunshea, F. R., & Warner, R. D. (2020). Effects of heat stress on animal physiology, metabolism, and meat quality: A review. *Meat science*, 162, 108025. <https://doi.org/10.1016/j.meatsci.2019.108025>
- Goo, D., Kim, J. H., Park, G. H., Delos Reyes, J. B., & Kil, D. Y. (2019). Effect of Heat Stress and Stocking Density on Growth Performance, Breast Meat Quality, and Intestinal Barrier Function in Broiler Chickens. *Animals: an open access journal from MDPI*, 9(3), 107. <https://doi.org/10.3390/ani9030107>
- Gregory, N. G. (2010). How climatic changes could affect meat quality. *Food Research International*, 43(7), 1866–1873. <https://doi.org/10.1016/j.foodres.2009.05.018>
- Huff Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle - lessons on mechanisms of meat tenderization. *Meat science*, 86(1), 184–195. <https://doi.org/10.1016/j.meatsci.2010.05.004>
- Jankowiak, H., Cebulska, A., & Bocian, M. (2021). The relationship between acidification (pH) and meat quality traits of polish white breed pigs. *European Food Research and Technology*, 247(11), 2813–2820. <https://doi.org/10.1007/s00217-021-03837-4>
- Jarosz, M., Olbert, M., Wyszogrodzka, G., Młyniec, K., & Librowski, T. (2017). Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF- κ B signaling. *Inflammopharmacology*, 25(1), 11–24. <https://doi.org/10.1007/s10787-017-0309-4>
- Kellner, T. A., Baumgard, L. H., Prusa, K. J., Gabler, N. K., & Patisience, J. F. (2016). Does heat stress alter the pig's response to dietary fat? *Journal of animal science*, 94(11), 4688–4703. <https://doi.org/10.2527/jas.2016-0756>
- Kielczykowska, M., Kocot, J., Paździor, M., & Musik, I. (2018). Selenium - a fascinating antioxidant of protective properties. *Advances in clinical and experimental medicine: official organ Wroclaw Medical University*, 27(2), 245–255. <https://doi.org/10.17219/acem/67222>
- Kim, T. W., Kim, C. W., Yang, M. R., No, G. R., Kim, S. W., & Kim, I. S. (2016). Pork Quality Traits According to Postmortem pH and Temperature in Berkshire. *Korean journal for food science of animal resources*, 36(1), 29–36. <https://doi.org/10.5851/kosfa.2016.36.1.29>
- Klont, R. (2005). Influence of Ultimate pH on Meat Quality and Consumer Purchasing Decisions. Retrieved from <https://www.thepigsite.com/articles/influence-of-ultimate-ph-on-meat-quality-and-consumer-purchasing->

- decisions#:~:text=The%20highest%20quality%20products%20tend,into%20lactic%20acid%20after%20slaughter
- Lehotayová, A., Bučko, O., Petrák, J., Mrázová, J., & Debrečeni, O. (2012). Effect of high ambient temperature on meat quality of pigs. *Research in Pig Breeding*, 6(2), 37–40. Retrieved from https://www.researchgate.net/profile/Andrea_Lehotayova/publication/295546165_Effect_of_high_ambient_temperature_on_meat_quality_of_pigs/links/5819998e08ae7c7cd6860992/Effect-of-high-ambient-temperature-on-meat-quality-of-pigs.pdf
- Liu, F., Cottrell, J. J., Furness, J. B., Rivera, L. R., Kelly, F. W., Wijesiriwardana, U., ... Dunshea, F. R. (2016). Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. *Experimental physiology*, 101(7), 801–810. <https://doi.org/10.1113/EP085746>
- Liu, Y., Yin, S., Tang, J., Liu, Y., Jia, G., Liu, G., ... Zhao, H. (2021). Hydroxy Selenomethionine Improves Meat Quality through Optimal Skeletal Metabolism and Functions of Selenoproteins of Pigs under Chronic Heat Stress. *Antioxidants (Basel, Switzerland)*, 10(10), 1558. <https://doi.org/10.3390/antiox10101558>
- Lv, C. H., Wang, T., Regmi, N., Chen, X., Huang, K., & Liao, S. F. (2015). Effects of dietary supplementation of selenium-enriched probiotics on production performance and intestinal microbiota of weanling piglets raised under high ambient temperature. *Journal of animal physiology and animal nutrition*, 99(6), 1161–1171. <https://doi.org/10.1111/jpn.12326>
- Ma, X., Wang, L., Shi, Z., Chen, W., Yang, X., Hu, Y., ... Jiang, Z. (2019). Mechanism of continuous high temperature affecting growth performance, meat quality, and muscle biochemical properties of finishing pigs. *Genes & nutrition*, 14, 23. <https://doi.org/10.1186/s12263-019-0643-9>
- Mani, V., Rubach, J. K., Sanders, D. J., Pham, T., Koltes, D. A., Gabler, N. K., & Poss, M. J. (2019). Evaluation of the protective effects of zinc butyrate in IPEC-J2 cells and grower pigs under heat stress. *Translational animal science*, 3(2), 842–854. <https://doi.org/10.1093/tas/txz023>
- Mayorga, E. J., Kvidera, S. K., Horst, E. A., Al-Qaisi, M., Dickson, M. J., Seibert, J. T., ... Baumgard, L. H. (2018). Effects of zinc amino acid complex on biomarkers of gut integrity and metabolism during and following heat stress or feed restriction in pigs. *Journal of animal science*, 96(10), 4173–4185. <https://doi.org/10.1093/jas/sky293>
- Morales, A., Grageola, F., García, H., Arce, N., Araiza, B., Yáñez, J., & Cervantes, M. (2014). Performance, serum amino acid concentrations and expression of selected genes in pair-fed growing pigs exposed to high ambient temperatures. *Journal of animal physiology and animal nutrition*, 98(5), 928–935. <https://doi.org/10.1111/jpn.12161>
- Mun, H. S., Rathnayake, D., Dilawar, M. A., Jeong, M. G., & Yang, C. J. (2022). Effect of ambient temperature on growth performances, carcass traits and meat quality of pigs. *Journal of Applied Animal Research*, 50(1), 103–108. <https://doi.org/10.1080/09712119.2022.2032084>
- Pearce, S. C., Gabler, N. K., Ross, J. W., Escobar, J., Patience, J. F., Rhoads, R. P., & Baumgard, L. H. (2013). The effects of heat stress and plane of nutrition on metabolism in growing pigs. *Journal of animal science*, 91(5), 2108–2118. <https://doi.org/10.2527/jas.2012-5738>
- Renaudeau, D., Anais, C., Tel, L., & Gourdine, J. L. (2010). Effect of temperature on thermal acclimation in growing pigs estimated using a nonlinear function. *Journal of animal science*, 88(11), 3715–3724. <https://doi.org/10.2527/jas.2009-2169>
- Renaudeau, D., Collin, A., Yahav, S., de Babilio, V., Gourdine, J. L., & Collier, R. J. (2012). Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal: an international journal of animal bioscience*, 6(5), 707–728. <https://doi.org/10.1017/S1751731111002448>
- Renaudeau, D., Frances, G., Dubois, S., Gilbert, H., & Noblet, J. (2013). Effect of thermal heat stress on energy utilization in two lines of pigs divergently selected for residual feed intake. *Journal of animal science*, 91(3), 1162–1175. <https://doi.org/10.2527/jas.2012-5689>
- Renaudeau, D., Gourdine, J. L., & St-Pierre, N. R. (2011). A meta-analysis of the effects of high ambient temperature on growth performance of growing-finishing pigs. *Journal of animal science*, 89(7), 2220–2230. <https://doi.org/10.2527/jas.2010-3329>
- Renaudeau, D., Kerdoncuff, M., Anais, C., & Gourdine, J. L. (2008). Effect of temperature level on thermal acclimation in Large White growing pigs. *Animal: an international journal of animal bioscience*, 2(11), 1619–1626. <https://doi.org/10.1017/S1751731108002814>
- Rezar, V., Salobir, J., Levart, A., Tomažin, U., Škrlep, M., BatorekLukač, N., & Čandek-Potokar, M. (2017). Supplementing entire male pig diet with hydrolysable tannins: Effect on carcass traits, meat quality and oxidative stability. *Meat science*, 133, 95–102. <https://doi.org/10.1016/j.meatsci.2017.06.012>
- Rhoads, R. P., Baumgard, L. H., Suagee, J. K., & Sanders, S. R. (2013). Nutritional interventions to alleviate the negative consequences of heat stress. *Advances in nutrition*, 4(3), 267–276. <https://doi.org/10.3945/an.112.003376>
- Romo-Valdez, J., Romo-Valdez, A., Montero-Pardo, A., Urias-Castro, C., Güémez-Gaxiola, H., & Romo-Rubio, J. (2019). Organic zinc supplementation and pigs productive performance in warm environment. *Abanico Veterinario*, 9(1), 1–9. <https://doi.org/10.21929/abavet2019.926>
- Sanz Fernandez, M. V., Pearce, S. C., Gabler, N. K., Patience, J. F., Wilson, M. E., Socha, M. T., ... & Baumgard, L. H. (2014). Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. *Animal: an international journal of animal bioscience*, 8(1), 43–50. <https://doi.org/10.1017/S1751731113001961>
- Sanz Fernandez, M. V., Stoakes, S. K., Abuajamieh, M., Seibert, J. T., Johnson, J. S., Horst, E. A., ... Baumgard, L. H. (2015). Heat stress increases insulin sensitivity in pigs. *Physiological Reports*, 3(8), e12478. <https://doi.org/10.14814/phy2.12478>
- Shakeri, M., Cottrell, J. J., Wilkinson, S., Le, H. H., Suleria, H., Warner, R. D., & Dunshea, F. R. (2019). Growth Performance and Characterization of Meat Quality of Broiler Chickens Supplemented with Betaine and Antioxidants under Cyclic Heat Stress. *Antioxidants (Basel, Switzerland)*, 8(9), 336. <https://doi.org/10.3390/antiox8090336>
- Shi, Z. B., Ma, X. Y., Zheng, C. T., Hu, Y. J., Yang, X. F., Gao, K. G., ... & Jiang, Z. Y. (2016). Effects of high ambient tem-

- perature on meat quality, serum hormone concentrations, and gene expression in the longissimus dorsi muscle of finishing pigs. *Animal Production Science*, 57(6), 1031–1039. <https://doi.org/10.1071/AN15003>
- Silva, V. A., Bertechini, A. G., Clemente, A., de Freitas, L., Nogueira, B., de Oliveira, B. L., & Ramos, A. (2019). Different levels of selenomethionine on the meat quality and selenium deposition in tissue of finishing pigs. *Journal of animal physiology and animal nutrition*, 103(6), 1866–1874. <https://doi.org/10.1111/jpn.13179>
- Slimen, I. B., Najar, T., Ghram, A., Dabbebi, H., Ben Mrad, M., & Abdrabbah, M. (2014). Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. *International journal of hyperthermia: the official journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group*, 30(7), 513–523. <https://doi.org/10.3109/02656736.2014.971446>
- Song, R., Foster, D. N., & Shurson, G. C. (2011). Effects of feeding diets containing bacitracin methylene disalicylate to heat-stressed finishing pigs. *Journal of animal science*, 89(6), 1830–1843. <https://doi.org/10.2527/jas.2010-3218>
- Traber, M. G., & Stevens, J. F. (2011). Vitamins C and E: beneficial effects from a mechanistic perspective. *Free radical biology & medicine*, 51(5), 1000–1013. <https://doi.org/10.1016/j.freeradbiomed.2011.05.017>
- Traore, S., Aubry, L., Gatellier, P., Przybylski, W., Jaworska, D., Kajak-Siemaszko, K., & Santé-Lhoutellier, V. (2012). Higher drip loss is associated with protein oxidation. *Meat science*, 90(4), 917–924. <https://doi.org/10.1016/j.meatsci.2011.11.033>
- Wang, F., & Zhang, J. (2019). Heat Stress Response to National-Committed Emission Reductions under the Paris Agreement. *International journal of environmental research and public health*, 16(12), 2202. <https://doi.org/10.3390/ijerph16122202>
- Węglarz, A. (2010). Meat quality defined based on pH and colour depending on cattle category and slaughter season. *Czech Journal of Animal Science*, 55(12), 548–556. <https://doi.org/10.17221/2520-CJAS>
- Yang, P., Hao, Y., Feng, J., Lin, H., Feng, Y., Wu, X., ... Gu, X. (2014a). The Expression of Carnosine and Its Effect on the Antioxidant Capacity of Longissimus dorsi Muscle in Finishing Pigs Exposed to Constant Heat Stress. *Asian-Australasian journal of animal sciences*, 27(12), 1763–1772. <https://doi.org/10.5713/ajas.2014.14063>
- Yang, P., Feng, Y., Hao, Y., Gu, X., Yang, C., & Cao, Z. (2014b). Effects of constant heat stress on performance, carcass traits, nutrition content and myofiber characteristics of Longissimus dorsi in finishing pigs. *Chinese Journal of Animal Nutrition*, 26(9), 2503–2512.
- Zhang, Z. Y., Jia, G. Q., Zuo, J. J., Zhang, Y., Lei, J., Ren, L., & Feng, D. Y. (2012). Effects of constant and cyclic heat stress on muscle metabolism and meat quality of broiler breast fillet and thigh meat. *Poultry science*, 91(11), 2931–2937. <https://doi.org/10.3382/ps.2012-02255>