Original scientific paper
Izvorni znanstveni članak

EFFECTS OF HEAT STRESS AND HIGH DIETARY ANTIOXIDANT SUPPLEMENTATION ON THE ILEAL DIGESTIBILITY OF NUTRIENTS AND CERTAIN MINERALS IN PIGS

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Abstract

Heat stress (HS) can impair the pigs' intestinal integrity, jeopardizing nutrient and mineral digestibility. Our study aimed to investigate HSs' impact on the ileal digestibility of nutrients and minerals in pigs and whether high dietary antioxidants (vitamins C and E, and micro-minerals Se and Zn) supplementation can improve their digestibility. A total of 24 Danbred barrows weighing 65.1 ± 2.81kg were distributed into four different groups, which were placed in thermo-neutral (TN) (19.5 ± 0.9°C, RH- 85.9±7.3%) and HS (28.9 ± 0.9°C, RH- 60.4 ± 4.3%) condition and fed with their respective dietary treatment. Pigs in the TN room only received the basal feed (B) (TC), and in the HS room, B (HC), elevated 1 (elevated dietary antioxidants) (HT1), and elevated 2 (dietary antioxidants doubled) (HT2) diets were given in *ad libitum*. Pigs were euthanized at the end of the trial for the collection of digesta from the ileum. Nutrient (crude protein (CP), fat, fiber, ash, organic matter, and nitrogen free extract (NFE)) and mineral (Ca, P, Zn, Se, and Na) content were measured from diets and digesta. Aside from crude ash ($P < 0.05$), HS did not significantly affect the ileal digestibility of other nutrients and minerals in pigs ($P > 0.05$). However, pigs fed the elevated 1 diet (HT1) had significantly greater digestibility ($P < 0.05$) of CP, crude ash, Zn, Se, and Na than HC. Further increase in vitamin and micro-mineral supplementation (HT2) did not improve the pigs' ileal digestibility of the measured nutrients and minerals. In conclusion, pigs can be resilient to HS adverse effects on the parameters studied. Antioxidant-fortified diet at elevated 1 level could improve pigs' nutrient and mineral digestibility under the HS challenge.

Keywords: Heat stress; dietary antioxidants; ileal digestibility; fattening pigs

Introduction

The digestibility of nutrients and minerals in pigs depends on their viable and healthy gastrointestinal tract (GIT). However, certain stressors, such as heat stress (HS), can jeopardize the functionality and integrity of the pigs' intestinal epithelium, which can have a detrimental impact on its digestive function (Pearce et al., 2013a; Liao and Nyachoti, 2017). Exposure of fattening pigs to environmental conditions above their thermal comfort (18-25°C) can cause HS. Based on literature, modern domesticated pigs are more sensitive to HS. This is due to their limited thermoregulation capacity, no sweat glands, dependence on panting and increased breathing rate to dissipate heat.

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Moreover, they produce more metabolic heat. Under such a challenge, the thermoregulatory response of pigs to HS shifts the splanchnic blood flow to the peripheral blood circulation, resulting in intestinal hypoxia and damage (Lian et al., 2020). HS also causes the accumulation of reactive oxygen species while reducing the levels of endogenous antioxidants creating an imbalance that leads to oxidative stress (OS) in the intestine (Cui and Gu, 2015). Intestinal hypoxia and OS can increase inflammation; also, it can lead to oxygen and nutrient deprivation to the enterocytes, which can harm its physiological function (Pearce et al., 2013a; Cui and Gu, 2015; Zeitouni et al., 2016). HSs' harm in the digestive functionality of the pigs' gastrointestinal tract have been reported in several studies. Kiefer et al. (2012), reported that upon exposure to HS condition (31 °C), barrows expressed poor digestibility of protein, phospohorus, calcium, and zinc. Pigs under such condition also had high percentage of nutrients excreted (nitrogen) in their feces and low percentage of nutrients and minerals retained (Patience et al., 2005; Renaudeau et al., 2013). Several dietary antioxidants, including vitamins C and E and micro-minerals Se and Zn, have been found to have a mitigation effect on the HS-induced intestinal OS and damage, which might positively impact the pigs' digestion and absorption of nutrients (Ortega and Szabó, 2021). However, high levels of inclusion and combining these dietary antioxidants in the feed of heat-stressed pigs have yet to be explored. Therefore, this research aimed to determine the effect of HS and high dietary antioxidants (vitamins C and E, Se and Zn) fortified diet on the ileal digestibility of nutrients and minerals in fattening pigs.

Materials and Methods

Animals and experimental diets

All experimental procedures were reviewed and approved by the University of Debrecen Animal Care Committee (Debrecen, Hungary – 9/2019/DEMÁB). A total of 24 Danbred hybrid barrows (65.1 ± 2.81kg) were assigned to one of two environmental conditions and one of 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 (12 pens, 2 pigs per pen), during which they were fed ad libitum (with basal feed) in a thermo-neutral (TN) environment (average 19.5 ± 1.5°C). Afterward, the temperature of the HS room was gradually raised to 30°C (heat increment, HI), and pigs in this period started receiving their respective dietary treatment. A week after the HI, the main period of the experiment commenced and 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). Elevated 1 and elevated 2 diets were formulated by providing vitamins C and E and micro-minerals Zn and Se in excess of the NRC recommendation (Table 3). The pigs were distributed among four treatment groups, which consisted of a combination of environmental and dietary treatments and was replicated three times wherein there were 2 pigs per replicate. The allocation of thermal and dietary treatments are as follows : 1) HS (28.9 ± 0.9°C, RH - 60.4 ± 4.3%) + control or basal diet (HC), 2) Thermo-neutral (19.5 ± 0.9°C, RH - 85.9±7.3%) + control or basal diet (TC, positive control), 3) HS + elevated 1 diet (HT1), and 4) HS + elevated 2 diet (HT2). Water and feed were given to the pigs *ad libitum*.

Table 1.Composition and calculated nutrient content of basal feed [a]

Ingredients	Inclusion rate (%)	Nutrient	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-Lys	0.30	SID Trp, %	0.14
DL-Met	0.01	Ca, %	0.59
L-Trp	0.03	digestible P, %	0.23
L-Thr	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 2. Nutrient content of the premixture (in 1kg of premixture)*

Nutrient	Inclusion rate	Composition
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 3. 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

Experimental procedure, chemical analysis, and calculation

On the 29th and 30th day of the experiment, six pigs from each treatment were slaughtered (three in one day from each treatment) after electrical stunning, and after opening the abdomen, the ileum section was separated and it's content was collected and stored at −20 °C for ileal digestibility measurement. Diets and ileal digesta were analyzed for dry matter (ISO 6496), crude ash (CA) (ISO 5984), crude protein (CP) by the Kjeldahl method (ISO 5983-2), crude fat (Cfat) using petroleum ether extraction (ISO 6942), and crude fiber (CF) with boiling samples alternating sulphuric acid and potassium hydroxide (ISO 6865). Acid insoluble ash was determined and used as internal marker. Calcium (Ca), phosphorus (P), sodium (Na), and zinc (Zn) analyses were carried out after 1.0000 gram samples were digested in a block digester (LABOR MIM, Budapest, Hungary) with 10 mL concentrated nitric acid at 60 °C for 30 min and 3 mL of 30% hydrogen peroxide (Sigma-Aldrich, Saint Louis, MI, USA) at 90 min at 120 °C. For selenium (Se) analysis, 0.5000 gram sample was measured into high-pressure digestion bombs with 5 mL concentration 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 filter paper (155 mm; Macherey-Nagel, Düren, Nordrhein-Westfalen, Germany). The analysis was carried out with the inductively coupled plasma optical emission spectrometry (ICP-OES) technique as describe by Mtei et al. (2019), using the 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 Scientiftic, 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. The ileal digestibility of nutrients and minerals was calculated using acid-insoluble ash as an internal marker using the following formula:

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

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)

Statistical analysis

Data were analyzed by GraphPad Prism 8.3 software (GraphPad Software Incorporated, San Diego, USA) using analysis of variance. Tukey's test examined differences between means, and differences between treatments were considered significant if $P<0.05$.

Results

The digestibility values observed in our study are high. This could be due to the concentration of the inert marker (acid insoluble ash, AIA) in our experimental diet. In the experimental diets that we formulated, the concentration of AIA was quite low: basal diet (0.077 g/kg), elevated diet 1 (0.041 g/kg), and elevated diet 2 (0.059 g/kg). It has been recommended that dietary AIA content should exceed 7.5 g/kg on a dry matter basis to get accurate measurements (Thonney et al., 1985). Although it is also important to note that dietary AIA of 2 g/kg could also give good results (Prawirodigdo et al., 2021). The low AIA content in our experimental diet could be the cause of the high ileal nutrients and mineral digestibility values in our study. Since it has been mentioned that such values can be generated when the concentration of AIA in the diet is low, subsequently, low AIA concentration could also be recovered from the digesta and faeces, which could affect the level of calculated digestibility values (Jones and De Silva, 1998; Sales and Janssens, 2003). However, in case of different treatments this will not affect the differences induced.

Ileal digestibility of nutrients

The ileal digestibility of crude protein, crude fat, crude fiber, organic matter and nitrogen-free extract was not affected by HS (Figure 1). Surprisingly, pigs in TC had lower crude ash digestibility ($P<0.05$) compared to other treatment groups. Nevertheless, supplementation of dietary antioxidants at an elevated 1 level (HT1) in the diet improves ($P<0.05$) the crude ash digestibility and crude protein digestibility despite the HS challenge.

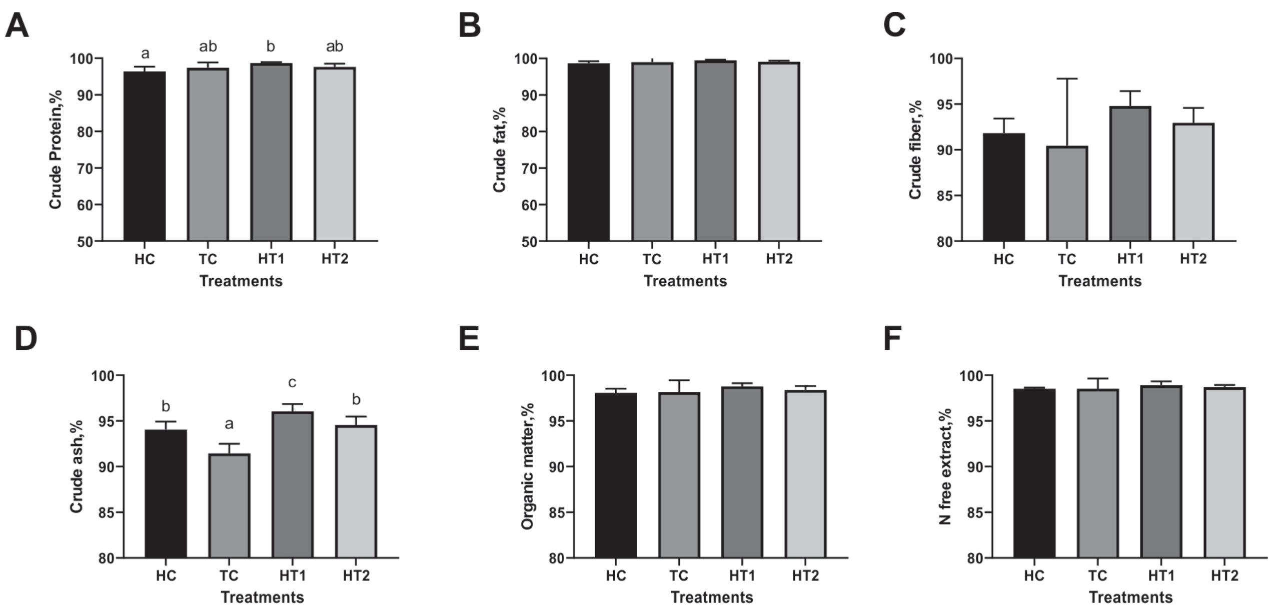

Figure 1. The effect of heat stress and high dietary antioxidant supplementation on the ileal digestibility of crude protein (A), crude fat (B), crude fiber (C), crude ash (D), organic matter (E), and nitrogen free extract (F) in fattening pigs. Values are means, with their standard deviation represented by vertical bars; [a,b] Means with the same letters do not differ ($P>0.05$). HC - HS + control or basal diet (HC), TC -Thermo-neutral + control or basal diet (positive control), HT1 - HS + elevated 1 diet, and HT2 - HS + elevated 2 diet.

Ileal digestibility of minerals

Heat stress did not affect (*P*>0.05) the ileal digestibility of the minerals studied (Figure 2). However, high dietary antioxidant supplementation at an elevated 1 level in the diet given to HT1 pigs 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 (*P*>0.05) influence their digestibility.

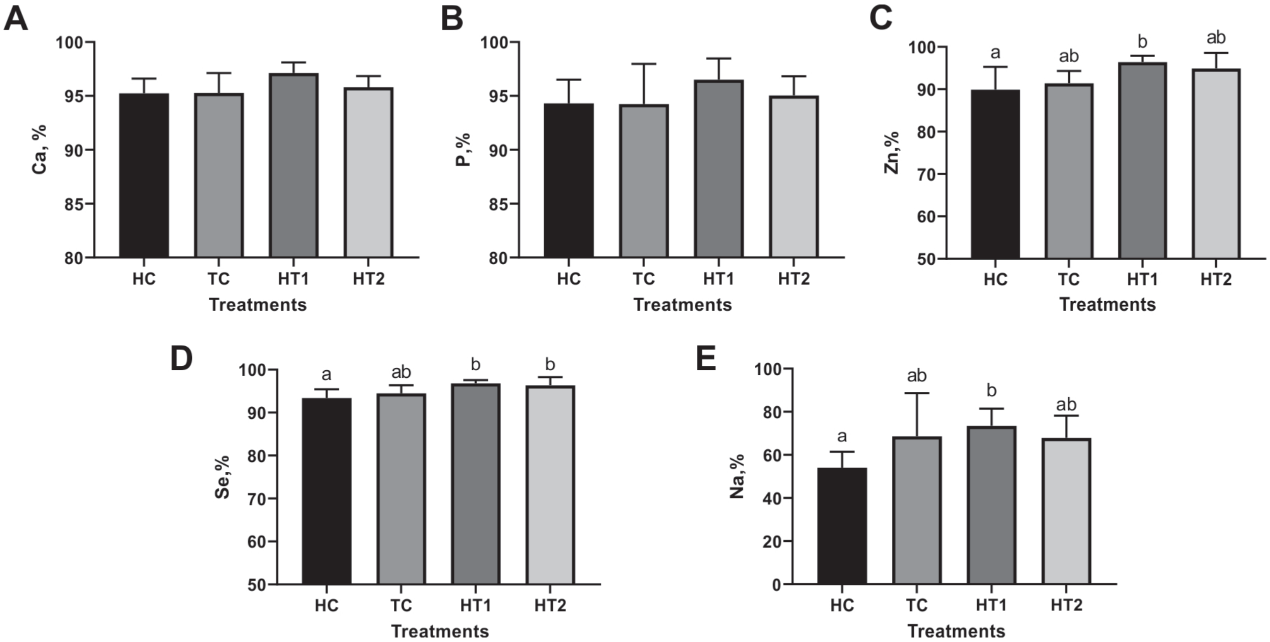

Figure 2. The effect of heat stress and high dietary antioxidant supplementation on the ileal digestibility of Ca (A), P (B), Zn (C), Se (D), and Na (E) in fattening pigs. Values are means, with their standard deviation represented by vertical bars; ^{a,b} Means with the same letters do not differ (*P*> 0.05). HC - HS + control or basal diet (HC), TC -Thermo-neutral + control or basal diet (positive control), HT1 - HS + elevated 1 diet, and HT2 - HS + elevated 2 diet.

Discussion

The susceptibility of fattening pigs to HS is a major concern, as the said stressor can negatively influence the integrity and functionality of the animals' gastrointestinal tract (Gabler and Pearce, 2015). Several studies have reported that HS causes a reduction in intestinal integrity and induces damage to the pigs' small intestine; thereby increasing the possibility of affecting its digestive and absorptive function (Yu et al., 2010; Pearce et al., 2013b; Pearce et al., 2015 Cui and Gu, 2015; Gabler et al., 2018). In the study of Hao et al. (2014), exposure of pigs to HS (30 °C) caused a reduction in nutrient digestibility. However, in the present study, HS did not (*P*>0.05) affect the digestibility of nutrients studied, except for the crude ash for which digestibility was higher (*P*<0.05) in pigs from the HS group than from the TC group. Our observation 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 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), 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 elevated 1 level in the pigs diet (HT1) 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 supplementation level also increased the ileal digestibility of Zn, Se, and Na ($P<0.05$) compared to the HC group. Although the difference between treatment groups was not determined, the digestibility of the other minerals (Ca and P) studied also show a similar trend. Although insignificant, pigs under heat stress fed diets containing further elevation of dietary antioxidant supplementation (HT2) had higher digestibility of nutrients and minerals than heat-stressed pigs fed the basal diet (HC). It is interesting that such higher level of dietary antioxidant supplementation (HT2) given to heat-stressed pigs resulted to comparable digestibility coefficients with HT1 pigs. Such observation signifies that higher level of supplementation does not always guarantee a better response from the animals. Several authors have reported similar observations. In the study of Tian et al. (2006), pigs fed diets with 0.1 mg/kg organic selenium supplementation had better digestibility of crude protein as oppose to pigs with no supplementation. Moreover, those pigs also performed slightly better digestibility of crude protein than pigs supplemented with higher level of selenium (0.3 mg/kg in the diet). Jiao et al. (2020), also had similar observations. The supplementation of organic zinc through the diet of pigs at 214.8 mg/kg (0.2% increase of Zn from the basal diet)led to better digestibility of nutrients and minerals of pigs than pigs fed the control diet (contained 179 mg/kg Zn). However, further increase in Zn supplementation (232.7 mg/kg, 0.3% increase of Zn from the basal diet) in the diet resulted to slightly lower digestibility of nutrients and minerals in pigs as opposed to pigs supplemented with 0.3% increase in zinc level.

Nevertheless, the improved digestibility of the aforementioned nutrients and minerals in pigs despite their exposure to HS condition could be attributed to the effectiveness of dietary antioxidants' in improving the integrity and functionality of the pigs' GIT (Cotrell et al., 2015; Celi et al., 2017). The dietary antioxidants (vitamins C and E, and micro-minerals Se and Zn) used in this study have their various role 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-mineralsis 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; 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), as it was observed in the present study involving pigs under HS challenge.

Conclusions

The ileal digestibility of nutrients and certain minerals between heat-stressed pigs and pigs under thermal comfort were comparable in this study. Nevertheless, elevated dietary antioxidant (vitamins C: 150 mg/kg, and E: 41 mg/kg, and micro-minerals Se: 0.21 mg/kg and Zn: 100 mg/kg) supplementation in the diet (elevated 1) of heat-stressed pigs improved the ileal digestibility of CP, crude ash, Zn, Se, and Na. Further increase in the level of dietary antioxidants in the diet (elevated 2) did not influence the digestibility of the nutrients and minerals studied. Therefore, pigs can be resilient to chronic HS's (~ 30 °C) detrimental effect on the ileal digestibility of nutrients and certain minerals. The inclusion of elevated dietary antioxidants at elevated 1 level in the diet of heat-stressed pigs could increase the ileal digestibility of several nutrients and minerals.

Acknowledgment

A.D.S.V.O. highly appreciates the funding from the Tempus Public Foundation, Stipendium Hungaricum Scholarship Programme. This research was funded by the European Union and the European Social Fund, grant number EFOP-362-16-2017-00001. The authors are thankful to the DSM Nutritional Products Hungary Ltd. (Újhartyán, Hungary) for providing the vitamin and mineral supplements.

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UTJECAJ TOPLINSKOG STRESA I DODATKA VISOKIH RAZINA ANTIOKSIDANSA NA ILEALNU PROBAVLJIVOST HRANJIVIH TVARI I ODREĐENIH MINERALA U SVINJA

Sažetak

Toplinski stres (HS) može narušiti integritet crijeva kod svinja i posljedično utječe na probavljivost hranjivih tvari. Cilj je ovog rada istražiti utjecaj toplinskog stresa HS na probavljivost hranjivih tvari i minerala u ileumu kod svinja i može li dodatak prehrani s visokim udjelom antioksidansa (vitamini C i E, te mikrominerali Se i Zn) poboljšati njihovu probavljivost. Ukupno 24 Danbred nazimica težine 65,1 ± 2,81 kg raspoređene su u četiri različite skupine, koje su smještene u termo-neutralne (TN) (19,5 ± 0,9°C, RH- 85,9±7,3 %) i HS (28,9 ± 0,9°C, RH - 60,4 ± 4,3 %) uvjete i hranjeni odgovarajućom hranom. Svinje u TN skupini primale su samo osnovni (bazalni) obrok (B) (TC), a u HS skupini, B (HC), povišenog sadržaja antioksidanasa 1 (HT1) i povišenog sadržaja antioksidanasa 2 (dijetni antioksidansi udvostručeni) (HT2), uz ad libitum hranjenje. Svinje su eutanazirane na kraju pokusa za prikupljanje digesta iz ileuma. Sadržaj hranjivih tvari (sirovih proteina (CP), masti, vlakana, pepela, organske tvari i ekstrakta bez dušika (NFE)) i minerala (Ca, P, Zn, Se i Na) mjeren je u hrani i probavnom sadržaju. Osim sirovog pepela (P < 0,05), HS nije značajno utjecao na ilealnu probavljivost drugih hranjivih tvari i minerala u svinja (P > 0,05). Međutim, svinje hranjene hranom s povišenim udjelom antioksidanasa 1 (HT1) imale su značajno veću probavljivost (P <0,05) CP, Zn, Se i Na nego HC. Daljnje povećanje dodataka vitamina i mikrominerala (HT2) nije poboljšalo probavljivost promatranih hranjivih tvari i minerala u ileumu svinja. Prehrana obogaćena antioksidansima na povišenoj razini 1 mogla bi poboljšati probavljivost hranjivih tvari i minerala svinja izloženih toplinskom stresu.

Ključne riječi: toplinski stress, antioksidansi, ilealna probavljivost, tov svinja

Received - primljeno: 16.12.2022.
Accepted - prihvaćeno : 26.04.2023.