Archiva Zootechnica 25:1, 116-129, 2022
DOI: 10.2478/azibna-2022-0008

Effect of dietary supplementation of some antioxidant combinations on nutrient digestibility in heat-stressed broilers

Mihaela Saracila*, Tatiana Dumitra Panaite, Arabela Elena Untea, Iulia Varzaru

*corresponding author: mihaela.saracila@yahoo.com; mihaela.saracila@ibna.ro

National Research-Development Institute for Animal Biology and Nutrition (IBNA), Calea Bucuresti, 1, Balotesti, 077015, Ilfov, Romania.

ABSTRACT

The paper investigated the effect of some dietary antioxidant's combinations on nutrient digestibility in heat-stressed broilers. The experimental study was conducted for 28 days on 120 Cobb 500 broilers (14 days of age) assigned into 4 groups (30 chicks/group) in cages and exposed to heat stress conditions (32 °C). The control group was fed a control diet (C) and the experimental groups were fed diets including 200 μg/kg diet chromium picolinate and: 0.25 g vitamin C (VC)/kg diet (Cr-VC), 0.025 g Zn/kg diet (Cr-Zn), and 10 g creeping wood sorrel powder (CWS)/kg diet (Cr-CWS). Our results showed that dietary combinations of supplemented antioxidants for chickens under heat stress had a positive effect on the digestibility of nutrients and nutrient deposits in liver tissue. Dietary combined effect of Cr and vitamin C or Cr and Zn potentiates the Fe deposition in broilers' liver while Cr-Zn improved the apparent digestibility of crude protein, crude fat, Fe and Zn.

Keywords: antioxidant, mineral, vitamin C, heat stress, nutrient digestibility.

INTRODUCTION

Heat stress- defined as a physiological condition in which the body is unable to maintain a balance between heat production and body heat loss, is undoubtedly a major factor in the economic losses in the poultry sector. These losses are the result of numerous damages caused by heat stress in the body of chickens such as: decreased digestion and absorption of nutrients, decreased production performance, decreased resistance of the body to disease, etc (Nawaz et al., 2021; Saracila et al., 2021b).

There is evidence that heat stress increases the need for vitamins and minerals for broilers (Calik, 2022; Livingston et al., 2022). Research has supported the use of Cr supplements as a nutritional strategy to improve growth performance (Ghazi et al., 2012), nutrient metabolism (Jahanian and Rasouli, 2015), antioxidant status, and stress response (Khan et al., 2016) in chickens exposed to heat stress. Significant improvements in birds growth have been reported when vitamin C was supplemented in diets of chickens raised under heat stress (Abudabos et al., 2017; Chand et al., 2017).

Zinc is a major component of various proteins (many of them enzymes) has many functions in chickens organism including cell proliferation, improvement of antioxidant status, providing defense against free radicals (Cao et al., 2015) and improvement of serum vitamin C and E levels (Sahin et al., 2006). Zinc protects the pancreatic tissue against oxidative stress (Horváth and Babinszky, 2018) so it can be presumed that it can improve the digestibility of nutrients.

Creeping wood sorrel (*Oxalis corniculata*, CWS) contains a wide range of phytochemicals with antioxidant properties such as flavonoids, tannins, phytosterols, polyphenols, glycosides (Sharma & Kumari, 2014). Recent studies (Saracila et al., 2020; 2021a; Untea et al., 2021) have shown that diet supplemented with Cr picolinate and CWS had a positive effect on meat quality from heat-stressed broilers.

The latest research studies show that the vitamins and/or minerals used in combination have more pronounced effects on the antioxidant status and performance of birds raised under heat stress than they had alone (Horváth and Babinszky, 2018). This observation is explained by the existence of the phenomenon of synergism between some of these nutrients. However, the topic of combining vitamins, minerals and phytoadditives in the diets of chickens raised under heat stress is little debated and should be further investigated.

The aim of the study was to investigate the effects of dietary supplementation of chromium and vitamin C, zinc, and creeping wood sorrel supplements on nutrient digestibility in heat-stressed broilers.

MATERIALS AND METHODS

Experiment design

The study was performed on 120 Cobb 500 broilers reared in an experimental hall (experimental protocol approved by the Ethical Commission of National Research and Development Institute for Biology and Animal Nutrition). At 14 days of age, the broilers were assigned to four groups (30 chicks/group) and housed in digestibility cages under controlled environmental conditions and monitored by a Viper Touch computer. The control group was fed a control diet (C) and the experimental groups were fed diets including 200 μg/kg diet chromium picolinate and: 0.25 g vitamin C (VC)/kg diet (Cr-VC), 0.025 g Zn/kg diet (Cr- Zn), and 10 g creeping wood sorrel powder (CWS)/kg diet (Cr-CWS).

Table 1. Diet formulation (%)

Ingredient	Grower(14-28d)				Finisher (28-42d)			
	C	Cr-VC	Cr-Zn	Cr-CWS	C	Cr-VC	Cr-Zn	Cr-CWS
Corn	36.63	36.63	36.63	35.63	40.64	40.64	40.64	39.64
Wheat	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Corn gluten	4.00	4.00	4.00	4.00	6.00	6.00	6.00	6.00
Soybean meal	30.20	30.20	30.20	30.20	23.95	23.95	23.95	23.95
Creeping wood sorrel (CWS)	-	-	-	1.00	-	-	-	1.00
Oil	4.30	4.30	4.30	4.30	4.72	4.72	4.72	4.72
Monocalcium phosphate	1.52	1.52	1.52	1.52	1.43	1.43	1.43	1.43
Calcium carbonate	1.38	1.38	1.38	1.38	1.31	1.31	1.31	1.31
Salt	0.38	0.38	0.38	0.38	0.33	0.33	0.33	0.33
Methionine	0.25	0.25	0.25	0.25	0.21	0.21	0.21	0.21
Lysine	0.29	0.29	0.29	0.29	0.36	0.36	0.36	0.36
Choline	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
A1 Premix	1.00	1.00*	1.00**	1.00***	1.00	1.00*	1.00**	1.00***
Total	100	100	100	100	100	100	100	100
ME, Kcal/kg	3128.99				3217.72			
CP, %	21.50				20.00			
EE, %	6.01				6.49			
CF, %	3.57				3.36			

* A1 premix + 20 mg CrPic/kg premix + 25 g vit. C/kg premix; ** A1 premix + 20 mg CrPic/kg premix + 2.5 g Zn/kg premix; *** A1 premix + 20 mg CrPic/kg premix + 1% creeping wood sorrel powder; Diet structure published previously by Saracila et al. (2021a).

During all experimental period (14-42 days), the chicks were subjected to heat stress (HS) conditions (32 ±1 °C). The light regimen was 23h light and 1h darkness, feed and water were administered *ad libitum*. Chromium supplement was used as Chromium picolinate, $Cr(C_6H_4NO_2)_3$ and purchased from Santa Cruz Biotechnology (USA). Vitamin C (99% purity) and Zn supplements were purchased from DSM Nutritional Products Romania SRL. The creeping wood sorrel plants were harvested from Ilfov county in their late vegetative stage, slow dried and grounded. Plant samples were characterized previously by Saracila et al., (2022) that revealed important concentration of crude protein (16.74%), crude fibre (19.36%) and ash (22.56%), high concentration of lutein and zeaxanthin (267.41μg/g) and vitamin E

(210.93μg/g) with a high total antioxidant capacity (170.70 μM Trolox). Regarding the mineral profile, creeping wood sorrel contains significant levels of Fe (243.68 mg/kg) and Zn (92.10 mg/kg).

Apparent absorption of the nutrients

The coefficients of apparent absorption of the nutrients were determined using the balance technique (weeks 4 and 6). The quantity of ingested feed and the excreted droppings were recorded for 5 consecutive days. After this, the droppings were homogenized, dried in the drying oven for 48 h, at 65°C and ground. Proximate composition analysis (dry matter at 65°C, DM; crude protein, CP; crude fat, EE; Ash and mineral determinations (Fe, Mn, Zn) were performed on feed and droppings samples.

The coefficients of apparent absorption of nutrients were calculated based on the results obtained from the chemical analysis of the feeds and droppings, corroborated with the daily feed intake and excreta. The coefficients of apparent absorption of trace minerals were determined using digestibility equations according to Schiemann (1981).

Birds slaughter and liver tissue collection

At 42 days, six broilers from each group were slaughtered by cervical dislocation. Samples of liver were collected (n=6) and stored at −80 ° C until further analysis.

Chemical analyses

The chemical proximate composition of feed, droppings and liver samples was assayed using the chemical methods from Commission of the European Communities (2009).

Trace minerals (Fe, Zn, Mn) concentrations were determined by flame atomic absorption spectrometry (FAAS) with a Thermo Electron-SOLAAR M6 Dual Zeeman Comfort (Cambridge, UK) spectrophotometer according to Untea et al., (2012). Mineral concentrations were expressed as mg/kg sample.

The total antioxidant capacity (T-AOC) of the sample was determined by the phosphomolybdenum method described by Prieto et al. (1999). The results were expressed as mmol equivalent ascorbic acid/kg DW and mmol equivalent vitamin E/kg DW.

Lutein and zeaxanthin assay was performed according to Varzaru et al., (2015) using a Perkin Elmer liquid chromatograph equipped with a Nucleodur C18 column.

Cholesterol content in liver tissue was performed by gas-chromatography using a Perkin-Elmer Clarus 500 gas chromatograph (Shelton, MA, USA) according to AOAC Official Method 994.10: Cholesterol in foods (1996) and described by Panaite et al., (2021).

Statistical analysis

The effect of dietary treatments on selected parameters of heat-stressed broiler was analysed by performing one-way analysis of variance (ANOVA one way) and Fisher LSD test as post hoc test using Statview for Windows (SAS, version 6.0). The differences between means were considered statistically significant at p<0.05.

RESULTS AND DISCUSSION

In the present study, the data from Table 2 showed that crude fat (EE) content of liver was significantly higher in Cr-VC and Cr-Zn groups compared to C and Cr-CWS. Contrary to our findings on liver crude fat, other authors have shown different results on deposit tissues (breast and thigh). For example, some authors showed that Cr-Zn and Cr-VC supplementation to the diet of heat stressed broiler decreased the content of crude fat in breast (Untea et al., 2021) and thigh meat (Saracila et al., 2021a).

Several previous studies reported that chronic heat stress can enhance fat synthesis and deposition in broilers (Lu et al., 2007, 2018), but the relationship between heat stress and the accumulation of lipids in the liver remains unclear.

Table 2. Proximate composition of liver tissue

Specification, %	C	Cr-VC	Cr-Zn	Cr-CWS	SEM	p-value
DM	26.16	26.44	26.95	26.77	0.203	0.5469
CP	17.58	17.63	17.24	17.91	0.136	0.3972
EE	3.11[a]	3.59[c]	4.13[b]	3.23[a]	0.088	<0.0001
Ash	1.30	1.31	1.34	1.36	0.010	0.176

[a–b] Means in the same column without a common superscript differ (p<0.05); C= Control diet; Cr-VC= basal diet + Cr +vitamin C (VC); Cr-Zn= basal diet + Cr+ Zn; Cr-CWS= basal diet +Cr+CWS; DM-dry matter; CP- Crude protein; EE- Ether extractives.

As other authors stated that Chromium supplements inhibit the lipogenesis process leading to a decreased fat accretion in tissues (Untea et al., 2019; 2021), and others considered that chromium had no effect on lipid metabolism (Balk et al., 2007). However, to our knowledge no results were found regarding the effect of Cr on crude fat content in the liver of heat-stressed broilers.

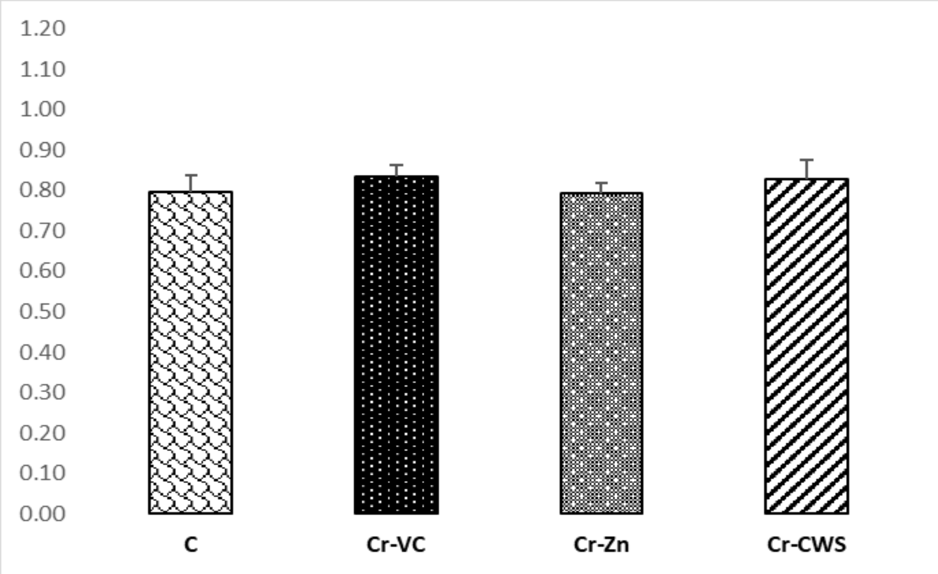

Figure 1. Cholesterol content in liver tissue

Figure 1 showed the cholesterol content in liver tissue. The dietary supplementation with Cr-VC, Cr-Zn, Cr-CWS did not affect the cholesterol content in the liver of heat- stressed broilers.

The bioactive nutrient content of liver tissue is presented in table 3. The lutein and zeaxanthin content in liver was significantly lower in the experimental groups compared to C group. In contrast, the results of a previous study (Saracila et al., 2022) performed under normal temperature showed an increased content of lutein and zeaxanthin in breast and thigh samples from chickens fed a diet supplemented with Cr and CWS. However, the total antioxidant capacity recorded in the liver was significantly higher in group Cr-VC, Cr-Zn and Cr-CWS compared to C. These observations indicate that vitamin C and Zn supplementation have led to increased antioxidant capacity in the liver. There are studies showing that exogenous antioxidants obtained from the diet such as vitamins (vitamins C, E, A) and minerals (Zn, Cu) are involved in the antioxidant defense mechanism of broilers, protecting against oxidative damage of liver especially under thermal stress (Surai et al., 2019; Seyidoglu and Aydin, 2020). Regarding the mineral profile, it was observed that the level of Fe was higher in the liver of the chicks from Cr-Zn group compared to C, Cr-VC and Cr-CWS. Although thermal stress causes a decrease in tissue mineral concentrations including iron, zinc, and selenium (Ghazi et al., 2012; Habibian et al., 2016), in this study the combination of Cr and vitamin C or Cr and Zn led to increased levels of Fe in liver, supporting the antioxidant status of broilers.

Table 3. Bioactive nutrient content of liver tissue

Bioactive nutrient	C	Cr-VC	Cr-Zn	Cr-CWS	SEM	P-value
Antioxidant profile						
Lutein and zeaxanthin, µg/g	9.61^{a}	6.67^{c}	4.65^{b}	6.25^{c}	0.500	<0.0001
TAC, mM ascorbic acid equivalent	65.82^{a}	76.62^{b}	85.09^{c}	80.29^{bc}	0.190	<0.0001
TAC, mM vitamin E equivalent	68.93^{a}	80.75^{b}	90.10^{c}	84.81^{bc}	2.089	<0.0001
Mineral profile (mg/kg)						
Iron	1137.27^{a}	1256.26^{b}	1115.93^{a}	1043.30^{c}	0.750	<0.0001
Manganese	10.95^{a}	9.91^{b}	10.03^{b}	10.11^{b}	0.122	0.003
Zinc	96.97^{a}	93.04^{a}	108.21^{b}	95.34^{a}	2.082	0.0357

$^{a-c}$ Means in the same column without a common superscript differ (p<0.05); C= Control diet; Cr-VC= basal diet + Cr +vitamin C (VC); Cr-Zn= basal diet + Cr+ Zn; Cr-CWS= basal diet +Cr+CWS; TAC= total antioxidant capacity.

The literature is controversial regarding the interaction between Cr and Fe. Some authors have shown that Cr and Fe are antagonistic minerals, and Cr supplementation affects Fe absorption (Pechova and Pavlata, 2007) while others have shown that Cr supplementation increases Fe and Zn levels in chicken meat (Untea et al., 2021). However, the Fe levels were significantly lower in the liver from Cr-CWS group compared to the other groups. There are two possible explanations for the obtained result. One possible explanation could be the intake of polyphenols (in our study achieved via CWS), which could inhibit Fe availability and consequently the deposition, as some studies have shown (Hurrell et al. 1999).

The second one could refer to the oxalic acid derivates (ex. oxalates) present in CWS in important quantity which are known in the literature to impair the availability of nonheme iron (van Dokkum, 1992; Ems et al., 2021). However, some literature studies refute this observation and show that the oxalates have no influence on iron absorption (Genannt Bonsmann et al., 2008; Milman, 2020). The Mn level decreased in the liver of the Cr-VC, Cr-Zn and Cr-CWS group compared to C. The results could be due to the antagonism between these vitamins and minerals or bioactive compounds that also affect the bioavailability of minerals (Akter et al., 2020). As expected, a significantly higher amount of Zn was detected in the liver of Cr-Zn group than in that of the other groups.

Table 4. Effect of dietary treatments on the apparent absorption of the nutrients (grower stage)

Variable	C	Cr-VC	Cr-Zn	Cr-CWS	SEM	p-value
Crude protein						
Ingested *	21.88[a]	20.99[a]	24.76[b]	20.98[a]	0.510	0.0147
Excreted *	5.97	6.03	6.04	6.31	0.172	0.9130
Absorbed *	15.90[a]	14.97[a]	18.73[b]	14.67[a]	0.455	0.0012
Absorption coefficient (%)	72.65[ab]	71.39[a]	75.52[b]	70.01[a]	0.689	0.0214
Crude fat						
Ingested *	5.52[a]	5.47[a]	6.66[b]	5.46[a]	0.146	0.0032
Excreted *	0.63	0.67	0.68	0.71	0.021	0.6597
Absorbed*	4.89[a]	4.80[a]	5.98[b]	4.75[a]	0.135	0.0008
Absorption coefficient (%)	88.58[ab]	87.89[a]	89.72[b]	87.13[a]	0.280	0.0201
Ash						
Ingested *	6.14a	4.86b	6.10a	5.25b	0.150	0.0005
Excreted *	2.77	2.76	2.71	3.15	0.084	0.2342
Absorbed *	3.36[a]	2.10[b]	3.39[a]	2.10[b]	0.149	<0.0001
Absorption coefficient (%)	54.72[a]	43.35[b]	55.44[a]	40.27[b]	1.637	<0.0001
Iron						
Ingested *	37.45	38.44	36.40	35.59	0.721	0.5566
Excreted *	23.67	22.19	22.21	25.65	0.652	0.1958
Absorbed *	13.79[a]	16.25[a]	14.19[a]	9.95[b]	0.679	0.0033
Absorption coefficient (%)	36.60[a]	42.42[a]	38.78[a]	28.12[b]	1.531	0.0021
Manganese						
Ingested *	9.71[ac]	8.91[a]	11.36[b]	10.08[ac]	0.257	0.0020
Excreted *	7.00	6.88	7.18	7.89	0.207	0.3260
Absorbed *	2.71[a]	2.03[a]	4.18[b]	2.19[a]	0.210	<0.0001
Absorption coefficient (%)	27.76[a]	23.16[a]	36.68[b]	21.99[a]	1.631	0.0011
Zinc						
Ingested *	11.58[a]	11.13[a]	13.80[b]	11.10[a]	0.313	0.0010
Excreted *	7.04	6.87	8.04	7.90	0.226	0.1570
Absorbed *	4.54[a]	4.25[a]	5.75[b]	3.20[c]	0.245	0.0003
Absorption coefficient (%)	38.99[a]	38.43[a]	41.54[a]	29.15[b]	1.475	0.0078

[a–c] Means in the same column without a common superscript differ ($p<0.05$); * - expressed as g/chick/day; C= Control diet; Cr-VC= basal diet + Cr +vitamin C (VC); Cr-Zn= basal diet + Cr+ Zn; Cr-CWS= basal diet +Cr+CWS.

Table 4 shows the apparent absorption of nutrients for broilers in the grower stage. It is observed that the chickens fed Cr-Zn showed higher amounts of ingested and digested crude protein, crude fat, Mn and Zn compared to other diets.

Similarly, Ahmed et al. (2005) recorded increased apparent protein metabolism in broiler chickens fed diets supplemented with Cr chloride ($CrCl_3$). The coefficient of apparent digestibility of crude fat was significantly higher in the Cr-Zn group compared to Cr-VC and Cr-CWS.

Mineral absorption can be influenced by many factors including environment conditions, diets, etc. In this study, the apparent absorption coefficient of ash was significantly higher in Cr-Zn compared to Cr-VC and Cr-CWS groups. Also, the apparent absorption coefficient of Mn was significantly higher in Cr-Zn group compared to the other groups. Manganese is an essential enzymatic co-factor with implications in improving broiler performance (Dalólio et al., 2021). It was observed that apparent absorption coefficient of Fe and Zn was significantly higher in Cr-Zn compared to Cr-CWS. Other authors (Amatya et al., 2004) have shown increases in Fe, Zn, Mn retention as a consequence of Cr supplementation for heat- stressed chickens.

Table 5 shows the apparent absorption of the nutrients for broilers in the finisher stage. It is perfectly assumed that heat stress also affects the nutrients intake and digestibility. However, in this study, it is observed that chickens fed Cr-Zn had higher amounts of ingested and digested crude protein, crude fat, Mn and Zn compared to other diets. The apparent digestibility coefficient of crude protein and crude fat was significantly higher in the experimental groups compared to C group. One possible explanation could be the antioxidant potential of these protective minerals and vitamin C which protect the pancreatic tissue against oxidative stress resulting in increased nutrient digestibility (Preuss et al. 1997).

The apparent digestibility of ash was significantly lower in the group fed a combination of Cr and CWS than the other groups. Similar with the results obtained in the balance performed in the grower stage, the apparent digestibility of Fe was significantly improved in Cr-Zn compared to group C, while that of Zn was significantly higher in Cr-Zn and Cr-CWS groups compared to C.

Table 5. Effect of dietary treatments on the apparent absorption of the nutrients (finisher stage)

Variable	C	Cr-VC	Cr-Zn	Cr-CWS	SEM	p-value
Crude protein						
Ingested *	20.47	22.38	23.71	22.48	0.633	0.3579
Excreted *	8.07	7.61	7.61	8.05	0.260	0.8788
Absorbed *	12.41[a]	14.77[b]	16.10[b]	14.43[ab]	0.451	0.0222
Absorption coefficient (%)	60.75[a]	66.12[b]	67.86[b]	64.40[bc]	0.700	0.0002
Crude fat						
Ingested *	6.18	6.52	6.59	6.67	0.175	0.7878
Excreted *	0.87	0.81	0.77	0.78	0.029	0.6848
Absorbed*	5.31	5.71	5.82	5.89	0.154	0.5754
Absorption coefficient (%)	86.02[a]	87.64[b]	88.26[b]	88.44[b]	0.284	0.0034
Ash						
Ingested *	7.27	7.18	7.43	6.95	0.193	0.8617
Excreted *	3.37	3.23	3.21	3.52	0.110	0.7612
Absorbed *	3.90[ab]	3.96[ab]	4.22[a]	3.43[b]	0.114	0.0889
Absorption coefficient (%)	53.76[a]	55.17[a]	56.76[a]	49.60[b]	0.793	0.0033
Iron						
Ingested *	37.99	39.97	41.05	40.22	1.073	0.8024
Excreted *	27.31	26.30	26.18	28.33	0.933	0.8527
Absorbed *	10.68[a]	13.67[bc]	14.86[b]	11.89[ac]	0.550	0.0230
Absorption coefficient (%)	28.34[a]	34.40[ab]	36.11[b]	30.07[ab]	1.193	0.0451
Manganese						
Ingested *	13.39	13.30	12.81	12.41	0.354	0.7677
Excreted *	9.06	8.31	8.31	8.71	0.251	0.6961
Absorbed *	4.34	4.99	4.50	3.70	0.231	0.2743
Absorption coefficient (%)	31.95	37.17	34.91	30.21	1.306	0.2462
Zinc						
Ingested *	12.59	12.59	14.74	14.20	0.401	0.1190
Excreted *	9.21	8.56	9.67	9.56	0.305	0.6017
Absorbed *	3.39[a]	4.03[ab]	5.08[b]	4.65[b]	0.195	0.0050
Absorption coefficient (%)	27.03[a]	32.16[ab]	34.35[b]	33.06[b]	1.068	0.0475

[a–c] Means in the same column without a common superscript differ ($p<0.05$); *expressed as g/chick/day; C= Control diet; Cr-VC= basal diet + Cr +vitamin C (VC); Cr-Zn= basal diet + Cr+ Zn; Cr-CWS= basal diet +Cr+CWS.

Conclusion

Our results showed that dietary combinations of supplemented antioxidants for chickens under heat stress had a positive effect on increasing the total antioxidant capacity in the liver. In conclusion, of all the other supplements, the best solution on attenuating the negative effects of HS by improving the digestibility of nutrients and deposits in liver tissue was the combination of Cr with Zn.

Acknowledgements

This work was funded by Romanian Ministry of Education and Research, project PN 19 09 0102 and National Research Development Project Projects to Finance Excellence (PFE) - 8/2021.

References

Akter, S., Netzel, M., Tinggi, U., Fletcher, M., Osborne, S. Sultanbawa, Y., 2020. Interactions Between Phytochemicals and Minerals in Terminalia ferdinandiana and Implications for Mineral Bioavailability. Front. Nutr. 7, 598219.

Amatya, J.L., Haldar, S., Ghosh, T.K., 2004. Effects of chromium supplementation from inorganic and organic sources on nutrient utilization, mineral metabolism and meat quality in broiler chickens exposed to natural heat stress. Animal Science, 79(2), 241-253.

Association of Official Analytical Chemists (AOAC). Official Method 994.10: Cholesterol in Foods-Direct Saponification-Gas Chromatographic Methods; Association of Official Analytical Chemists: Arlington, VA, USA, 1996.

Balk, E.M., Tatsioni, A., Lichtenstein, A.H., Lau, J. & Pittas, A.G., 2007. Effect of chromium supplementation on glucose metabolism and lipids a systematic review of randomized controlled trials. Diabetes Care 30, 2154-2163.

Calik, A., Emami, N.K., White, M.B., Walsh, M.C., Romero, L.F., Dalloul, R.A., 2022. Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, Part I: Growth performance, body composition and intestinal nutrient transporters. Poultry Science, 101857.

Cao, Y., Roursgaard, M., Kermanizadeh, A., Loft, S., Møller, P., 2015. Synergistic effects of zinc oxide nanoparticles and fatty acids on toxicity to caco-2 cells. International Journal of Toxicology, 34(1), 67-76.

Chand, N., Naz, S., Maris, H., Khan, R. U., Khan, S., Qureshi, M.S., 2017. Effect of betaine supplementation on the performance and immune response of heat stressed broilers. Pakistan Journal of Zoology, 49(5).

Dalólio, F.S., Albino, L.F.T., da Silva, J.N., Fireman, A.K.A.T., Burin, Á.M., Busanello, M., Ribeiro, V., 2021. Dietary chromium-methionine supplementation and broiler (22–43 days) responses during heat stress. 1. Growth performance and carcass yield, metabolisable energy and serum biochemistry. Animal Production Science, 61(6), 586-595.

Ems, T., St Lucia, K., & Huecker, M. R. (2021). Biochemistry, iron absorption. In StatPearls [internet]. StatPearls Publishing.

Genannt Bonsmann, S.S., Walczyk, T., Renggli, S., Hurrell, R.F., 2008. Oxalic acid does not influence nonhaem iron absorption in humans: a comparison of kale and spinach meals. European journal of clinical nutrition, 62(3), 336–341.

Ghazi, S.H., Habibian, M., Moeini, M.M., Abdolmohammadi, A.R., 2012. Effects of different levels of organic and inorganic chromium on growth performance and immunocompetence of broilers under heat stress. Biological trace element research, 146(3), 309-317.

Habibian, M., Ghazi, S., & Moeini, M.M., 2016. Effects of dietary selenium and vitamin E on growth performance, meat yield, and selenium content and lipid oxidation of breast meat of broilers reared under heat stress. Biological trace element research, 169(1), 142-152.

Horváth, M., & Babinszky, L., 2018. Impact of selected antioxidant vitamins (Vitamin A, E and C) and micro minerals (Zn, Se) on the antioxidant status and performance under high environmental temperature in poultry. A review, Acta Agriculturae Scandinavica, Section A — Animal Science, 68,3, 152-160.

Hurrell, R.F., Reddy, M., Cook, J.D., 1999. Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. Br J Nutr. 81:289–95.

Jahanian, R., & Rasouli, E., 2015. Dietary chromium methionine supplementation could alleviate immunosuppressive effects of heat stress in broiler chicks. Journal of Animal Science, 93(7), 3355-3363.

Khan, A.Z., Kumbhar, S., Hamid, M., Afzal, S., Parveen, F., Liu, Y., & Huang, K., 2016. Effects of selenium-enriched probiotics on heart lesions by influencing the mrna expressions of selenoproteins and heat shock proteins in heat stressed broiler chickens. Pakistan Veterinary Journal, 36(4).

Livingston, M.L., Pokoo-Aikins, A., Frost, T., Laprade, L., Hoang, V., Nogal, B., Phillips, C., Cowieson, A.J., 2022. Effect of heat stress, dietary electrolytes, and vitamins E and C on growth performance and blood biochemistry of the broiler chicken. Front. Anim. Sci. 3:807267.

Lu, Q., Wen, J., Zhang, H., 2007. Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. Poultry science, 86(6), 1059-1064.

Lu, Z., He, X., Ma, B., Zhang, L., Li, J., Jiang, Y. & Gao, F., 2018. Serum metabolomics study of nutrient metabolic variations in chronic heat-stressed broilers. British Journal of Nutrition, 119(7), 771-781.

Milman, N.T., 2020. A review of nutrients and compounds, which promote or inhibit intestinal iron absorption: making a platform for dietary measures that can reduce iron uptake in patients with genetic haemochromatosis. Journal of Nutrition and Metabolism, 2020.

Nawaz, A.H., Amoah, K., Leng, Q.Y., Zheng, J.H., Zhang, W.L., & Zhang, L., 2021. Poultry response to heat stress: its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. Frontiers in veterinary science, 8, 699081.

Panaite, T.D., Nour, V., Saracila, M., Turcu, R.P., Untea, A.E., Vlaicu, P.A., 2021. Effects of linseed meal and carotenoids from different sources on egg characteristics, yolk fatty acid and carotenoid profile and lipid peroxidation. Foods, 10, 1246.

Pechova, A., & Pavlata, L., 2007. Chromium as an essential nutrient: a review. Veterinární medicína, 52(1), 1.

Preuss, H.G., Grojec, P.L., Lieberman, S., Anderson, R.A., 1997. Effects of different chromium compounds on blood pressure and lipid peroxidation in spontaneously hypertensive rats. Clinical nephrology, 47(5), 325-330.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269 (2), 337-341.

Sahin, K., Onderci, M., Sahin, N., Gursu, M.F., Khachik, F., & Kucuk, O., 2006. Effects of lycopene supplementation on antioxidant status, oxidative stress, performance and carcass characteristics in heat-stressed Japanese quail. Journal of Thermal Biology, 31(4), 307-312.

Saracila, M., Panaite, T. D., Tabuc, C., Soica, C., Untea, A., Varzaru, I., Criste, R. D., 2020. Maintaining intestinal microflora balance in heat-stressed broilers using dietary creeping wood sorrel (Oxalis corniculata) powder and chromium (chromium picolinate). Spanish journal of agricultural research, 18(3), 19.

Saracila, M., Panaite, T.D., Mironeasa, S., Untea, A.E., 2021a. Dietary supplementation of some antioxidants as attenuators of heat stress on chicken meat characteristics. Agriculture, 11, 638.

Saracila, M., Panaite, T.D., Papuc, C.P., Criste, R.D., 2021b. Heat stress in broiler chickens and the effect of dietary polyphenols, with special reference to willow (*Salix spp.*) bark supplements—A Review. Antioxidants, 10, 686.

Saracila, M., Untea, A.E., Panaite, T.D., Varzaru, I., Oancea, A., Turcu, R.P., & Vlaicu, P.A., 2022. Creeping wood sorrel and chromium picolinate effect on the nutritional composition and lipid oxidative stability of broiler meat. Antioxidants, 11(4), 780.

Schiemann, R., 1981. Methodische Richtlinien zur Durchführung von Verdauungsversuchen für die Futterwertschätzung. Archiv für Tierernährung. 31 (1), 1-19.

Seyidoglu, N., & Aydin, C., 2020. Stress, natural antioxidants and future perspectives. in (ed.), the health benefits of foods - current knowledge and further development. IntechOpen.

Sharma, R.A., & Kumari, A., 2014. Phytochemistry, pharmacology and therapeutic application of *Oxalis corniculata Linn*. –a review. Int J Pharm Pharm Sci, 3, 6-12.

Surai, P.F., Kochish, I.I., Fisinin, V.I., & Kidd, M.T., 2019. Antioxidant defence systems and oxidative stress in poultry biology: An Update. Antioxidants (Basel, Switzerland), 8(7), 235.

Untea, A.E., Panaite, T.D., Dragomir, C., Ropota, M., Olteanu, M., & Varzaru, I., 2019. Effect of dietary chromium supplementation on meat nutritional quality and antioxidant status from broilers fed with Camelina-meal-supplemented diets. animal, 13(12), 2939-2947.

Untea, A.E., Varzaru, I., Turcu, R.P., Panaite, T.D., & Saracila, M., 2021. The use of dietary chromium associated with vitamins and minerals (synthetic and natural source) to improve some quality aspects of broiler thigh meat reared under heat stress condition. Italian Journal of Animal Science, 20(1), 1491-1499.

Untea, A.E., Criste, R.C., Vladescu, L., 2012. Development and validation of a microwave digestion–FAAS procedure for Cu, Mn and Zn determination in liver. Revista de Chimie. 63(4), 341-346.

van Dokkum, W., 1992. Significance of iron bioavailability for iron recommendations. Biol Trace Elem Res 35, 1–11.

Varzaru, I., Untea, A.E., Van, I., 2015. Distribution of nutrients with benefic potential for the eyes in several medicinal plants. Rom. Biotechnol. Lett., 20, 10773–10783.