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EFFECT OF DIETARY SUPPLEMENTATION OF CHROMIUM AND ZINC ON PERFORMANCE, SERUM BIOCHEMICAL PARAMETERS, CARCASS DEVELOPMENT, AND INTESTINAL MICROFLORA BALANCE IN BROILER CHICKENS REARED UNDER HEAT STRESS

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Abstract

The present study evaluates the effect of dietary chromium and zinc (Cr-Zn) supplementation on performance, serum biochemical parameters, carcass development and intestinal microflora of broilers reared under high heat stress (HS). An experiment was carried out on 60 Ross 308 broiler chickens, assigned to two experimental groups (C and Cr-Zn) with 30 chickens/group and maintained under high heat stress (32°C). Compared to the control diet (C), the experimental diet included the addition of a premix with 20 mg chromium picolinate +2.5 g Zn/kg premix (Cr-Zn). Dietary Cr-Zn did not affect the performance and serum biochemical parameters of broiler chickens reared under HS conditions. The use of Cr-Zn in broiler diet led to a significant reduction of Enterobacteriaceae, E. coli, staphylococci in the caecal and intestinal content. Both in the caecum and in the intestinal contents of Cr-Zn broiler chickens, the number of lactobacilli was significantly higher than in the C broilers. The combination of Zn and Cr in broiler feeding has a positive impact on maintaining the balance of intestinal microflora during heat stress

***Key words**: broiler, chromium, heat stress, intestinal microflora balance, zinc.*

INTRODUCTION

Heat stress has adverse effects on general health, growth performance, physiology, etc. (Xu et al., 2018). High ambient temperatures compromise performance and productivity by reducing feed intake and nutrient utilization, growth rate and meat quality, resulting in economic losses (Freeman, 1987). Given the negative consequences of heat stress, the problem of heat stress quickly became a point of particular interest in the livestock sector (Lara & Rostagno, 2013). That is why the need for well-developed strategies to mitigate the negative effects of thermal stress was imposed. During heat stress, a reduction in plasma and tissue levels of vitamins (vitamin C, E, folic acid) and minerals (Zinc) has been observed, which may be associated with reduced food consumption and high-water consumption in broilers (Abudabos et al., 2018). The decrease in the concentration of vitamins and minerals leads to cell membrane damage (Sahin et al., 2001), as well as the generation of free radicals that can affect homeostasis mechanisms leading to pathological changes (Teeter et al., 2005), tissue damage, as well as adverse effects on erythrocytes (Adenkola & Ayo, 2009). In addition, intestinal balance is impaired associated with intestinal diseases (Saracila et al., 2021). Minerals play the central role of all metabolic functions in broiler chickens (Alagawany et al., 2021). Many studies have shown the positive effects of mineral supplementation (e.g., selenium, chromium, zinc, etc.) in the diet of chickens under heat stress conditions (Saracila et al., 2021; Untea et al., 2021). Mineral supplementation is important to reduce heat stress and maintain performance and production in the poultry sector. Studies have shown the significant effects of minerals on improving performance parameters, reducing heat shock proteins and lipid peroxidation (Rao, 2016). Improved performance, anti-oxidant and immune responses were reported in chicken fed higher concentrations of Cr and Zn (Burrell et al., 2004). Chromium (Cr) has been used to increase feed efficiency, improve body weight and organs in broilers; therefore, Cr is considered a popular mineral supplement as a result of acting a dynamic role in improving the retention of other essential elements in the blood and decreasing their excretion (Sahin et al., 2017). Zinc (Zn) is one of the essential nutrients that is a cofactor for 200 enzymes. Thus, it has a central role in antioxidant capacity, growth, immunity and nutrient digestion through interaction with other minerals in the gut (Naz et al., 2016). The present study evaluates the effect of dietary supplementation with chromium and zinc (Cr-Zn) on the performance, serum biochemical parameters, carcass development and intestinal microflora of broilers raised under high heat stress (HS).

MATERIALS AND METHODS

The experiment was carried out in accordance with the animal welfare principles set out by Directive 2010/63/EU. The experimental protocol was approved by the Ethics Commission of INCDBNA-IBNA Balotesti (no. 4775/02.08.2019). The experiment was carried out on a number of 60 chickens from the Ross 308 hybrid for six weeks (1-42 days). At the age of 1 day, the chicks were divided according to the average body weight and divided into two groups (C and Cr-Zn), with 30 chicks/group. They were housed in experimental halls equipped with a three-level Zucammi battery, in digestibility cages (cage dimensions 65 x 75 x 45 cm). Chicks were reared under heat stress with 32°C constant temperature. Microclimate parameters (ventilation, %; humidity, %; temperature, °C; carbon dioxide, ppm and ammonia, ppm) were controlled using the Viper Touch computer (Big Dutchman, Germany). The light program was ensured according to the growth age of the chicks (23 h light/1 h dark). Water and feed were given ad libitum. The diet structure was in accordance with the nutritional requirements (NRC, 1994) and the nutritional requirements of the hybrid Ross 308. Compared with the control diet (C group), the experimental diet (Cr-Zn group) included 200 µg chromium picolinate (Cr)/kg diet+0.025 g Zn/kg diet (Cr-Zn). The diet structure is presented in Table 1. Chromium supplement was used in the premix in the form of chromium picolinate ($Cr(C_6H_4NO_2)_3$) (Santa Cruz Biotechnology, CA, USA) and Zinc was purchased from DSM Nutritional Products Romania SRL.

At the end of the experiment (42 days), 6 chicks were randomly selected from each group. From each group, blood samples were collected aseptically from the sub axial region, respectively the brachial vein, in vacutainers with anticoagulant (Li-heparin) and prepared as described by Saracila et al. (2018). The obtained supernatant (serum) was used to determine biochemical parameters (glucose, cholesterol, triglycerides, urea, albumin, phosphorus, phosphorus, iron, alanine aminotransferase and aspartate aminotransferase, gamma GT). Analyses were performed using kits according to the manufacturer's instructions.

After blood sampling, the broiler chickens were slaughtered by cervical dislocation. The carcasses were eviscerated, and then the organs (gizzard, liver, heart, and spleen) and the breast and thigh samples were collected. Both carcasses and organs were weighed using a Sartorius balance in order to calculate the relative weight of the carcass and organs, according to the following calculation formula:

$$\text{Relative weight of organ/carcass (\%)} = \frac{\text{Live bodyweight x organ/carcass weight}}{100}$$

Consequently, the entire gut has been removed from the oesophagus to the cloaca. Samples of intestinal and caecal contents were collected in aseptic medium in sterilised plastic tubes and stored at -20ºC until the bacteriological assays (*Enterobacteriaceae, Escherichia coli*, staphylococci, lactobacilli, *Salmonella* spp.).

The bacteriological analyses were performed according to the assays described by Saracila et al. (2020; 2021). For counting bacterial colonies was used Scan 300, Interscience (France). The results were reported as log base 10 colony-forming units (CFU) per gram of caecal/intestinal contents.

Table 1. Diet formulation

Ingredient	Starter (1-14 days)		Grower (15-28 days)		Finisher (29-42 days)	
	C	Cr-Zn	C	Cr-Zn	C	Cr-Zn
	%					
Corn	32.73	32.73	36.63	36.63	40.64	40.64
Wheat	20	20	20	20	20	20
Corn gluten	2	2	4	4	6	6
Soybean meal	36.17	36.17	30.2	30.2	23.95	23.95
Sunflower oil	3.85	3.85	4.3	4.3	4.72	4.72
Monocalcium phosphate	1.68	1.68	1.52	1.52	1.43	1.43
Calcium carbonate	1.5	1.5	1.38	1.38	1.31	1.31
Salt	0.39	0.39	0.38	0.38	0.33	0.33
Methionine	0.33	0.33	0.25	0.25	0.21	0.21
Lysine	0.3	0.3	0.29	0.29	0.36	0.36
Choline	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral premix*	1	1**	1	1**	1	1**
TOTAL	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Metabolisable energy, kcal/kg	3039.79		3128.99		3217.72	
Chemical composition- calculated (%)						
Crude protein	23.00		21.50		20.00	
Ether extractives	5.48		6.01		6.49	
Crude fibre	3.77		3.57		3.36	
Calcium	0.96		0.87		0.81	
Phosphorus	0.77		0.70		0.65	

*1 kg premix contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg Vit. B1; 400 mg/kg vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vit. B6; 4 mg/kg vit. B7; 100 mg/kg vit. B9; 1.8 mg/kg vit. B12; 2000 mg/kg vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.
**Vitamin-mineral premix + 2.5 g Zn/ kg premix+ 20 mg Cr picolinate/kg premix
Where: C- conventional diet; Cr-Zn- conventional diet + 2.5 g Zn / kg premix + 20 mg Cr picolinate/kg premix.

Statistical analysis

The statistical analyses were performed using Prism-GraphPad software v. 9.03 (San Diego, CA, USA).

The effect of dietary treatments on tested parameters was analysed using one-way analysis of variance (ANOVA). Significant differences among treatment means were determined at $p<0.05$ by Tukey's multiple-range test.

RESULTS AND DISCUSSIONS

Table 2 presents the results of the determination of the primary chemical composition and the bacterial and mycological analysis of the compound feeds.

The results of the determinations regarding the chemical composition of the feeds showed that they were balanced from an energy-protein point of view.

With regard to the bacteriological and mycological analysis of the combined feeds, the values fall within the stipulated maximum limits, regulated, published in the Official Gazette of Romania no. 362/2003.

In all feed compounds, *Salmonella* spp. was absent (Table 2).

In Table 3 is presented the effect of tested compound feeds on the productive performance of chickens raised under heat stress. It is observed that none of the tested compound feeds had a significant influence on the production parameters.

Consistent with this, many studies suggest that Cr in various forms (chromium picolinate, chromium chloride) behaves better in combination with minerals such as Zn in terms of body weight gain (Sahin & Sahin, 2002; Perai et al., 2013; Attia et al., 2015) especially under stress conditions such as high or low temperature and humidity.

Some studies support the synergistic action of Cr and other antioxidants under stress conditions; by mutually amplifying their actions, they lead to enhanced performance in birds (Sahin et al., 2001; Haq et al., 2017; Al-Sultan et al., 2019).

Table 2. Mineral composition and bacteriological and mycological analysis of compound feeds

Item	Starter (1-14 days)		Grower (15-28 days)		Finisher (29-42 days)	
	C	Cr-Zn	C	Cr-Zn	C	Cr-Zn
	%					
Mineral composition						
Ash, %	6.27	6.50	6.29	5.66	7.02	6.15
Calcium, %	0.89	0.88	0.89	0.90	0.89	0.90
Phosphorus, %	0.84	0.95	0.94	0.95	0.81	0.82
Cu, mg/kg	8.06	7.79	7.13	6.46	7.44	7.18
Fe, mg/kg	354.7	358.2	343.2	302.3	301.6	333.4
Mn, mg/kg	105.1	104.4	89.0	94.3	115.9	102.6
Zn, mg/kg	110.3	124.0	106.1	114.6	109.0	118.2
TAC, mM equivalent ascorbic acid	42.7	43.5	43.3	44.0	42.8	44.5
TAC, mM equivalent vit. E	43.3	44.8	44.5	46.6	45.3	47.2
Bacteriological and mycological analysis						
TNG, Col/g	57 x 10^3	37.6 x 10^4	49 x 10^3	39x10^4	32.5 x 10^3	32.4 x 10^4
Total coliforms /g	40	1.4	2	0.9	0.3	1.4
E. coli/g	11.5	0	0.9	0	0	0
Salmonella Col/g	-	-	-	-	-	-
TNF, Col/g	7750	3500	1725	3500	2570	2500

Maximum permissible level (MPL): (MO 362 bis/2003): TNG (Total number of germs): max. 15x 106 col/g (SR 13178-1); total coliforms: max. 3000 col/g (SR 13178-2); *E. coli*: max. 100 col/g (SR 13178-2); Salmonella sp.: 0 col/g (SR EN 12824); TNF (total number of fungi): max. 5 x 104 col/g (STAS 6953-81). Where: SR= Romanian Standard; STAS= State Standards; SR EN= European Standards TAC= Total antioxidant capacity.

Table 3. Effect of the tested compound feeds (1-42 days) on productive performance (average values± SD)

Parameter	Days of age	C	Cr-Zn	General effects of diets
		$\bar{X} \pm S_{\bar{X}}$		
Body weight (g)	1	46.4±2.9	46.4±3.5	ns
	14	446.1±43.3	450.2±44.2	ns
	28	1213.0±152.3	1216.0±152.0	ns
	42	1988.0±347.0	1935.0±399.1	ns
Average daily feed intake (g/day)	1-14	34.9±16.9	34.5±17.1	ns
	15-28	77.4±9.8	78.7±10.5	ns
	29-42	102.0±3.6	99.3±2.1	ns
	1-42	71.4±29.8	70.8±29.7	ns
Body weight gain (g/broiler/day)	1-14	28.6±3.1	28.8±3.1	ns
	15-28	54.7±9.9	54.7±10.4	ns
	29-42	55.5±28.6	51.4±28.9	ns
	1-42	46.2±8.3	44.9±9.5	ns
Feed conversion ratio (g feed/g gain)	1-14	1.22±0.04	1.20±0.03	ns
	15-28	1.42±0.02	1.45±0.04	ns
	29-42	1.83±0.1	1.90±03	ns
	1-42	1.53±0.05	1.56±0.1	ns

ns: non-significant.

Among other things, differences between the results of the studies may be due to the source, the level of inclusion, the bioavailability of Zn and chromium in the chicken diet.

Sahin et al. (2003) showed that Cr (400 mg Cr/kg diet) added to the diet of chickens (21-42 days old) raised in heat stress led to obtaining a higher final weight, average weight gain, average daily consumption and better specific consumption.

Other authors showed that 1.50 mg/kg Cr-nicotinic increased FCR of 4% in broilers exposed to heat stress (Toghyani et al., 2012).

Table 4 presents the effect of dietary treatments on serum biochemical parameters.

Table 4. Serum biochemical parameters (average values ± SD)

Parameter	C	Cr-Zn	General effects of diets
Energy profile			
Glycaemia, mg/dL	247.1±33.35	213.2±17.49	ns
Cholesterol, mg/dL	144.8±22.8	132.9±6.79	ns
Triglycerides, mg/dL	34.50±8.53	50.53±14.21	ns
Renal metabolism			
Albumin (mg/dL)	1.00	1.00	ns
Urea (mg/dL)	4.87±0.89	4.63±0.44	ns
Mineral profile			
Phosphorus (mg/dL)	5.69±0.51	5.59±0.21	ns
Iron (µg/dL)	80.78±11.57	77.31±3.90	ns
Hepatic parameters			
ALT (TGP), U/L	3.41±0.62	3.59±0.94	ns
AST (TGO), U/L	453.0±112.8	309.2±60.65	ns
Gama GT, U/L	15.23±4.74	16.36±6.14	ns

Where: ALT- Alanine aminotransferase; AST- aspartate aminotransferase; ns: non-significant.

Results showed that dietary Cr-Zn did not affect serum biochemical parameters of broilers reared under HS condition. Contrary, Sahin et al. (2002) showed that dietary chromium and zinc supplementation decreased serum glucose and cholesterol concentrations and increased protein concentrations in laying hens reared under low ambient temperature.

Table 5. Effect of the tested compound feeds (1-42 days) on carcass development and relative organ weight[1]

Parameter	C	Cr-Zn	General effects of diets
$\bar{X} \pm S_{\bar{X}}$ (g)			
Live body weight	2357.50±0.02^{a}	2155.83±1.20^{b}	*
Carcass weight	83.34±20.01	82.20±1.52	ns
Breast weight	16.58±8.16	19.87±1.63	ns
Thigh weight	19.36±2.63	20.37±0.96	ns
Gizzard	1.61±0.18	1.67±0.33	ns
Liver	1.51±0.26	0.09±0.09	ns
Heart	0.32±0.02^{a}	0.42±0.07^{b}	*
Spleen	0.04±0.01	0.06±0.03	ns

[1]Percent from Live body weight; * $^{a–b}$ Means within a column with no common superscript differ ($p<0.05$).

Table 5 shows that under conditions of heat stress, supplementing the diet of chickens with Cr+Zn led to a significantly lower live body weight compared to those fed with C. Also, within the same group, a higher relative weight of the heart was recorded compared to group C. Otherwise, the dietary treatments did not influence the weight of the carcass, the relative weight of the breast, thigh, liver and spleen.

Table 6 shows the effect of the dietary treatments on the caecal microbiota of chickens raised under heat stress. The data obtained in this study showed that the inclusion of Cr+Zn had a significant effect against the tested pathogens (*Enterobacteriaceae*, *E. coli*, staphylococci) in the cecum of chickens raised under heat stress (Table 6). Thus, the number of *Enterobacteriaceae* colony-forming units was significantly lower in Cr-Zn compared to C. Also, the chickens that included Cr+Zn in the diet had a significantly lower number of *E. coli* and staphylococci compared to those who were given the conventional diet. At the same time, the number of lactobacilli was significantly higher in chickens from Cr-Zn compared to group C.

Table 6. Effect of the tested compound feeds on caecal microbiota (log_{10} CFU*/g caecal content)

Parameter	C	Cr-Zn	General effects of diets
$\bar{X} \pm S_{\bar{X}}$			
Enterobacteriaceae	11.39±0.009[a]	11.37±0.005[b]	****
E. coli	10.16±0.019[a]	10.10±0.008[b]	****
Staphylococci	8.91±0.009[a]	8.66±0.015b	****
Lactobacili	10.99±0.007[a]	11.13±0.010[b]	****
E. coli:lactobacilli	0.87±0.001[a]	0.85±0.001[b]	****

[a–b]Means within a column with no common superscript differ (p<0.05).

In Table 7 is presented the effect of diets tested on the intestinal microbiota. The number of colony-forming units of *Enterobacteriaceae*, *E. coli* and staphylococci was significantly lower in the intestinal contents of the chickens from the Cr-Zn group compared to C.

The addition of Cr+Zn to the chicken diet led to the detection of a higher number of lactobacilli in the intestinal contents compared to those fed the C diet. Regarding the *E. coli* : lactobacilli ratio on the cecal content, it was significantly lower in the groups whose diet was supplemented with Cr+Zn compared to the C group.

These observations are important because heat stress is known to favour the growth of pathogenic bacteria over beneficial ones (Yadav & Jha, 2019). The previous results might be due to the antioxidant mechanism of Cr and Zn supplements, through which attenuates these adverse effects on gut microflora. Some authors showed that Zn can be used as anti-oxidative stress agent, down-regulating ROS production and accumulation through several mechanisms including inhibition of oxidation of macro-molecules such as (DNA)/ribonucleic acid (RNA) and proteins as well as inhibition of inflammatory response (Prasad & Bao, 2019).

Table 7. Effect of the tested compound feeds on intestinal microbiota (log_{10} CFU*/g intestinal content)

Parameter	C	Cr-Zn	General effects of diets
$\bar{X} \pm S_{\bar{X}}$			
Enterobacteriaceae	7.46±0.001[a]	7.43±0.004[b]	****
E. coli	6.14±0.004[a]	6.11±0.004[b]	****
Staphylococci	5.85±0.006[a]	5.78±0.019[b]	****
Lactobacili	7.37±0.005[a]	7.40±0.004[b]	****
E. coli:lactobacilli	0.84±0.001[a]	0.82±0.001[b]	****

[a–b]Means within a column with no common superscript differ (p<0.05).

Chromium can play a secondary antioxidant role (Farag et al., 2017; Krol et al., 2017) and also improves the immune system of chicks raised under heat stress (Dalólio et al., 2018).

There is a strong relation between immune system and the intestinal health in chickens (Adedokun & Olojede, 2019), so improving the immune system also maintains the balance of intestinal microflora and prevents the adhesion of pathogenic bacteria to the mucosa.

In addition, Cr can interact with gut microbiota. Feng et al. (2019) showed that the use of Cr and micronutrients as dietary supplements can provide significant protection for intestinal microflora.

CONCLUSIONS

The diet supplementation with Cr and Zn did not significantly affect the production parameters of heat-stressed broilers and biochemical parameters of the serum. The inclusion of Cr and Zn supplements in broiler chickens' diet reduced the number of tested pathogens (*Enterobacteriaceae*, *E. coli*, staphylococci) and increased the abundance of beneficial bacteria (lactobacilli).

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REFERENCES

Abudabos, A.M., Al-Owaimer, A.N., Hussein, E.O., & Ali, M.H. (2018). Effect of natural vitamin C on performance and certain haemato-biochemical values in broiler chickens exposed to heat stress. *Pakistan Journal of Zoology*, *50*(3).

Adedokun, S.A., & Olojede, O.C. (2019). Optimizing gastrointestinal integrity in poultry: the role of nutrients and feed additives. *Front Vet. Sci., 5*, 348.

Adenkola, A.Y., Ayo, J.O., Sackey, A.K.B., & Adelaiye, A.B. (2009). Haematological and serum biochemical changes in pigs administered with ascorbic acid and transported by road for four hours during the harmattan season. *J. Cell Anim. Biol, 3*(2), 21-28.

Alagawany, M., Elnesr, S.S., Farag, M.R. Tiwari, R., Yatoo, M.I., Karthik, K., & Dhama, K. (2021). Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health–a comprehensive review. *Veterinary Quarterly, 41*(1), 1-29.

AL-Sultan, S., Abdel-Raheem, S., Abd-Allah, S., & Edris, A. (2019). Alleviation of chronic heat stress in broilers by dietary supplementation of novel feed additive combinations. *Slov. Vet. Res., 56*, 269-279.

Attia, K.M., Tawfeek, F.A., Mady, M.S., & Assar, A.H. (2015). Effect of chromium, selenium and vitamin C on productive performance and blood parameters of local strain Dokki in Egypt summer conditions. *Egypt. Poult. Sci., 35*, 311–329.

Burrell, A.L., Dozier, W.A., Davis, A.J., Compton, M.M., Freeman, M.E., Vendrell, P.F. & Ward, T.L. (2004) Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. Br *Poult Sci., 45*, 225–263.

Dalólio, F.S., Albino, L.F.T., Silva, J.N., Campos, P.H.R. F., Moreira, J., & Ribeiro, V. Junior. (2018). Dietary chromium supplementation for heat-stressed broilers. *World's Poult Sci J., 74*, 101-116.

Farag, M.R., Alagawan, M., Abd El-Hack, M.E., Arif, M., Ayasan, T., Dhama, K., Patra, A., & Karthik, K. (2017). Role of chromium in poultry nutrition and health: beneficial applications and toxic effects. *Int J Pharmacol., 13*, 907-915.

Feng, P., Ye, Z., Kakade, A., Virk, A., Li, X., & Liu, P. (2019). A review on gut remediation of selected environmental contaminants: possible roles of probiotics and gut microbiota. *Nutrients 11*, 22.

Freeman, M.L., Scidmore, N.C., Malcolm, A.W., & Meredith, M.J. (1987). Diamide exposure, thermal resistance, and synthesis of stress (heat shock) proteins. *Biochemical pharmacology, 36*(1), 21-29.

Haq, Z., Jain, R.K., Khan, N., Dar, M.Y., Ali, S., Gupta, M., & Varun, T.K. (2016). Recent advances in role of chromium and its antioxidant combinations in poultry nutrition: A review. *Veterinary World, 9*, 1392–1399.

Król, B., Słupczyńska, M., Kinal, S., Bodarski, R., Tronina, W., & Mońka, M. (2017). Bioavailability of organic and inorganic sources of chromium in broiler chicken feeds. *J. Elem., 22*, 283-294.

Lara, L.J., & Rostagno, M.H. (2013). Impact of heat stress on poultry production. *Animals, 3*(2), 356-369.

Naz, A., Chowdhury, A., Mishra, B. K., & Gupta, S. K. (2016). Metal pollution in water environment and the associated human health risk from drinking water: A case study of Sukinda chromite mine, India. Human and Ecological Risk Assessment: *An International Journal, 22*(7), 1433-1455.

Perai, A.H., Kermanshahi, H., Nassiri Moghaddam, H., & Zarban, A. (2015). Effects of chromium and chromium+vitamin C combination on metabolic, oxidative, and fear responses of broilers transported under summer conditions. *Int. J. Biometeorol., 59*, 453-462.

Prasad, A.S., & Bao, B. (2019). Molecular Mechanisms of Zinc as a Pro-Antioxidant Mediator: Clinical Therapeutic Implications. *Antioxidants (Basel), 6*, 8(6), 164.

Rao, S.N. (2016). The role of heat shock proteins in kidney disease. *Journal of translational internal medicine, 4*(3), 114-117.

Sahin, K., Kucuk, O., Sahin, N., & Ozbey, O. (2001). Effects of dietary chromium picolinate supplementation on egg production, egg quality, and serum concentrations of insulin, corticostrerone and some metabolites of Japanese quails. *Nutr. Res., 21*, 1315-1321.

Sahin, K., & Sahin, N. (2002). Effects of chromium picolinate and ascorbic acid dietary supplementation on nitrogen and mineral excretion of laying hens reared in a low ambient temperature (7°C). *Acta Vet. Brno., 71*, 183–189.

Sahin, K., Sahin, N., & Kucuk, O. (2003). Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32°C). *Nutrition Research, 23*, 225–238.

Sahin, N., Hayirli, A., Orhan, C., Tuzcu, M., Akdemir, F. A.T.I.H., Komorowski, J.R., & Sahin, K. (2017). Effects of the supplemental chromium form on performance and oxidative stress in broilers exposed to heat stress. *Poultry science, 96*(12), 4317-4324.

Sahin, N., Onderci, M. & Sahin, K. (2002). Effects of dietary chromium and zinc on egg production, egg quality, and some blood metabolites of laying hens reared under low ambient temperature. *Biol. Trace Elem. Res., 85*, 47–58.

Saracila, M., Panaite, T.D., Vlaicu, P.A., Tabuc, C., Palade, M.L., Gavris, T., & Criste, R.D. (2018). Dietary Willow Bark Extract for Broilers Reared Under Heat Stress. *Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Animal Science & Biotechnologies*, *75*(2).

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), e0612-e0612.

Saracila, M., Panaite, T.D., Papuc, C.P., & Criste, R.D. (2021). Heat stress in broiler chickens and the effect of dietary polyphenols, with special reference to Willow (Salix spp.) bark supplements—a review. *Antioxidants, 10*(5), 686.

Toghyani M, Toghyani M, Shivazad M, Gheisari A, & Bahadoran R. (2012). Chromium supplementation can alleviate the negative effects of heat stress on growth performance, carcass traits, and meat lipid oxidation of broiler chicks without any adverse impacts on blood constituents. *Biol. Trace Elem. Res., 146*(2), 171-180.

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.

Xu, Y., Lai, X., Li, Z., Zhang, X., & Luo, Q. (2018). Effect of chronic heat stress on some physiological and immunological parameters in different breed of broilers. *Poultry science,* 97(11), 4073-4082.

Yadav S., & Jha R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *J. Anim. Sci. Biotechnol*., 10, 1-11.