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

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ABSTRACT

The effect of chromium associated with vitamins and minerals (synthetic or natural ingredients) on broiler meat nutritional quality, subjected to heat stress conditions were evaluated. The 6-week feeding trial was conducted on 120 unsexed 1-day-old Cobb 500 broilers, assigned to 4 treatments and housed in digestibility cages at 32°C constant temperature. Compared to the control diet (C), the experimental diets were supplemented with 200 μg/kg diet chromium picolinate (Cr), as follows: Cr-AA has 0.25 g ascorbic acid/kg diet; Cr-Zn has 0.025 g zinc/kg diet; Cr-WS has 10 g creeping wood sorrel/kg diet. The results of the study showed that essential amino acids concentrations determined in the thigh meat samples tend to increase in groups supplemented with chromium and ascorbic acid and chromium and zinc, especially sulphur containing amino acids (methionine and cystine) which increased for experimental groups (methionine: 1.25% (C) vs. 1.77% (Cr-AA); 1.58% (Cr-Zn); 1.64% (Cr-WS) and cystine: 0.46% (C) vs. 0.52% (Cr-AA); 0.54% (Cr-WS)). Among the compounds with biological value, vitamin E and lutein & zeaxanthin concentration registered the highest increase for chromium and zinc supplemented group (vitamin E: 35.49 mg/kg (C) vs. 59.05 mg/kg (Cr-Zn) and lutein & zeaxanthin: 0.66 mg/kg (C) vs. 1.36 mg/kg (Cr-Zn)). The peroxidation process was delayed under nutritional supplements influence, the primary oxidation parameters (peroxide value) from the Cr-WS group being significantly lower compared to the control group. The same trend was maintained for the secondary oxidation parameters, the Cr-WS group highlighting reduced values for p-anisidine and TBARS concentration compared to the C group (p-anisidine: 17.22 (C) vs. 9.29 (Cr-WS) and TBARS: 0.15 mg/kg (C) vs. 0.11 mg/kg (Cr-WS)).

In conclusion, sulphur-containing amino acids (methionine and cysteine) concentrations increased for experimental groups, in the thigh meat samples, the liposoluble compounds (vitamin E, lutein & zeaxanthin) concentration registered the highest increase for chromium and zinc supplemented group and the peroxidation process was more pronounced delayed under chromium and wooden sorrel combination.

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Introduction

The effects of heat stress on poultry are related to decreased productivity in terms of depression of feed intake which affects the growth rate. Also, the high temperature has an influence on thyroid activity, reduced values of plasma protein or parameters of antioxidant defense system (vitamins and minerals) in serum (Sahin et al. 2002) or quality of products (Sahin et al. 2017). There are a number of strategies used by broiler breeders to attenuate the negative effects of heat stress. Among the strategies related to the microclimate conditions in the breeding halls, there are also the strategies regarding the broiler diets, namely supplementing them with vitamins and minerals (Dalólio et al. 2018). Dietary manipulation has been considered a method for alleviating the high-temperature effect on animals productivity. Under heat stress conditions, the vitamins and minerals are mobilised from tissues in order to meet requirements and as consequence, their excretion is increased (Ghazi et al. 2012).

Chromium is an essential element, with an important contribution in the metabolism of carbohydrates,
proteins and lipids and also the preferred mineral for temperature stress conditions (Sahin et al. 2017). It potentiates insulin activity with strong implications in carbohydrate and protein metabolism and has a positive effect on lipid peroxidation (Untea et al. 2019). Chromium supplements were used to alleviate cold or heat stress effects in several studies on poultry. The influence of chromium supplements followed some directions: the productivity parameters; serum thyroid hormones, insulin and corticosterone (Sahin et al. 2002); cell preservation, antioxidant activity and immune response. All these physiological functions have particular importance under heat stress conditions for animal homeostasis and their thermoregulatory capacity (Dalólio et al. 2018).

In addition to chromium, there are bioactive compounds such as ascorbic acid that combat the effects of heat stress. Unlike humans, birds are able to synthesise vitamin C but environmental stressors can alter its utilisation (Karami et al. 2018). Vitamin C supplementation of animal diets helps the system immunity, regulates body temperature, minimises stress hormones, improves production performance through feed efficiency. Combined with other compounds with antioxidant activity, ascorbic acid behaves as a co-antioxidant with synergistic effects. Its presence in diets potentiates the absorption of chromium and correlates very well against symptoms caused by heat-stressed broilers (Shakeri et al. 2020). It was observed that dietary zinc used in poultry diets improved growth rate and feed efficiency under heat stress conditions (Kucuk 2008). Zinc participates in the antioxidant defence system by potentiating the synthesis of metallothionein, which is recognised as a free radical scavenger (Sahin et al. 2009). Studies on zinc association with chromium revealed positive effects on performance, immune responses and meat quality of quails under heat stress conditions (Rouhalamini et al. 2014). The interaction effects of chromium, zinc and ascorbic acid on antioxidant enzyme activity were observed in poultry reared under heat stress conditions (Karami et al. 2018).

*Oxalis corniculata*, commonly called creeping wood sorrel, is an herbaceous plant from *Oxalidaceae* family, used in traditional medicine. The creeping wood sorrel is a good source of vitamin C, carotene and essential fatty acids, but has also phenolic compounds, flavonoids, tannins, phytosterols, amino acids and volatile oils. According to Muhammad et al. (2012), the plant leaves have a high mineral content such as sodium, potassium, calcium, nitrogen and magnesium. Due to its chemical composition, this plant has important properties such as antioxidants, anti-inflammatory, antimicrobial, anti-diarrheal and others related to them (Sarkar et al. 2020), which could alleviate broiler heat stress. Although it is a promising plant material for animal feed, studies on its use being very limited. Subhani et al. (2018) indicated a decrease in production performances of Hubbard broilers fed with creeping wood sorrel during Afatoxicosis depending on the rate of inclusion in diets (250 mg to 500 mg/kg). In another study (Gupta et al. 2012) it was shown a moderation of the anxiety parameters in mice, due to the presence of flavonoids and tannins from the plant (Table 1).

As a nutritional strategy for alleviating the heat stress effects on the meat quality of broilers, other two nutritional compounds are known as powerful antioxidants (ascorbic acid and zinc) were added to the diets in order to potentiate the chromium effect. The purpose of this study was to evaluate the impact of proposed nutritional solutions on growth performance and some broiler thigh meat nutritional aspects such as quality of protein and antioxidant status.

### Table 1. Ingredients and chemical composition of diets.

| Diet composition, % (fed basis) | Starter (0 to 14 days) | Grower (14 to 28 days) | Finisher (28 to 42 days) |
|-------------------------------|------------------------|------------------------|------------------------|
| Total                         | 100                    | 100                    | 100                    |
| Metabolizable energy, kcal/kg | 3039.00                | 3128.00                | 3217.00                |
| Crude protein                 | 23.00                  | 21.50                  | 20.00                  |
| Crude fat                     | 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                   |
| Available phosphorus           | 0.48                   | 0.43                   | 0.41                   |
| Lysine                        | 1.44                   | 1.29                   | 0.16                   |
| Methionine                    | 0.69                   | 0.61                   | 0.32                   |
| Tryptophan                    | 0.25                   | 0.22                   | 1.19                   |

*Premix C: 1 kg contains: 1,100,000 IU/kg vit. A; 200,000 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; 600 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.

*Premix Cr-AA contains premix C structure + 20 mg Cr/kg premix and 25 g ascorbic acid/kg premix;**

**Premix Cr-Zn contains premix C structure + 20 mg Cr/kg premix and 25 g Zn/kg premix;***

***Premix Cr-WS contains premix C structure + 20 mg Cr/kg premix (~1% creeping wood sorrel replaced 1% corn).
Material and methods

The experimental procedures were approved by the Ethical Committee of the National Research Development Institute for Biology and Animal Nutrition, in accordance with the Romanian legislation (Law 206/2004, ordinance 28/31.08.2011, law 43/11.04.2014, Directive 2010/63/EU).

Experimental design

A total of 120 unsexed 1-day-old Cobb 500 broilers, obtained from a commercial hatchery were subjected to a heat stress in a completely randomised trial. All broilers (initial body weight of 46.36 g ± 0.35 g) were randomly allocated to 4 dietary treatments (C; Cr-AA; Cr-Zn and Cr-WS), with 6 replicate pens/treatment and 5 chicks/pen. The broilers were housed under controlled environmental conditions in digestibility cages. The feeding trial (0–42 d) was conducted in heat stress conditions (32 ± 0.47°C) maintained during all experimental periods, 24 h/day. Three-tiered Zucammi cages (dimensions 65 cm x 75 cm x 45 cm, one cage per replicate) were used for birds housing and a ViperTouch computer was used for monitoring environmental parameters. The temperature and lighting programs were consistent with the recommendations of broiler commercial management guide, as follows: average temperature 32°C; humidity 62.97 ± 5.09%; ventilation/broiler 0.64 ± 0.18%; CO₂ level 888.48 ± 101.31 ppm; 23-h light/1-h darkness.

Throughout the experimental period, the following parameters were monitored: body weight (BW, g), average daily feed intake (ADFI, g feed/broiler/day), average daily weight gain (ADWG, g/broiler/day) and feed conversion ratio (FCR, g feed/g gain). Individual measurements on body weight were performed weekly in order to calculate ADWG (as the ratio between total weight and number of experimental days) and ADFI was calculated by the difference between daily basis and leftovers. FCR represents the ratio between total feed consumed and total live weight. No mortality was recorded during the overall trial period.

Temperature humidity index (THI) is a measurement tool for heat stress and the THI formulae (Kang et al. 2020) used dry-bulb temperature (Tdb) as ambient air temperature and wet-bulb temperature (Twb) estimated using the method suggested by Stull (2011). Based on data recorded during the experimental period, the THI value was 29.72 indicating severe heat stress for non-sweating animals like broilers (Lallo et al. 2018).

Diet formulation

The four dietary treatments were based on corn and soybean meal, according to nutritional requirements of the Cobb 500 management guide. Compared to the control diet (C), the other three diets were supplemented with 200 μg/kg diet chromium picolinate associated with vitamins or minerals (synthetic or natural sources), as follows: first experimental group (Cr-AA) was supplemented with 0.25 g ascorbic acid/kg diet; the second experimental group (Cr-Zn) was supplemented with 0.025 g zinc/kg diet; the third experimental group (Cr-WS) was supplemented with 10 g creeping wood sorrel/kg diet. The broilers had free access to the feed and water.

All chemical additives were included in the premix, by replacing the corresponding amount of support. The creeping wood sorrel was included directly into the feed mill replacing the corresponding amount of corn.

Tissue sampling

At the end of the feeding trial (42 d), according to the experimental procedures, 6 broilers from each treatment (1 per each replicate) were randomly selected and slaughtered and muscle tissue samples (thigh meat) were collected for further analysis. Each sample was divided into several parts and stored in plastic bags, labelled according to each origin group (C; Cr-AA; Cr-Zn and Cr-WS) in order to determine the amino acid profile; the concentrations of compounds with biological value and antioxidant potential (vitamin E, lutein and zeaxanthin, iron (Fe), zinc (Zn), polyphenols, antioxidant capacity) and the oxidative stability products. The samples were stored in the freezer at −80°C until the assessment time.

Chemical analysis

The Oxalis corniculata (creeping wood sorrel) included in the experimental diet (Cr-WS) was harvested in the late vegetative stage (44.62°N, 26.12°E). For maximum retention of bioactive compounds, this raw material was dried at ambient temperature for 3 weeks and then grounded in order to be included in the broiler diet (Saracila et al. 2020).

The estimations of total phenolic (TP) content (Folin Ciocalteu method) and total antioxidant capacity (phosphomolybdenum method) were performed according to the methods described by Untea et al. (2020).
The amino acids profile of thigh meat samples was performed using a reversed-phase high-performance liquid chromatography (RP-HPLC) method on HyperSil BDS C18 column, with silica gel, dimensions 250 × 4.6 mm, particle size 5 μm (Thermo-Electron Corporation, Waltham, MA), according to the method described by Varzaru et al. (2013).

Trace mineral concentrations were determined by flame atomic absorption spectrometry (FAAS) as described by Untea et al. (2012) after microwave digestion.

The oxidative stability products evaluated at slaughter time were determined following three parameters of the primary lipid degradation products: the peroxide value (PV) and the values of conjugated dienes and trienes (CD and CT), and two indices of the secondary peroxidation products: the TBARS value and the p-anisidine value (p anis). The lipid oxidation products were spectrophotometrically determined by using a V-530 Jasco (Japan Servo Co. Ltd., Japan) spectrophotometer, according to the methods described by Untea et al. (2020).

Data analysis

XLStat (Addinsoft, New York, USA) was used for performing statistical analysis. The significant difference between groups for various variables was tested by one-way ANOVA, followed by Tuckey post-hoc test. A probability level below 5% was considered a significant and statistical trend for 5–10%. For productive performance, the experimental unit was considered the pen. For meat quality evaluation, each slaughtered animal was considered an experimental unit.

In order to determine the relationships between bioactive compounds which determine the meat quality and lipid degradation products, a principal component analysis (PCA) was performed using XLStat (Addinsoft, New York, USA). Meat quality parameters were introduced as active variables and for increasing the quality of interpretation, the degradation parameters were introduced as supplementary variables. Based on data used as variables, principal components were generated and the first three of them were considered to represent 76.5% of the total variability (eigenvalues) and by using three factors we obtained a good quality projection of the initial multi-dimensional table.

Results

Data from Table 2 present the concentrations of compounds with biological value and antioxidant potential from broilers diets.

Compared to the control group, the final weight of broilers, weight gain, consumption and conversion rate were not statistically different. Regarding muscle and organ yields, no significant differences were noticed, except heart, where the experimental groups registered decreased values (Table 3).

Amino acids profile determination in thigh meat samples revealed a tendency of increasing essential amino acids in groups supplemented with chromium and ascorbic acid (Cr-AA) and chromium and zinc (Table 3).

Table 2. Bioactive compounds with antioxidant activity of experimental diets.

| Specification | C | Cr-AA | Cr-Zn | Cr-WS |
|---------------|---|-------|-------|-------|
| Vit E, mg/kg  | 40.13 | 39.81 | 39.36 | 45.17 |
| Lut&zeax, mg/kg | 8.97 | 9.15 | 9.46 | 10.15 |
| TP, mg/g GAE  | 1.71 | 1.81 | 1.83 | 2.51 |
| TAC, mM AAE   | 42.83 | 43.91 | 44.52 | 45.30 |

Abbreviations: Vit E: Vitamin E; lut&zeax: lutein & zeaxanthin; TP: total polyphenols; TAC: total antioxidant capacity.
Cr-AA (20 mg Cr/kg premix and 25 g ascorbic acid/kg premix); Cr-Zn (20 mg Cr/kg premix and 2.5 g Zn/kg premix); Cr-WS (20 mg Cr/kg premix and 1% creeping wood sorrel).

Table 3. Growth performance and carcass traits of broiler chickens fed chromium associated with vitamins and minerals (synthetic and natural source).

| Specification | C | Cr-AA | Cr-Zn | Cr-WS | SEM | p-value |
|---------------|---|-------|-------|-------|-----|---------|
| Growth parameters | | | | | | |
| BW, g (42 d) | 1987.9700 | 2014.1300* | 1935.4300 | 2015.4700** | 30.5890 | .7660 |
| ADWG, g/broiler/day | 46.2300 | 46.8500* | 44.9800 | 46.8800** | 0.7280 | .7660 |
| ADFI, g/broiler/day | 71.4200 | 71.1500* | 70.8300 | 72.0700** | 0.7280 | .7660 |
| FCR, g feed / g gain | 1.5400 | 1.5200* | 1.5700 | 1.5400** | 0.0100 | .7039 |
| Muscle yields | | | | | | |
| Breast yield, % | 20.8200 | 23.0400 | 21.9700 | 21.4400 | 0.3340 | .1052 |
| Thigh yield, % | 18.7700 | 19.1700 | 19.3400 | 19.5200 | 0.2770 | .8238 |
| Organ yields | | | | | | |
| Gizzard, % | 1.2500 | 1.3600 | 1.3700 | 1.4100 | 0.0310 | .2966 |
| Liver, % | 1.4300 | 1.4800 | 1.4400 | 1.4100 | 0.0240 | .8196 |
| Heart, % | 0.4500* | 0.3700* | 0.3700* | 0.3700* | 0.0130 | 0.0459 |
| Spleen, % | 0.0700 | 0.0600 | 0.0700 | 0.0800 | 0.0040 | .3383 |
| Bile, % | 0.0900 | 0.0800 | 0.0900 | 0.1000 | 0.0070 | .9451 |

Means within a row with no common superscript differ (p < .05). Abbreviations: BW: body weight; ADWG: average daily weight gain; ADFI: average daily feed intake; FCR: feed conversion ratio.
Cr-AA (20 mg Cr/kg premix and 25 g ascorbic acid/kg premix); Cr-Zn (20 mg Cr/kg premix and 2.5 g Zn/kg premix); Cr-WS (20 mg Cr/kg premix and 1% creeping wood sorrel).
*Data reported previously by Saracila, Panait, Tabuc, Soica, Untea, Varzaru, et al. (2020); **Data reported previously by Saracila, Panait, Tabuc, Soica, Untea, Ayasan, et al. (2020).
(Cr-Zn). Methionine concentrations were increased (p < .0001) in all experimental groups compared to the C group, and the highest value was registered for the Cr-AA group, with 41.8% more than the C group value. The second sulphur-containing amino acid, cysteine, was positively influenced (p < .05) for Cr-AA and Cr-WS groups. No significant differences were noticed for lysine, but threonine concentrations were increased (p = .0071) for all experimental groups (Table 4).

Among the compounds with biological value and antioxidant potential determined in the thigh meat samples (Table 5), the highest increase (p < .05) of vitamin E, lutein and zeaxanthin concentration was in the group that included chromium and zinc (Cr-Zn). A significant increase (p = .0004) was obtained for iron content in a group with chromium and creeping wood sorrel (Cr-WS), followed by the Cr-AA group, compared to the C group. At the same time, the Cr-WS group revealed a rich concentration (p = .0080) of polyphenols and higher antioxidant capacity, even if the latter was not statistically assured (p > .05), compared to the C group.

The results obtained for the oxidative stability products of thigh meat samples are presented in Table 6. As can be seen, although there was an improvement in all primary oxidation parameters, the peroxide value from the Cr-WS group was significantly lower (p < .05) compared to the control group. The same trend was maintained for the secondary oxidation parameters, the Cr-WS group highlighting this time also significantly reduced values for p-anisidine (p < .05) and TBARS concentration (p < .05) compared to the C group.

Principal component analysis (PCA) was conducted in order to explore the relationship between bioactive nutrients and lipid peroxidation parameters from meat. In Table 7 are presented the results of eigenvalues. The correlation between the original set of data and each of the eight principal components.

**Table 4. Amino acids profile in thigh meat samples, %.**

| Specification | C | Cr-AA | Cr-Zn | Cr-WS | SEM | p-value |
|---------------|---|-------|-------|-------|-----|---------|
| Aspartic acid | 4.6440 | 4.6350 | 4.7040 | 4.5540 | 0.1240 | .8618 |
| Glutamic acid | 12.6540 | 12.9040 | 12.9940 | 12.7270 | 0.3152 | .8625 |
| Serine | 4.0640 | 4.2570 | 3.7960 | 4.0530 | 0.1151 | .0918 |
| Glycine | 3.4290 | 3.0410 | 2.9260 | 2.6340 | .0134 | .0096 |
| Threonine | 3.1210 | 3.7680 | 3.8090 | 3.6200 | .1234 | .0071 |
| Arginine | 5.7150 | 5.8970 | 5.9020 | 5.8330 | 0.1290 | .5971 |
| Alanine | 4.1700 | 4.4300 | 4.3950 | 4.0740 | 0.1004 | .0508 |
| Tyrosine | 1.9320 | 2.1320 | 2.3200 | 1.8600 | 0.0816 | .0072 |
| Valine | 1.8010 | 1.8760 | 1.7830 | 1.7630 | 0.1314 | .9965 |
| Phenyalanine | 2.1870 | 2.4730 | 2.6040 | 2.3660 | 0.1138 | .3851 |
| Isoleucine | 3.2360 | 3.5000 | 3.5680 | 3.3520 | 0.1419 | .3851 |
| Leucine | 5.0490 | 5.0640 | 5.0540 | 5.0220 | 0.0508 | .9965 |
| Phenylalanine | 6.7350 | 6.4070 | 6.4590 | 6.5140 | 0.1098 | .2157 |
| Lysine | 0.4630 | 0.5230 | 0.4780 | 0.5390 | 0.0110 | .0387 |
| Methionine | 1.2490 | 1.7710 | 1.5790 | 1.6380 | 0.0510 | <.0001 |
| E aa | 31.4880 | 33.4720 | 33.5380 | 32.5080 | 0.2720 | .0888 |
| NE aa | 28.8980 | 29.2670 | 28.8140 | 28.0420 | 0.3160 | .4351 |
| Total aa | 60.3870 | 62.7930 | 62.3730 | 60.5500 | 0.2050 | .0060 |

*Means within a row with no common superscript differ (p < .05).

**Table 5. Concentrations of compounds with biological value and antioxidant potential, determined in thigh meat samples.**

| Specification | C | Cr-AA | Cr-Zn | Cr-WS | SEM | p-value |
|---------------|---|-------|-------|-------|-----|---------|
| Vitamin E, mg/kg | 35.4920 | 48.5210 | 39.0490 | 44.7400 | 6.1200 | .0221 |
| Lutein & Zeaxanthin, mg/kg | 0.6604 | 0.5969 | 1.3647 | 0.8792 | 0.0065 | <.0001 |
| Fe, mg/kg | 31920 | 30.4830 | 28.8230 | 31.7660 | 0.4921 | .0004 |
| Zn, mg/kg | 62.7310 | 61.9060 | 62.2630 | 63.7080 | 0.5095 | .1219 |
| TP, mg/g GAE | 0.8430 | 0.7879 | 0.7992 | 0.8785 | 0.0165 | .0800 |
| TAC, mm AAE | 15.9730 | 16.3690 | 16.0450 | 17.3590 | 0.5041 | .1055 |
| *Means within a row with no common superscript differ (p < .05).

**Table 6. Oxidative stability products evaluated at slaughter time.**

| Products | C | Cr-AA | Cr-Zn | Cr-WS | SEM | p-value |
|----------|---|-------|-------|-------|-----|---------|
| PV, mg/kg | 0.2770 | 0.2436 | 0.2391 | 0.1839 | 0.0170 | .163 |
| CD, µmol/g | 7.3281 | 6.3134 | 6.0040 | 6.1848 | 1.0857 | .8142 |
| CT, µmol/g | 2.8385 | 2.3274 | 2.2733 | 2.1096 | 0.4841 | .6993 |
| Secondary oxidation products | 17.2200 | 12.5870 | 13.6470 | 13.7200 | 1.1144 | .0069 |
| TARS, mg/kg | 0.1460 | 0.1129 | 0.1193 | 0.1103 | 0.0080 | .183 |
| *Means within a row with no common superscript differ (p < .05).

**Table 7. Results from Principal component analysis (PCA) for the eight principal components.**

| Eigen value | Variability, % | Cumulative, % |
|-------------|----------------|---------------|
| F1 | 2.8775 | 35.9685 | 35.9685 |
| F2 | 1.7728 | 21.5347 | 57.5032 |
| F3 | 1.5209 | 21.5347 | 35.9685 |
| F4 | 0.5661 | 7.0578 | 83.5907 |
| F5 | 0.5345 | 6.9310 | 90.5217 |
| F6 | 0.4335 | 5.4186 | 95.9404 |
| F7 | 0.1931 | 2.4136 | 98.3540 |
| F8 | 0.1317 | 1.6460 | 100.0000 |

*Principal component analysis (PCA) was conducted in order to explore the relationship between bioactive nutrients and lipid peroxidation parameters from meat. In Table 7 are presented the results of eigenvalues. The correlation between the original set of data and each of the eight principal components. The correlation circle (Figure 1) shows a projection of the considered variables in the factors space. The factors are situated far from the centre so the results obtained can be interpreted and they are dispersed in all four quadrants.
Table 8. Correlations between variables and factors.

|       | F1      | F2      | F3      |
|-------|---------|---------|---------|
| Ea a  | 0.7417  | 0.4536  | 0.2236  |
| NE aa | 0.4108  | -0.0082 | 0.7903  |
| vit E | 0.8169  | -0.0294 | -0.4062 |
| Lut&zeax | 0.7008 | -0.1570 | -0.5594 |
| TP    | -0.6647 | -0.0204 | -0.4872 |
| TAC   | 0.4173  | 0.7489  | -0.1329 |
| Fe    | -0.2732 | 0.7871  | -0.2731 |
| Zn    | -0.5563 | 0.5574  | 0.1972  |
| PV    | -0.0517 | -0.6994 | 0.2132  |
| CD    | -0.5562 | -0.3803 | 0.1788  |
| CT    | -0.4589 | -0.4745 | 0.2626  |
| p anis | 0.0237 | -0.7270 | 0.4868  |
| TBARS | -0.1546 | -0.7553 | 0.2677  |

E a a: Essential amino acids; NE a a: Non-essential amino acids; Vit E: Vitamin E; Lut&zeax: Lutein & zeaxanthin; TP: total polyphenols; TAC: total antioxidant capacity; PV: Peroxide Value; CD: Conjugated Dienes; CT: Conjugated Trienes; p anis: P-anisidine Value.

Figure 1. Correlation circle from principal component analysis.

Discussion

Data from the Table 2 shows increased concentrations of bioactive compounds with antioxidant potential in the Cr-WS diet. The results can be considered in the expected range, taking into account the valuable chemical composition of creeping wood sorrel (Saracila et al. 2020).

Chromium is a trace element involved in carbohydrates, lipids, protein and nucleic acid metabolism and its growth-promoting effect was reported previously (Ali et al. 2018). Improved productive parameters in stress conditions under chromium or combinations of chromium with other antioxidants were published in terms of weight gain or feed efficiency (Perai et al. 2014; Ali et al. 2018). In a study on broilers reared in normal environmental conditions, Subhani et al. (2018) published feed consumption values significantly lower than broilers fed with a conventional diet. Unlike the results reported, no significant differences were noticed between productive parameters, in the present study. The different effects recorded in our study can be attributed to the chromium source, level of supplements inclusion in the diets or reared conditions.

From the amino acids profile determined, the most important are the essentials, those amino acids which cannot be synthesised in the animal organism and must be supplied by the diets. Quantity and quality of ingested protein are determining factors in achieving the amino acids requirements in humans. Animal origin food is a simple and efficient way to prevent deficiencies in the elderly and children (Dasgupta et al. 2005; Grillenberger et al. 2003). The scientific literature is lack regarding the effect of dietary chromium on the amino acids profile of animal origin products. Chromium picolinate supplements had positive effects on the lipid and protein metabolism of pigs, improving pork quality from amino acids point of view (Untea et al. 2017). Toghyani et al. (2012) studied the effects of organic or inorganic chromium supplements on the meat quality of broilers raised in heat stress conditions. They observed a significant increase in protein concentrations in breast samples. A similar study (Haq et al. 2018) showed that chromium yeast with or without antioxidants association increased the protein content and decreased the fat concentrations. The authors showed that the combined effect of chromium and vitamin C potentiates the protein deposition in broilers’ breasts. The same observation was noticed in the present study regarding the methionine and cysteine concentrations (essential amino acid for animals and humans) which were increased both for experimental groups supplemented with chromium and vitamin C (synthetic source (Cr-AA) or natural complex mixture of bioactive compounds (Cr-WS)). Threonine is the third limiting amino acid in poultry nutrition, after lysine and methionine (Qaisrani et al. 2018). At the same time, threonine is also an essential amino acid for humans, being involved in the physiological process, including absorption and digestion of nutrients or immune function (Lee and Kim 2019). Lien et al. (2001) suggest that Cr supplementation increases the activity of insulin, which stimulates amino acid transport and protein synthesis in muscle cells.
Chromium is known in scientific literature to have great potential in alleviating the heat stress effects on birds (Sahin et al. 2017; Huang et al. 2016; Orhan et al. 2019). Chromium and vitamin C are considered synergistic (Watts 1989) elements with antioxidant potential and their association might have a positive effect on the oxidative status of chicken. In the particular case of thermal stress conditions, chromium and zinc requirements are increased, due to chromium mobilisation from tissues and zinc accumulation in the liver which can lead to a marginal deficiency of chromium and zinc (Onderci et al. 2003). Due to the antioxidant potential of those two metals, the pancreatic tissue is protected against oxidative stress and a proper digestive enzymes secretion can produce a higher nutrient digestibility (Preuss et al. 1997).

To study the effect of supplements on oxidative stability, thigh meat samples were subjected to analytical determinations of lipid degradation products. In the oxidation process of fats, in the incipient phase, the formation of hydroperoxides exceeds their decomposition rate, a process reversed in the following phases of oxidation. Monitoring hydroperoxide concentrations depending on the time can show whether lipid degradation is increasing or not. This information can be used as a criterion for the acceptability of a food product (Shahidi and Zhong 2005). Primary oxidation products values indicate a delayed peroxidation process under nutritional supplements influence. The most pronounced effect on oxidative stability of meat samples was registered by the association of chromium with wooden sorrel. The plants are complex mixtures of bioactive antioxidant compounds and many times, their synergistic activity exceeds the activity of the individual compounds (Vertuani et al. 2004). In the case of secondary products, in the final stage of peroxidation, the values recorded for experimental groups were decreased compared to control, but significant differences were calculated only for groups supplemented with chromium and wooden sorrel. The P-anisidine value indicates the aldehydes formed as secondary products in the lipid peroxidation process (Tompkins and Perkins 1999). Deterioration of animal origin foods is principally caused by oxidising of polyunsaturated fatty acids and forms hydroperoxides, which are odourless and tasteless but in the final stage of peroxidation, hydroperoxides are decomposed in specific off-odour and off-flavour compounds mainly aldehydes (Papastergiadis et al. 2012). TBARS value is the most used indicator of oxidative stress, measuring the concentrations of malondialdehyde (the main biomarker of lipid peroxidation). The spectrophotometric measurement of the pink-coloured reaction product of MDA with 2-thiobarbituric acid (TBA) is the most commonly used method for lipid peroxidation quantification. Nowadays, the lack of specificity of MDA spectrophotometric determination is the subject of debates being accepted that other substances present in food matrices can form a pink colour complex with TBA, the result obtained being overestimated (Rakotondramavo et al. 2019).

PCA is a multivariate data analysis tool frequently used in order to reduce the number of interrelated variables, but keeping most of the variation from the original set of data (Peyvasteh et al. 2020). The results of Table 6 show that 76.5% of the total variation is explained by the first three principal components. First PCA is strongly correlated with three original variables. It increases with essential amino acids, vitamin E and lutein & zeaxanthin. It means that these parameters vary together, if one of them increases, the other ones tend to increase too. The second PCA increases with antioxidant capacity and minerals and the third one are related to non-essential amino acids. According to correlation circle and squared cosines, the horizontal axis is linked with antioxidant capacity, minerals and lipid peroxidation parameters while the vertical axis with essential amino acids, total polyphenols, vitamin E and lutein & zeaxanthin. On the horizontal axis, antioxidant capacity and minerals are negatively correlated with lipid peroxidation parameters (opposite side of the centre), significant negative correlations \( \rho \times 0.05 \) being recorded between antioxidant capacity vs PV, CD, CT, TBARS and Fe vs. PV, p anis, TBARS. Total polyphenols are negatively correlated with lipophilic compounds (vitamin E and lutein & zeaxanthin). Strong correlations \( \rho = 0.7550 \) were noticed between lipophilic antioxidants and also lipid peroxidation parameters were correlated at a significant level \( \rho < .05 \). Based on the overall results, PCA proved to be a useful statistical tool for identifying the most active variables and highlighting the relationships among the studied parameters.

**Conclusion**

In the present study, no significant differences were noticed between productive parameters. Compared to the control group, sulphur-containing amino acids (methionine and cysteine) concentrations determined in the thigh meat samples increased for experimental groups. Among the compounds with biological value and antioxidant potential, the liposoluble compounds (vitamin E, lutein & zeaxanthin) concentration
registered the highest increase for the chromium and zinc supplemented group. The peroxidation process was delayed under nutritional supplements influence, but chromium and the wooden sorrel combination produced the most pronounced effect.

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Data availability statement
The data that support the findings of this study are openly available in [repository name e.g., “figshare”] at http://doi.org/10.1080/1828051X.2021.1978335.

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