The Performance and Haematological Indices of Broiler Chickens Fed Chromium Picolinate, and Vitamin C Supplemented Diets

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors FBA, OAA and CAC designed the study. Authors OAA and ODO performed the statistical analysis. Authors FBA, OAA and ODO wrote the protocol. Authors FBA and ODO wrote the first draft of the manuscript. All authors managed the analyses of the study. Authors FBA, OAA, ODO and SOA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigates the out-turn of ChromiumPicolinate (CrPic) and vitamin C dietary supplementation on the performance characteristics and haematological indices of broiler chickens.

Study Design: The completely randomised design was used for this study.

Methodology: Six hundred and forty day-old Cobb 500 broiler chickens were randomly assigned to eight dietary treatments (10 birds/replicate). A basal diet was fractionated into eight equal parts and labelled diets 1 to 8. Diets 1 to 4 were supplemented with 0, 0.4, 0.8 and 1.2 mg/kg CrPic, respectively. The diets 5 to 8 were supplemented with 200 mg/kg vitamin C; 0.4 mg CrPic+200 mg vitamin C; 0.8 mg CrPic+200 mg vitamin C and 1.2 mg CrPic+200 mg Vitamin C, respectively.

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Results: The final body weight (FBW) and relative growth rate (RGR) of the birds fed diets 2, 3, 5, 6, 7 and 8 were significantly (P<0.05) higher to those fed the control diet and diet 4. The CrPic supplementation at 0.4 and 0.8 mg/kg levels improved (P<0.05) the FBW and RGR of the birds, compared to the control. The vitamin c supplementation (200mg/kg) improved (P<0.05) the FBW and RGR of the birds. The haematological indices were stable (P>0.05) across the diets. However, the granulocytes count increased by the CrPic supplementations, compared to the control while lymphocytes count increased (P<0.05) by vitamin C supplementation.

Conclusion: The growth of the broiler chicken are enhanced by 0.4 to 0.8 mg/kg CrPic, 200 mg/kg vitamin C and combination of CrPic and vitamin C dietary supplementations without affecting main haematological indices of the birds.

Keywords: Avian; blood; chromium; growth; supplements; vitamin C.

1. INTRODUCTION

The tropical regions with high ambient temperature and humidity were reported to be more susceptible to high heat stress, compared to the polar or temperate regions [1]. Besides, the meteorological factors such as temperature and humidity are among the major factors exerting significant influence on the production performance and haematological parameters of the domestic birds [2]. Particularly, in Nigeria, the provision of protection against heat which is usually in temporary light shades and radiation shield is usually inadequate [3].

The adverse effects of heat stress cause depressed growth rate and feed intake in broiler chickens [4]. However, the bodyweight of the broiler chickens raised under the heat-stressed environment is improved with dietary supplements and antioxidants [5,1].

Presently, Chromium (Cr) is not yet generally considered as essential microelements for poultry, but it is thought that this trace element may play a beneficial nutritional and physiological role [6]. Cr plays an essential role in the activation of certain enzymes and in stabilising the protein and nucleic acid [6]. Khan et al. [7] had earlier reported the improved weight gain, and reduce the stress reactions in birds fed diets supplemented with Chromium. Despite these reported potentials of dietary Cr in poultry production, its’ dietary inclusion recommended levels in poultry has not been given by the National Research Council. Besides the beneficial effects of Cr, there is also a need for studies on the potentially toxic effects of wrong or inappropriate dosage in poultry. In addition, since Cr, when combined with other antioxidants (e.g. vitamin C) was reported to improve the stress influenced performance characteristics in broiler chickens [8,9]. Vitamin C is also an antioxidant that improves the stress suppressible performance characteristics by reducing the plasma corticosterone level and adrenocorticotropic hormone [8,10]. Vitamin C supplementation reduces the respiratory quotient in heat-stressed broiler chickens by supporting or enhancing increased the fatty acid oxidation over the increase in the protein-derived gluconeogenesis [10].

Therefore, this study is aimed at investigating the out-turn of Chromium Picolinate (CrPic) and vitamin C dietary supplementation on the performance characteristics and haematological indices of broiler chickens.

2. MATERIALS AND METHODS

This feeding trial was carried out at the Avian Unit of The Federal University of Technology, Akure (FUTA) Teaching and Research Farm (TRF), during the peak of the dry season (i.e. between January and February 2020). The experimental pen’s daily temperature-humidity index (THI) was 34.08°C±1.36. The THI was calculated (Tao and Xin, 2003) using the formula:

THI= 0.85*T_{db}+0.15*T_{wb}

Where T_{db} = dry bulb temperature (°C); T_{wb} = wet bulb temperature (°C).

2.1 Chromium Picolinate and Vitamin C Source

AK Scientific, Union City, CA, USA, produced the Chromium picolinate powder (purity level = 98%). The L-ascorbic acid powder (purity level = 100% pure (USP/FCC grade) was produced by the Avondale Laboratories (Supplies and Services) Limited, Banbury, England.

2.2 Experimental Diets and Animals

A basal diet each was prepared for the starter (age 1-3 weeks) and the finisher (age 4-6 weeks) phases (Table 1) and analysed for proximate
The basal diets were sundered equally into eight parts and labelled diets 1 to 8 and supplemented as follows:

- Diet 1: Control
- Diet 2: 0.4 mg CrPic supplementation
- Diet 3: 0.8 mg CrPic supplementation
- Diet 4: 1.2 mg CrPic supplementation
- Diet 5: 200 mg Vitamin C supplementation
- Diet 6: 0.4 mg CrPic + 200 mg Vitamin C supplementation
- Diet 7: 0.8 mg CrPic + 200 mg Vitamin C supplementation
- Diet 8: 1.2 mg CrPic + 200 mg Vitamin C supplementation

Six hundred and forty day-old Cobb 500 broiler chickens were allocated randomly into the eight experimental diets/treatment groups (80 birds/treatment; 10 birds/treatment). The birds were raised in independent pens (200 x 100 cm) having concrete floor covered with wood shavings as litter. The temperature of the experimental house was maintained at 32°C±2 for the initial first week and reduced by 2°C after each following weeks till the house temperature was 24°C±2. The lightning (23 hours/day) was provided, and unrestricted access to feed and water were given to the birds throughout the experimental period.

2.3 Growth Performance
The body weights of the broiler chickens were measured on a weekly interval. The body weight gain was calculated by subtracting the birds’ initial body weight from their final body weight and the initial body weight. The feed intake was also estimated by subtracting the quantity of feed given from the feed leftover. The relative growth rate (RGR) was estimated using the following formula [12]:

$$RGR = \left( \frac{w2 - w1}{(w1 + w2)/2} \right) \times 100.$$ 

$W_1$: Body weight at the onset of the experiment; $W_2$: Bodyweight the end of the experiment.

The birds’ viability percentage ($V\%$) were recorded daily and calculated as follows:

$$V\% = \left( \frac{\text{Total number of live animals at the end}}{\text{Total number of animals at the start}} \right) \times 100.$$

2.4 Blood Sample Collection and Analysis
On day 42 of the experiment, three birds per replicate were randomly chosen, labelled, and phlebotomised with syringe and needle via the wing vein. About 4 ml of blood was pass out into Ethylenediaminetetraacetic acid bottles for haematological indices examination. The haematological studies were performed within 120 minutes post bleeding [13]; for red blood cells (RBC), packed cell volume (PVC), haemoglobin concentration (Hbc), white blood cells (WBC), granulocytes (GRA), lymphocyte (LYM) and monocytes (MON).

| Ingredients (%) | Starter feed | Finisher diet |
|-----------------|--------------|---------------|
| Maize           | 52.35        | 59.35         |
| Rice bran       | 0.00         | 6.00          |
| Maize bran      | 7.00         | 0.00          |
| Soybean meal    | 30.00        | 24.00         |
| Soy oil         | 3.00         | 3.00          |
| Fish meal       | 3.00         | 3.00          |
| Limestone       | 0.50         | 0.50          |
| Bone meal       | 3.00         | 3.00          |
| Salt            | 0.30         | 0.30          |
| Premix          | 3.00         | 3.00          |
| Methionine      | 0.30         | 0.30          |
| Lysine          | 0.25         | 0.25          |

| Nutrient composition (%) | |
|--------------------------|--|
| *Crude protein           | 22.18         | 20.03 |
| Metabolizable energy (Kcal/kg) | 3018.89  | 3108.10 |
| Methionine               | 0.68          | 0.66  |
| Lysine                    | 1.36          | 1.24  |
| Available phosphorus     | 0.45          | 0.33  |
| Calcium                   | 1.01          | 0.99  |
2.5 Data Analysis

All data were subjected to analysis of variance from General Linear Model stratagem for complete randomised design with 4 CrPic levels x 2 Vitamin C levels factorial setting of treatments. The data were checked for CrPic, Vitamin C and interaction of CrPic with Vitamin C. When the treatment out-turn was significant (P<0.05), means were differentiated using Duncan's multiple range test using SPSS.

3. RESULTS AND DISCUSSION

The effects of Chromium picolinate and vitamin C dietary supplementation on the performance characteristics of broiler chickens are shown in Table 2. The final weight and relative growth rate of the broiler chickens fed diets 2, 3, 5, 6, 7 and 8 were significantly (P<0.05) higher than those fed the diets 1 (control) and 4. The improved final weight and relative growth rate recorded in the broiler chickens fed diet 2 (0.4 mg/kg CrPic), diet 3 (0.8 mg/kg CrPic), diet 5 (200 mg/kg vitamin C), diet 6 (0.4 mg/kg CrPic+200 mg/kg vitamin C) diet 7 (0.8 mg/kg CrPic+200 mg/kg vitamin C) and diet 8 (1.2 mg/kg CrPic+200 mg/kg vitamin C), compared to those fed the control diet and diet 4 (1.2 mg/kg CrPic) suggests, CrPic supplementation at 0.4 to 0.8 mg/kg and 200 mg/kg vitamin C supplementation have growth performance-enhancing effects on the broiler chickens.Quite a few studies show that chromium picolinate dietary supplementation has a promoting effect on the growth performance of chickens [8,14,6]. This result further unfolds another beneficial biological activity of Chromium used as a dietary supplement [7]. CrPic improves growth by increasing insulin sensitivity, initiation of microRNA translation and consequently, the improvement in the stimulation of muscle protein synthesis [15]. However, the depressed growth performance recorded in the broiler chickens fed relatively high diet 4 (1.2mg/kg CrPic) suggests the possible toxic and negative effects of dietary supplementation of CrPic at high dosage [9]. However, the unimpaired growth performance recorded in the broiler chickens fed the 1.2 mg/kg CrPic+200mg/kg vitamin C supplemented diet suggests the suitability of vitamin C when used as a dietary supplement in removing the negative and toxic effects of CrPic at 1.2 mg/kg level [8]. This is further explained by the significant (P<0.05) interaction of CrPic and vitamin C supplementation for the final body weight and relative growth rate in the broiler chickens. The vitamin C supplementation (200mg/kg) improved the final body weight and relative growth rate, compared to the control. It was reported that vitamin C dietary supplementation in poultry improved the growth performance characteristics that are sensitive to oxidative stress [16], by reducing the corticosterone and adrenocorticotropic hormone concentration in the plasma [10]. The feed intake of the broiler chickens fed diet 4 (1.2 mg/kg CrPic reduced (P<0.05), compared to those fed the rest diets. The 1.2 mg/kg CrPic supplementation reduced (P<0.05) the feed intake of the birds; while vitamin C supplementation (200 mg/kg) improved their feed intake. The depressed feed intake recorded in the broiler chickens fed diet 4 also depicts the negative, toxic and appetite suppressive effects of CrPic at 1.2mg/kg supplementation [6]. The interaction of CrPic and vitamin C supplementation was significant (P<0.05) for the feed intake. The viability of the birds was not affected (P>0.05) by the dietary treatments.

Table 3 shows that the haematological indices of the birds were not (P>0.05) affected by CrPic and vitamin C supplementation, except for the granulocytes and monocytes counts. The granulocyte count at 0.8 mg/kg CrPic supplementation was similar (P>0.05) to 0.4 mg/kg and 1.2 mg/kg but higher (P<0.05) than the control. The lymphocytes count improves by 200 mg/kg vitamin C supplementation. The stability of most blood indices across the various dietary treatments in this study shows the supports of the CrPic and vitamin C at the levels used in this study for normal blood formation. Granulocytes (neutrophils, eosinophils, and basophils) are phagocytes and possess granules of enzymes which digest the invading microbes. The variation in the granulocytes counts, as being recorded in the broiler chickens fed varying levels of CrPic supplementation did not follow a specific pattern, while the observed increased in monocytes count due to vitamin C dietary supplementation suggest the immunomodulatory effect of the supplement in the broiler chickens [17]. It was earlier hypothesized that vitamin C modulates the activities and number of cell sticking molecules in monocytes [18].
Table 2. Effects of Chromium Picolinate, and vitamin C dietary supplementation on the performance characteristics of broiler chickens

| Diets | CrPic (mg/kg) | Vitamin C (mg/kg) | Initial weight (kg/bird) | Final body weight (kg/bird) | Relative growth rate | Feed intake (kg/bird) | Viability |
|-------|---------------|-------------------|--------------------------|----------------------------|----------------------|-----------------------|-----------|
| 1     | 0.00          | 0.00              | 0.03                     | 1.84<sup>a</sup>           | 192.68<sup>a</sup>  | 3.47<sup>a</sup>      | 94.44     |
| 2     | 0.40          | 0.00              | 0.03                     | 2.16<sup>a</sup>           | 194.00<sup>a</sup>  | 3.69<sup>a</sup>      | 100.00    |
| 3     | 0.80          | 0.00              | 0.03                     | 2.12<sup>a</sup>           | 193.76<sup>a</sup>  | 3.65<sup>a</sup>      | 100.00    |
| 4     | 1.20          | 0.00              | 0.03                     | 0.83<sup>c</sup>           | 184.44<sup>c</sup>  | 1.37<sup>c</sup>      | 100.00    |
| 5     | 0.00          | 200.00            | 0.03                     | 2.06<sup>a</sup>           | 193.79<sup>a</sup>  | 3.62<sup>a</sup>      | 100.00    |
| 6     | 0.40          | 200.00            | 0.03                     | 2.09<sup>a</sup>           | 193.72<sup>a</sup>  | 3.63<sup>a</sup>      | 100.00    |
| 7     | 0.80          | 200.00            | 0.03                     | 2.19<sup>a</sup>           | 194.12<sup>a</sup>  | 3.78<sup>a</sup>      | 100.00    |
| 8     | 1.20          | 200.00            | 0.03                     | 2.08<sup>a</sup>           | 193.79<sup>a</sup>  | 3.70<sup>a</sup>      | 100.00    |
| SEM.  | 0.00          | 0.09              | 0.64                     | 0.16                       | 0.69                 |                      |
| P-value | 0.55         | 0.00              | 0.00                     | 0.00                       | 0.46                 |                      |

| Diets | CrPic (mg/kg) | Vitamin C (mg/kg) | Initial weight (kg/bird) | Final body weight (kg/bird) | Relative growth rate | Feed intake (kg/bird) | Viability |
|-------|---------------|-------------------|--------------------------|----------------------------|----------------------|-----------------------|-----------|
| 0.0   | 0.03          | 1.95<sup>a</sup>  | 193.23<sup>a</sup>       | 3.54<sup>a</sup>           | 97.22                |                      |
| 0.4   | 0.03          | 2.13<sup>a</sup>  | 193.86<sup>a</sup>       | 3.66<sup>a</sup>           | 100.00               |                      |
| 0.8   | 0.03          | 2.16<sup>a</sup>  | 193.94<sup>a</sup>       | 3.71<sup>a</sup>           | 100.00               |                      |
| 1.2   | 0.03          | 1.46<sup>c</sup>  | 189.11<sup>c</sup>       | 2.54<sup>c</sup>           | 100.00               |                      |
| SEM.  | 0.00          | 0.04              | 0.14                     | 0.07                       | 1.38                 |                      |
| P-value | 0.58         | 0.00              | 0.00                     | 0.00                       | 0.41                 |                      |

| CrPic x Vitamin C | SEM | P-value | SEM | P-value |
|-------------------|-----|---------|-----|---------|
|                   | 0.02| 0.00    | 0.21| 0.11    |
|                   | 0.58| 0.00    | 0.00| 0.42    |

Means with a different superscript in the same column are significantly (P<0.05) different; CrPic: Chromium picolinate.
### Table 3. Effects of Chromium Picolinate, and vitamin C dietary supplementation on the haematological indices of broiler chickens

| Diets | Cr Pic (mg/kg) | Vitamin C (mg/kg) | PCV (%) | RBC (x10^6/l) | HBC (g/dl) | MCHC (g/dl) | MCV (fl) | MCH (pg/cell) | WBC (x10^9/l) | GRA (x10^9/l) | LYM (x10^9/l) | MON (x10^9/l) |
|-------|----------------|------------------|---------|---------------|-----------|-------------|--------|-------------|--------------|-------------|-------------|-------------|
| 1     | 0.00           | 0.00             | 34.00   | 2.70          | 11.33     | 33.20       | 126.40 | 42.13       | 2.08         | 0.69        | 1.34        | 0.02        |
| 2     | 0.40           | 0.00             | 32.33   | 2.83          | 10.77     | 32.88       | 122.47 | 40.82       | 3.36         | 1.25        | 2.07        | 0.04        |
| 3     | 0.80           | 0.00             | 32.66   | 3.43          | 10.89     | 33.21       | 98.65  | 32.88       | 4.93         | 2.26        | 2.63        | 0.08        |
| 4     | 1.20           | 0.00             | 34.00   | 3.33          | 11.33     | 33.40       | 102.64 | 34.21       | 4.40         | 1.48        | 2.82        | 0.09        |
| 5     | 0.00           | 200.00           | 33.67   | 3.03          | 11.22     | 32.91       | 114.70 | 38.23       | 3.53         | 0.77        | 2.76        | 0.00        |
| 6     | 0.40           | 200.00           | 33.66   | 2.80          | 10.73     | 33.16       | 118.66 | 39.26       | 5.06         | 2.06        | 3.02        | 0.07        |
| 7     | 0.80           | 200.00           | 33.00   | 2.80          | 11.00     | 33.37       | 119.29 | 39.76       | 5.20         | 2.07        | 3.05        | 0.07        |
| 8     | 1.20           | 200.00           | 31.33   | 3.13          | 10.44     | 33.14       | 106.71 | 35.57       | 5.63         | 1.34        | 4.23        | 0.09        |
| SEM.  | 0.11           | 0.40             | 0.13    | 0.14          | 4.76      | 1.58        | 0.34   | 0.17        | 0.23         | 0.01        |             |             |
| P-value | 0.75           | 0.77             | 0.71    | 0.99          | 0.85      | 0.86        | 0.12   | 0.13        | 0.10         | 0.55        |             |             |
| 0.0   | 33.83          | 2.86             | 11.27   | 33.35         | 120.55    | 40.18       | 2.80   | 0.73ab      | 2.05         | 0.12        |             |             |
| 0.4   | 33.00          | 2.81             | 10.75   | 33.02         | 120.57    | 40.04       | 4.21   | 1.66abc     | 2.54         | 0.05        |             |             |
| 0.8   | 32.83          | 3.11             | 10.94   | 33.29         | 108.97    | 36.32       | 5.06   | 2.16ab      | 2.84         | 0.07        |             |             |
| 1.2   | 32.83          | 3.23             | 10.88   | 33.27         | 104.67    | 34.89       | 5.01   | 1.41abc     | 3.53         | 0.07        |             |             |
| SEM.  | 0.86           | 0.25             | 0.27    | 0.33          | 10.42     | 3.46        | 0.61   | 0.30        | 0.40         | 0.02        |             |             |
| P-value | 0.78           | 0.62             | 0.60    | 0.91          | 0.62      | 0.63        | 0.06   | 0.03        | 0.11         | 0.19        |             |             |
| 0.00  | 33.25          | 3.07             | 11.08   | 33.17         | 112.14    | 37.51       | 3.69   | 1.42        | 2.22         | 0.05        |             |             |
| 200.00| 32.92          | 2.94             | 10.84   | 33.14         | 114.84    | 38.21       | 4.85   | 1.56        | 3.26         | 0.06        |             |             |
| SEM   | 0.61           | 0.18             | 0.19    | 0.23          | 7.37      | 2.44        | 0.42   | 0.21        | 1.61         | 0.01        |             |             |
| P-value | 0.70           | 0.60             | 0.41    | 0.94          | 0.82      | 0.84        | 0.07   | 0.65        | 0.01         | 0.95        |             |             |

Means with a different superscript in the same column are significantly (P<0.05) different; CrPic: Chromium picolinate; PCV: Packed cell volume; RBC: Red blood cell; HBC: Haemoglobin concentration; MCV: Mean cell volume; MCH: Mean cell haemoglobin; WBC: White blood cells; GRA: Granulocytes; LYM: Lymphocytes; MON: Monocytes; SEM: Standard error of the mean.
4. CONCLUSION

The 0.4-0.8 mg/kg CrPic dietary supplementations improved the final body weight and relative growth rate of the broiler chickens. Besides, the 200 mg vitamin C supplementation enhanced the final body weight, relative growth rate and the feed intake of the broiler chickens. The vitamin 200 mg vitamin C supplementation produced immune-modulatory effects on the lymphocyte counts of the broiler chickens.

ETHICAL APPROVAL

This work was approved by the Research and Ethics Committee of the Animal Production and Health Department, The Federal University of Technology, Akure, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhao Y, Ducharne A, Sultan B, Braconnot P, Vautard R. Estimating heat stress from climate-based indicators: present-day biases and future spreads in the CMIP5 global climate model ensemble. Environmental Research Letters. 2015;10(8):084–013

2. Ayo JO, Obidi JA, Rekwot PI. Effects of heat stress on the well-being, fertility and humidity of chickens in the Northern Guinea Savanah Zone of Nigeria: ISRN Veterinary Science. 2011;838606. DOI:10.5402/2011/838606.

3. Ayo JO, Oladele SB, Fayomi A. Effect of heat stress on livestock production: a review. Nigerian Veterinary Journal. 1996;1:58–68.

4. Sahin N, Sahin K, Küçük O. Effects of vitamin E and vitamin A supplementation on performance, thyroid status and serum concentrations of some metabolites and minerals in broilers reared under heat stress (32°C). Veterinarni Medicina. 2001;46(11-12):286–292.

5. Donkoh A. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. International Journal of Biometeorology. 1989;33(4):259–265.

6. Ognik K, Drazbo A, Stepniowska A, Kozlowski K, Listos P, Jankowski J. The effect of chromium nanoparticles and chromium picolinate in broiler chicken diet on the performance, redox status and tissues histology. Animal Feed Science and Technology. 2020;259:114326. DOI:10.1016/j.anifeedsci.2019.114326

7. Khan RU, Naz S, Dhama K, Saminathan M, Tiwari R, Jeon GJ, Laudadio V, Tufarelli V. Modes of action and beneficial application of Chromium in poultry nutrition, production and health: A review. International Journal of Pharmacology. 2014;10(7):357-367.

8. Sahin K, Sahin N and Kucuk O. 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. Nutrition Research. 2003;23:225-238.

9. Haq Z, Jain RK, Khan N, Dar MY, Ali S, Gupta M, Varun TK (2016). Recent advances in role of Chromium and its antioxidants combinations in poultry nutrition. A review. Veterinary World. 2016;9(12):1392-1399. DOI:10.14202/vetworld.2016.1392-1399.

10. Lin H, Jiao HC, Buyse J, Decuypere E. Strategies for preventing heat stress in poultry. World’s Poultry Science Journal. 2006;62(1):71-85. DOI:10.1079/ WPS200585

11. AOAC. Association of official analytical chemistry, official methods of analysis (16th Ed.). Washington, DC; 1995.

12. North MO. Commercial chicken production manual, 2nd edn. AVI. Publishing Company, Inc, Westpost Connecticut;1981.

13. Shastry GA. Veterinary clinical pathology, 2nd edn. CBS Publishers and Distributors, New Delhi, India; 1983.

14. Hajializadeh F, Ghahri H, Talebi A. Effects of supplemental chromium picolinate and chromium nanoparticles on performance and antibody titers of infectious bronchitis and avian influenza of broiler chickens under heat stress condition. Veterinary Research Forum. 2017;8:259-264.

15. O’Connor PM, Kimball SR, Suryawan A, Bush JA, Nguyen HV, Jefferson LS, Davis TA. Regulation of translation initiation by insulin and amino acids in skeletal muscle of neonatal pigs. American
Journal of Physiology, Endocrinology and Metabolism. 2003;285(1):40-53.

16. Lin H, Buyse J, Sheng QK, Xie, YM, Song JL. Effects of ascorbic acid supplementation on the immune function and laying performance of heatstressed laying hens. Journal of Feed, Agriculture and Environment. 2003;1:103-107.

17. Lording and Friend. Interpretation of laboratory results. Australian Veterinary Practitioner. 1991;21:188-193.

18. Preedy VR, Watson RR, Sherma Z. Dietary components and immune function (Nutrition and Health). Totowa NJ: Humana Press. 2010;36-52.