Comprehensive Diagnosis of Selenium and Iodine Deficiencies in Golden Manchurian Quails and Gray–Speckled Guinea Fowls Risen in the Astrakhan Region

Pavel Polkovnichenko1*, Vladimir Vorobiev2, Dmitry Vorobiev3, Vladimir Safonov3

1Department of Veterinary and Sanitary Expertise of Animal Husbandry and Crop Production, Astrakhan State University, Astrakhan, Russia; 2Department of Veterinary Medicine, Astrakhan State University, Astrakhan, Russia; 3Laboratory of Environment Biogeochemistry, Vernadsky Institute of Geochemistry and Analytical Chemistry, Moscow, Russia.

Abstract | This study presents the results of comprehensive diagnosis of combined selenium–iodine hypomicroelementosis in quails and guinea fowls adapting to new biogeochemical conditions. This study focused on golden Manchurian quails, Coturnix japonica (Temminck and Schlegel, 1849), and gray–speckled guinea fowls, Numida meleagris (Linnaeus, 1758), imported to the Astrakhan region in 2015 from the Krasnodar Krai, Russia. The major components of terrestrial ecosystem (soil, water, and plants) in the Astrakhan region were found to be low in selenium, iodine and cobalt. As a result, quails and guinea fowls received a diet with insufficient amounts of therein. All subjects were examined for microelements, physiological, hematological, and biochemical changes, and pituitary–thyroid hormones. In addition, the study examined antioxidant indicators and markers of free radical oxidation. The comprehensive examination revealed selenium deficiency and iodine deficiency in the body of examined quails and guinea fowls. The experimental birds were found to have elevated levels of blood corpuscles, glucose, diene conjugates, and malonic aldehyde. Levels of antioxidant vitamins (E, A, C) were found to be low. Based on the above, it can be concluded that golden Manchurian quails and gray–speckled guinea fowls raised in conditions of selenium deficiency and iodine deficiency can experience latent symptoms of hypomicroelementosis when fed a diet based on local plants. The consequence is a drop in egg productivity. Thus, farmers specialized at breeding quails and guinea fowls should considers feeds enriched with selenium and iodine when the local ecosystem lacks a sufficient amount of therein.

Keywords | Quails, Guinea fowls, Selenium, Iodine, Hypomicroelementosis.

INTRODUCTION

The primary goal of poultry farming is to provide high-quality dietary food to the population. Therefore, more and more attention is paid to quails and guinea fowls. These types of birds are known for laying nutrient–dense eggs that can be used in the prevention of various diseases. Quail meat is low in calories and can be consumed by people who follow a strict diet. The guinea fowl meat with its high nutrient content helps in maintaining the immunity. Because quail and guinea fowl are fast growing and highly productive birds, the number of farms breeding these types of birds continues to grow. Yet, the ever–increasing demand for eggs and meat from these birds is not fully met.
for quails and guinea fowls is bird feed. This may pose a challenge since birds with high metabolic rates have increased sensitivity to important trace element deficiencies in plant-based feeds. The consequences include a decline in immune function, oxidative stress, and latent forms of hypomicroelementosis, conditions caused due to the insufficient intake of essential trace elements. Normally, these health issues do not have pronounced clinical manifestations, and indicators such as body temperature, pulse, and respiratory rate are within the normal range. However, there are evidences that birds suffering from trace element deficiency experience decrease in productivity, encounter reproductive problems (Corrie, 1996; Fisinin, 2012; Tsurik and Bezborodov, 2015; Temiraev et al., 2016, Vorobiev et al., 2018), and have difficulty adapting to new conditions (Fisinin, 2012; Tsurik and Bezborodov, 2015; Vorobiev et al., 2018). Thus, it is important to diagnose the latent form of hypomicroelementosis in birds in time. However, the fact that this condition proceeds without clinical symptoms significantly complicates the diagnosis and necessitates the conduct of clinical biochemical study, including at the molecular-cellular level (Rodionova et al., 2010; Kostin, 2017).

The negative effects of stress can cause pathological changes in the body of birds. It was established that reactions such as free radical oxidation and antioxidant defense are indicative for that kind of process (Belyaev, 2011). The free radical oxidation involves radical cations of selenium, iodine, manganese, zinc, copper, cobalt, molybdenum, and iron-sulfur clusters (Rodionova et al., 2010, Vorobiev, 2013). When there is a shortage of selenium in the body, the activity of antioxidant enzymes decreases. This interferes with the neutralization of hydroperoxides and lipid peroxides and results in the development of oxidative stress (Weiss et al., 1990). Selenium affects the thyroid function and its deficiency can aggravate the functional and structural changes in the thyroid gland. Since selenium works in conjunction with iodine, the low selenium level can cause the iodine absorption to worsen, resulting in iodine deficiency (Weiss et al., 1990; Braun et al., 1991). Selenium supports the conversion of thyroid hormone thyroxine (T4) into its active analogue triiodothyronine (T3). The latter participates in the synthesis of thyroglobulin, which accelerates the conversion of carotene into vitamin A (Struev and Simakhov, 2006). Other consequences of selenium deficiency are reproductive failure, increased propensity to inflammatory diseases, and decrease in immune function. The iodine deficiency, on the other hand, affects the thyroid hormone production, resulting in metabolic abnormalities.

Overall, selenium deficiency and iodine deficiency are conducive to asymptomatic hypomicroelementosis and slower metabolism. This is the reason why it is impossible to reach the full genetic potential of poultry birds (Vorobiev, 2013; Vorobiev et al., 2018).

The molecular and cellular mechanisms underlying the pathogenesis of asymptomatic hypomicroelementosis in birds are poorly studied. There is insufficient evidence about the diagnosis of latent hypomicroelementosis in poultry birds, especially quails and guinea fowls. Studies only provide sketchy information about selenium and iodine deficiencies in chickens and some other domesticated birds (Kostin, 2017; Gorelik and Derkho, 2013; Kolesnik and Derkho, 2015; Popova et al., 2015).

In recent years, Russian farmers have been actively importing quails and guinea fowls into the Lower Volga region, where terrestrial ecosystems were found to have low levels of selenium and iodine (Samokhin, 2008; Vorobiev, 2013). The insufficient intake of selenium and iodine can negatively affect the adaptive capacity and productivity of birds. However, there are no comprehensive studies on hypomicroelementosis induced by deficiency of combined selenium and iodine in imported quails and guinea fowls. As a result, there are no evidence about many important health indicators for birds that were imported to the Astrakhan region, such as microelement profile, metabolism, hematological parameters, free radical oxidation, antioxidant activity, pituitary-thyroid function, trace element content of feed, and egg productivity. This study aims to close this gap. Following the above mentioned, the main hypothesis of this study implies that low level of selenium and iodine in the main components of terrestrial ecosystems of the Lower Volga region (soils, water, plants) and local plant forages predetermines the development of selenium-iodine deficiency in acclimatized agricultural birds, which negatively affects metabolism and many physiological and biochemical parameters of birds, reducing their productivity.

This study aimed to perform a comprehensive diagnostics of combined selenium-iodine hypomicroelementosis in quails and guinea fowls acclimatized in biogeochemical conditions of terrestrial ecosystems in the Astrakhan region.

MATERIALS AND METHODS

This study focused on golden Manchurian quails, Coturnix japonica (Temminck and Schlegel, 1849), and gray-speckled guinea fowls, Numida meleagris (Linnaeus, 1758), imported to the Astrakhan region in 2015 from the Krasnodar Krai, Russia. Between 2015 and 2017, samples from multiple sources were collected using sampling methods according to Kovalski and Maclyanaya (1982). For comparison, samples were taken from farms both in the Astra-
khan and Krasnodar regions. Overall, the study included 36 soil samples, 15 water samples, 31 samples collected from plants and plant-based feed to measure the trace element content, and 186 tissue samples from internal organs of quails and guinea fowls farmed in the Astrakhan region (experimental birds). The comparison tissue samples were taken from 10 quails and 10 guinea fowls raised in the Krasnodar Krai (control birds). Data from Vorobiev et al. (2018) was used to assess the selenium and iodine content in the ecosystem components of the control region (Krasnodar Krai). All animal experiments and diagnostic tests were carried out in accordance with the European Community Council Directives (86/609/EEC) and the Declaration of Helsinki.

A comprehensive diagnostic study of combined selenium and iodine deficiency was carried out in 2016 at the farm in the Kamyzyak district, Astrakhan region. The study used 20 four-month-old quails (10 females and 10 males, weighing 298 ± 7.1 g and 261 ± 8.3 g, respectively) and 20 nine-month-old guinea fowls (10 females and 10 males, weighing 2.9 ± 0.31 kg and 2.8 ± 0.56 kg, respectively). Five females and males of four-month-old quails (weighing 296 ± 6.4 g and 264 ± 7.2 g, respectively) and five females and males of nine-month-old guinea fowls (weighing 3.1 ± 0.42 kg and 2.7 ± 0.48 kg, respectively) from a farm in Labinsky District, Krasnodar Krai, from where agricultural birds were brought to Astrakhan Region, were studied as control birds.

Quails were kept in battery cages with 5 birds in each cage, whereas guinea fowls were housed in the floor space of a typical house with 10 birds in each. Experimental birds were fed based on recommendations of the All-Russian Research and Technological Institute of Poultry. The following measurements were taken from the birds through clinical examination: body temperature, pulse rate, respiratory rate, and egg productivity. The content of metal elements such as Cu, Mn, Zn, and Co in tissue samples was measured by electrochemically by atomic absorption (Briske, 1982) using a SHITACHI 180-50 spectrophotometer. The selenium content was determined fluorometrically according to Nazarenko and Ermakov (1971). Iodine determination was performed by the rhodanate-nitrite method. The hematological analysis provides information about the following measurements: red blood cell (RBC) count, white blood cell (WBC) count, leukocyte formula, erythrocyte sedimentation rate (ESR), hemoglobin, glucose, alkali reserve, total protein, uric acid, and total lipids. All experiments were performed using generally accepted methods (Kondrakhin et al., 2004). For this, blood samples were collected by puncture of the brachial vein before feeding. The total calcium and inorganic phosphate in blood plasma were determined according to the method of Kahlitsky (1980). The vitamin E content in the blood serum samples was estimated by Emmerie and Engel reaction with ferrous dipyriddyI complex using the Minichrom chromato-graph equipped with scanning UV detector. The vitamin A content was measured by the reaction with antimony chloride (Dvinskaya et al., 1979). The vitamin C content was determined as described by Petrova et al. (1987).

Diene conjugate (DC) levels in the blood serum of birds were detected by UV spectroscopy (Platzer et al., 1970). Malondialdehyde (MDA) levels were estimated by the method of Buzlama et al. (1997) The catalase (CAT) activity was measured as described by Korolyuk (1998). The superoxide dismutase (SOD) enzyme activity was measured using the method of Paglia and Valentine (1967). The function of the endocrine system was evaluated through measuring thyroid-stimulating hormone (TSH), total thyroxine (T4) and total triiodothyronine (T3) on the Uniplan analyzer using the enzyme linked immunosorbent assay as described by Matreshina et al. (1998). For this, ELISA-AT-T and Adrenocorticotrophic Hormone (ACTH) ELISA kits (Biomerica, Inc.) were employed. The results were read on a vertical scanning spectrophotometer at 450 nm.

Statistical data processing of was carried out using Microsoft Excel Pro and Statistica. The Student’s t-test was used to measure the reliability of difference between the two means. Differences were considered significant at P<0.05.

RESULTS AND DISCUSSION

MICROELEMENT CONTENTS OF INTERNAL ORGANS AS A DIAGNOSTIC TOOL

It is important to measure the amount of trace elements in soil, water and plants in the study site because these concentrations define the trace element content of bird feed. Comparing biogeochemical characteristics of the Astrakhan region and sites with chernozem soils (Vorobiev, 1993; Matveev et al., 1997; Ermakov et al., 2008; Kostin, 2017), it was found that the major components of terrestrial ecosystem (soil, water, and plants) in the Astrakhan region are low in selenium, iodine and cobalt. No deficiency of manganese, zinc, iron, and copper was detected. Hence, the bird feed made from local plants will have low contents of selenium (between 0.04 ± 0.006 and 0.12 ± 0.08 mg/kg), cobalt (between 0.01 and 8.9 ± 0.37 mg/kg) and iodine (between 0.01 and 0.07 ± 0.004 mg/kg).

Since plants absorb essential elements directly from soil, the level of selenium, iodine, copper, manganese, zinc and
### Table 1: Levels of Cobalt, Selenium, and Iodine in Organs and Tissues of Experimental and Control Quails (mg/kg of dry weight)

| Organs and tissues | Astrakhan region | | Krasnodar Krai | |
|--------------------|------------------|-----------------|-----------------|-----------------|
|                    | Co               | Se              | I               | Co              | Se              | I               |
| Muscles            |                  |                 |                 |                 |                 |                 |
| Males              | 0.48±0.03        | 0.10±0.02       | 0.06±0.005      | 0.54±0.03       | 0.18±0.003*     | 0.28±0.016*     |
| Females            | 0.51±0.02        | 0.07±0.006      | 0.12±0.04       |                 |                 |                 |
| Liver              | 0.7±0.03         | 0.26±0.05       | 0.32±0.08       | 0.59±0.01       | 0.41±0.015*     | 0.59±0.004*     |
| Males              | 0.6±0.04         | 0.29±0.03       | 0.26±0.005      |                 |                 |                 |
| Females            | 1.5±0.07         | 0.12±0.05       | 0.28±0.09       | 1.22±0.04       | 0.32±0.016*     | 0.36±0.007*     |
| Intestinal wall    | 1.4±0.02         | 0.215±0.07      | 0.14±0.007      |                 |                 |                 |
| Cardiac muscle     | 0.47±0.02        | 0.14±0.06       | 0.22±0.08       | 0.51±0.06       | 0.19±0.008*     | 0.32±0.09*      |
| Spleen             | 0.79±0.03        | 0.15±0.04       | 0.13±0.02       | 0.99±0.003      | 0.38±0.006*     | 0.18±0.01*      |
| Feathers           | 0.16±0.03        | 0.29±0.06       | 0.09±0.06       | 0.46±0.007      | 0.28±0.02*      | 0.18±0.04*      |
| Ovaries            | 0.5±0.04         | 0.11±0.02       | 0.17±0.04       |                 |                 |                 |
| Whole egg          | 0.95±0.03        | 0.29±0.02       | 0.19±0.03       | 0.98±0.045      | 0.49±0.008*     | 0.72±0.008*     |
| Egg white          | 0.9±0.03         | 0.13±0.02       | 0.19±0.04       | 0.98±0.09       | 0.67±0.054*     | 0.52±0.014*     |
| Egg yolk           | 0.8±0.01         | 0.20±0.03       | 0.28±0.01       | 0.92±0.004      | 0.48±0.05*      | 0.64±0.03*      |
| Egg shell          | 0.91±0.04        | 0.19±0.003      | 0.08±0.007      | 0.93±0.02       | 0.46±0.013*     | 0.22±0.03*      |
| Testicles          | 0.9±0.01         | 0.024±0.007     | 0.61±0.09       | 0.94±0.005      | 0.29±0.003*     | 0.55±0.003*     |
| Sperm              | 0.9±0.02         | 0.25±0.05       | 0.54±0.08       | 1.06±0.07       | 0.57±0.014*     | 0.68±0.007*     |

* – significant difference relative to experimental value, *P*<0.05.

### Table 2: Levels of Cobalt, Selenium, and Iodine in Organs and Tissues of Experimental and Control Guinea Fowls (mg/kg of dry weight)

| Organs and tissues | Astrakhan region | | Krasnodar Krai | |
|--------------------|------------------|-----------------|-----------------|-----------------|
|                    | Co               | Se              | I               | Co              | Se              | I               |
| Muscles            |                  |                 |                 |                 |                 |                 |
| Males              | 0.28±0.04        | 0.29±0.009      | 0.16±0.03       | 0.33±0.003      | 0.52±0.031*     | 0.31±0.005      |
| Females            | 0.26±0.03        | 0.03±0.002       | 0.15±0.01       |                 |                 |                 |
| Liver              | 0.58±0.03        | 0.38±0.05       | 0.38±0.05       | 0.61±0.003      | 0.58±0.014*     | 0.84±0.006*     |
| Males              | 0.52±0.06        | 0.34±0.06       | 0.44±0.02       |                 |                 |                 |
| Females            | 0.68±0.01        | 0.16±0.08       | 0.18±0.04       | 0.64±0.005      | 0.61±0.007*     | 0.28±0.008*     |
| Intestinal wall    | 0.63±0.03        | 0.21±0.05       | 0.29±0.06       |                 |                 |                 |
| Cardiac muscle     | 0.19±0.07        | 0.10±0.06       | 0.18±0.04       | 0.21±0.006      | 0.78±0.008*     | 0.29±0.004*     |
| Spleen             | 0.17±0.02        | 0.12±0.07       | 0.17±0.06       |                 |                 |                 |
| Feathers           | 0.36±0.06        | 0.22±0.008      | 0.11±0.08       | 0.41±0.003      | 0.19±0.005*     | 0.19±0.009*     |
| Ovaries            | 0.6±0.38         | 0.09±0.003      | 0.4±0.05        | 0.21±0.007      | 0.65±0.005*     | 0.62±0.004*     |
| Egg white          | 0.2±0.11         | 0.07±0.006      | 0.24±0.04       | 0.24±0.003      | 0.26±0.004*     | 0.48±0.008*     |
| Egg yolk           | 0.23±0.53        | 0.42±0.09       | 0.31±0.06       | 0.47±0.008      | 0.49±0.016*     | 0.51±0.008*     |
| Egg shell          | 0.91±0.08        | 0.03±0.006      | 0.22±0.04       | 0.28±0.005      | 0.41±0.005*     | 0.55±0.003*     |
| Testicles          | 0.4±0.04         | 0.19±0.005      | 0.4±0.01        | 0.26±0.004      | 0.42±0.061*     | 0.48±0.001*     |
| Sperm              | 0.4±0.03         | 0.12±0.006      | 0.5±0.02        | 0.39±0.009      | 0.95±0.003*     | 0.32±0.005*     |

* – significant difference relative to experimental value, *P*<0.05.
Cobalt in plants is dependent upon their content in the soil ($r = +0.59$ to $r = +0.65$). The type and physiology of the plant are also important. One of the crucial markers for hypomicroelementosis in quails and guinea fowls are microelements, which occur in insufficient concentrations in the terrestrial ecosystem of the breeding farm.

Estimating the content of trace elements in tissue samples from experimental birds (Tables 1–2), it was found that studied elements were present in the body of quails and guinea fowls in the following decreasing order: Zn > Mn > Cu > Co > Se ≥ I. Similar results were obtained for other poultry birds (Vorobiev et al., 2018). The selenium and iodine levels were found to vary greatly amongst samples (Table 1).

In female quails, for example, selenium was found in the following organs and tissues, as arranged in order of decreasing concentration: liver > whole egg > spleen > intestinal wall > egg yolk > blood > egg shells > egg white > feathers > blood ≥ ovaries ≥ muscles ≥ heart muscle. Organs and tissues of male quails can be arranged in the order of decreasing selenium content as follows: feathers ≥ semen ≥ testicles > heart muscle > intestinal wall > spleen > muscles > blood. The iodine content in the examined female quails decreased in the following order: egg yolk ≥ liver > blood > egg white > ovaries > spleen ≥ intestinal wall > heart muscles > muscles > egg shells. For male quails, this order is as follows: testicles > sperm > liver > intestinal wall > blood ≥ spleen > heart muscle > feather > muscles.

The results of the microelement analysis for female and male guinea fowls are similar (Table 2).

Organs and tissues of female guinea fowls can be arranged in the order of decreasing selenium content as follows: egg yolk > liver > intestinal wall > blood > cardiac muscle > spleen ≥ egg shell > muscles > feathers. For iodine, the decreasing order is liver ≥ ovaries > egg yolk ≥ egg white ≥ shell ≥ intestinal wall > blood > muscles > feathers.

The overall accumulation of microelements in guinea fowls and quails farmed in the Astrakhan region was somewhat similar given the deficiency of I, Se, and Co in the terrestrial ecosystem. However, there is a difference in microelement assimilation. Quails accumulate significantly more deficient microelements (Se, J and Co) than guinea fowls (P < 0.05). This can be explained by the fact that quails that have higher metabolic rate (Fisinin, 2012). Other factors include feed storage, and feed composition, and fodder cultivation method.

The comparison of microelement contents between experimental and control birds showed that birds in the Astrakhan region received significantly less diet selenium and iodine (P < 0.05). This finding correlates with the low amount of selenium and iodine in the terrestrial ecosystem of the region (Tables 1–2). The low selenium and iodine levels are one of the biomarkers for hypomicroelementosis. It is likely that selenium deficiency negatively affects the synthesis of antioxidant enzymes, especially glutathione peroxidase, the molecule of which contains the given trace element. The iodine deficiency negatively affects the synthesis of thyroxine (T4), which increases cardiac output and nerve excitability, and regulates the plumage growth and normal development of feathers (Kostin, 2017).

What is interesting is that the cobalt content of organs and tissues in experimental birds is comparable to that in controls (Tables 1–2). At the same time, the amount of cobalt in terrestrial ecosystem of the Astrakhan region is significantly lower when compared to Krasnodar Krai. Therefore, there is no positive correlation between cobalt content in the environment and cobalt content in the body of the examined birds. This coincides with the previous study (Kostin, 2017), where concentrations of trace elements were examined on pigeons living in conditions of cobalt deficiency.

Based on the above, it can be stated that quails and guinea fowls raised in biogeochemical conditions similar to those recorded in the Astrakhan region will have enough essential microelements, such as zinc, manganese, copper and cobalt. At the same time, they will suffer from iodine and selenium deficiencies. The expected consequences are oxidative stress and asymptomatic hypomicroelementosis.

**Physiological and biochemical parameters as diagnostic tools**

The analysis of blood revealed that RBC count and WBC count in adult quails and guinea fowls was higher than normal (Table 3) (Motuzko et al., 2008).

The RBC count for adult quails was found to be in the range of 5.97 ± 0.08 million/µL x 10^12/L. The RBC count for adult guinea fowls was in the range of 7.39 ± 0.09 million/µL x 10^12/L. The WBC count for adult quails was found to be in the range of 14.02 ± 1.27 thousand/µL x 10^12/L. The blood glucose level was found to be in the range of 13.9 ± 1.4 thousand/µL x 10^12/L. The blood glucose levels varied in quails and guinea fowls within the range of 15.06 ± 1.04 mmol/L and 13.1 ± 1.7 mmol/L (Table 4), respectively.

These are the above-normal figures (Kondrakhin et al., 2004; Motuzko et al., 2008). At the same time, the values of alkali reserve (354 ± 11.9 mg% and 333 ± 14.9 mg%), selenium (0.09 ± 0.006 mg/L and 0.22 ± 0.06 mg/L), iodine (0.21 ± 0.005 mg/L 0.16 ± 0.06 mg/kg), and cobalt...
Table 3: Hematological Profile of Experimental Birds

| Parameter                      | Quails (n=10) | Guinea Fowls (n=10) |
|-------------------------------|---------------|----------------------|
|                               | Males         | Females              |
| Red blood cells, mln/µL (10¹²/L) | 5.97±0.05     | 5.93±0.08            |
|                               |              |                      |
| Hemoglobin, g/L               | 118±3.4       | 124±5.8              |
|                               |              |                      |
| White blood cells, thousand/µL x 10⁹ | 14.02±0.27   | 14.25±0.93           |
|                               |              |                      |
| Sed rate, mm/h                | 1.8±1.06      | 2.1±1.07             |

Table 4: Biochemical Profile of Experimental Birds

| Parameter | Quails (n=10) | Guinea Fowls (n=10) |
|-----------|---------------|----------------------|
| Total protein, g/L | 65.06±3.08     | 51.1±2.51            |
| Total lipids, g/L  | 7.53±0.02      | 6.23±0.03            |
| Glucose, mmol/L    | 15.6±1.44      | 13.1±1.72            |
| Alkali reserve, mg% | 354±11.9       | 333±14.9             |
| Uric acid, mmol/L  | 0.34±0.02      | 0.38±0.01            |
| Calcium, mmol/L    | 5.03±0.14      | 3.6±0.09             |
| Phosphorus, mmol/L | 1.91±0.03      | 1.81±0.06            |
| Selenium, mg/L     | 0.09±0.006     | 0.16±0.06            |
| Zinc, mg/L         | 69.4±2.55      | 50.7±1.24            |
| Copper, mg/L       | 3.4±0.19       | 5.55±0.37            |
| Magnesium, mg/L    | 3.89±0.02      | 4.19±0.08            |
| Cobalt, mg/L       | 0.61±0.09      | 0.54±0.03            |
| Iodine, mg/L       | 0.14±0.05      | 0.22±0.06            |

(0.6 ± 0.01 mg/L and 0.5 ± 0.02 mg/L) in the blood samples from experimental quails and guinea fowls, respectively, were below the normal (Motuzko et al., 2008; Vorobiev et al., 2018; Vorobiev, 2013; Statsenko, 2018). Levels of selenium (0.29 ± 0.002 mg/L and 0.77 ± 0.005 mg/L) and iodine (0.38 ± 0.05 mg/L and 0.29 ± 0.004 mg/L) in the control blood samples were significantly higher when compared to those in samples from experimental birds (P <0.05). Note that hemoglobin, total protein, total lipids, zinc, manganese, phosphorus, and calcium in the blood of experimental birds hit the lowest limit of normal, whereas the copper content was within the normal range (Kondrakhin et al., 2004; Motuzko et al., 2008; Rodionova et al., 2010; Kolesnik and Derkho, 2015; Temiraev et al., 2016; Statsenko, 2018).

Based on the results of biochemical analysis, it can be concluded that biochemical profile is suitable for determining hypomicroelementosis in golden Manchurian quails and gray-spotted guinea fowls caused by the combined effect of selenium and iodine deficiencies.

**Free Radical Oxidation and Antioxidant Activity in the Blood as Diagnostic Tools**

The indicators of lipid peroxidation (LPO) and antioxidant defense against continues stress, incl. microelement deficiency, serve as biomarkers of adaptation and homeostasis. The antioxidant defense system consists of exogenous (Ca, vitamins E, A, and C, Se, etc.) and endogenous (CAT, SOD, and selenium-containing GPx) antioxidants (Lankin et al., 2001). Exposure to diet-induced stress is associated with a sharp decline of vitamins, such as A, D and B2, in poultry. In stressful conditions, domesticated birds need for 4 times more vitamins E and C and potassium (Papazyan et al., 2009; Fisinin, 2012). The vitamin E plays a protective role in lipid peroxidation and is closely linked to selenium (Lankin, 2001; Rodionova et al., 2010; Vorobiev, 2013).

The comparison of indicators of free radical oxidation and antioxidant activity (Kondrakhin et al., 2004; Papazyan et al., 2009; Rodionova et al., 2010; Fisinin, 2012; Kolesnik and Derkho, 2015; Kosti, 2017; Statsenko, 2017) between experimental and control birds revealed that the levels of antioxidant vitamins (Table 5) in poultry exposed to combined selenium and iodine deficiency were significantly lower than in controls (P <0.05).

The amount of free radical oxidation products, such as diene conjugates (DC) and malondialdehyde (MDA), and carbohydrates in the blood of experimental quails and guinea fowls was significantly higher than in controls (P <0.05). This is in line with previous studies. The CAT, SOD and GPx activities in experimental poultry were sig...
Table 5: Lipid Peroxidation and Antioxidant Status of Experimental and Control Birds

| Indicator                        | Astrakhan region               | Guinea Fowls               | Krasnodar Krai               | Guinea Fowls               |
|---------------------------------|--------------------------------|----------------------------|----------------------------|----------------------------|
|                                 | Quails (n=10)                  | Guinea Fowls (n=10)        | Quails (n=10)               | Guinea Fowls (n=10)        |
| Vitamin E, µmol/L               | 0.006±0.0003                   | 0.003±0.0002               | 0.008±0.0003*               | 0.005±0.0001               |
| Vitamin A, µmol/L               | 0.86±0.002                     | 0.74±0.006                 | 0.92±0.003                  | 0.99±0.004*                |
| Vitamin C, mg%                  | 1.01±0.05                      | 1.02±0.04                  | 1.16±0.06*                  | 1.06±0.011                 |
| Total lipids, g/L               | 7.53±0.02                      | 6.23±0.03                  | 6.81±0.25*                  | 7.75±0.109                |
| Dienes conjugates, absorbance units/mg lipid | 0.477±0.02                   | 0.452±0.01                 | 0.301±0.01*                 | 0.219±0.008*               |
| Malondialdehyde, µmol/L         | 1.93±0.02                      | 1.62±0.06                  | 1.36±0.08*                  | 1.36±0.02*                |
| Catalase, µmol/L of H₂O₂ per min x 10^1 | 49.68±0.73                  | 33.9±0.85                  | 55.12±2.16*                 | 42.8±1.09*                |
| Superoxide dismutase, units/min | 112±3.12                      | 109±2.22                   | 133±5.57*                  | 122±8.53*                 |
| Glutathione peroxidase, µmol/L of GSH per min x 10^3 | 6.93±0.44                   | 8.07±0.0009                | 8.05±0.16*                 | 8.36±0.012*               |

* - significant difference relative to experimental value, P<0.05.

Table 6: The Pituitary-Thyroid Function in Experimental Birds

| Hormone                                | Quails (n=10) | Guinea Fowls (n=10) |
|----------------------------------------|---------------|---------------------|
| Thyroid-stimulating hormone, µIU/mL    | Females       | Males               |
|                                        | 0.55±0.01     | 0.54±0.03           |
| Total thyroxine, nmol/L                | 7.97±0.32     | 7.98±0.04           |
| Total triiodothyronine, nmol/L         | 2.51±0.08     | 2.48±0.06           |

Based on the above results, it can be concluded that the examined species of poultry have a latent form of hypomicoelementosis, which was caused by the combined effect of selenium and iodine deficiencies.

**Pituitary-Thyroid Hormone Levels as Diagnostic Tools**

To confirm the diagnosis of latent combined selenium-iodine hypomicoelementosis in the examined birds, TSH, T4, and total T3 levels were measured. It is known that thyroid hormones are responsible for post-stress recovery, enhance oxygen consumption by the tissues of the body, and are closely linked to iodine and selenium. Although iodine deficiency in the body was previously found to inhibit egg productivity in different species of poultry (Fisinin, 2012; Gorelik and Derkho, 2013; Kolesnik and Derkho, 2015; Vorobiev et al., 2018), there are no comprehensive studies on the diagnosis of hypomicoelementosis in quails and guinea fowls using pituitary-thyroid function as a reference. Therefore, it is impossible to fully compare data obtained in this study with those in the existing literature. However, the results are comparable with those concerning the pituitary-thyroid function of other species of poultry. For instance, it was found that a situation where there are a relatively high TSH level and relatively low T4 and T3 levels is typical for various species of poultry (Samokhin, 2008; Kolesnik and Derkho, 2015; Temiraev et al., 2016), including quails and guinea fowls (Table 6).

It should be noted that overall, quails had higher hormone level than guinea fowls (P <0.05). Perhaps, this is due to the fact that quails have better metabolism (Fisinin, 2012). It is believed that egg productivity is influenced by the thyroid function. In other words, egg productivity is associated with the basal metabolic rate, regulated by T4, T3, iodine, and selenium (Fisinin, 2012; Gorelik and Derkho, 2013; Kolesnik and Derkho, 2015; Vorobiev et al., 2018). Thus, given the role of selenium and iodine in the functioning of the pituitary-thyroid system, it is logical to assume that the shortage of these microelements in the terrestrial ecosystem and diet can result in a decrease of egg productivity. For quails, the value of egg productivity was in the range of 23.2 ± 0.9 eggs per month, guinea fowls 27.5 ± 1.1 eggs per month. These figures only relate to experimental birds.
CONCLUSIONS

The comprehensive biogeochemical analysis of terrestrial ecosystems in the Astrakhan region showed low concentrations of selenium, iodine and cobalt in soil, water, and vegetation. As a result, the local diet of quails and guinea fowls contains insufficient amounts of these essential trace elements. Thus, the etiology and pathogenesis of combined selenium and iodine deficiency in the examined birds were caused by the shortage of selenium and iodine in the major components of terrestrial ecosystem and diet. The comprehensive examination revealed physiological and biochemical changes in the body of quails and guinea fowls imported to the Astrakhan region. There were the elevated levels of blood corpuscles, glucose, diene conjugates, and malonic aldehyde. Levels of antioxidant vitamins (E, A, C) in experimental birds were found to be low. The CAT, SOD, and GPx activates decreased in response to combined selenium and iodine deficiency. At the same time, levels of hemoglobin, total protein, total lipids, P, Ca, Zn and Mn were at the lowest limit of normal. Based on the above, it can be concluded that golden Manchurian quails and gray-speckled guinea fowls raised in conditions of selenium deficiency and iodine deficiency can experience latent symptoms of hypomicroelementosis when fed a diet based on local plants. The consequence is a drop-in egg productivity. Physiological and biochemical parameters of blood, indicators of metabolism status, levels of POL and AOS, and the activity of pituitary-thyroid system (TTG, T4, T3) provide an opportunity for correction and prevention of this asymptomatic disease of the studied birds, which are in biogeochemical conditions of Se and J deficit in the main components of ecosystems. It allows for a scientifically grounded diagnosis and choice of the missing trace elements in plant foods and bodies of quails and guinea fowls in order to apply them for improvement of metabolism and increase of integrative functions of egg production in birds.

Thus, farmers specialized at breeding quails and guinea fowls should consider feeds enriched with selenium and iodine when the local ecosystem lacks a sufficient amount of therein. It will improve the metabolism of quails and guinea fowls, increase egg production and weight of eggs of farm birds, which is a cost-effective measure. According to the previous study, good results were obtained by enriching feed with organic preparations DAFS-25 in a dose of 1.6 mg/kg and YODDAR in the amount of 50 g/t. (Polkovnichenko et al., 2019).

Further research in this area can provide a scientific basis for the compilation of bird diets that can increase their productivity.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

AUTHORS CONTRIBUTION

Pavel Polkovnichenko: Conceptualization, Validation, Writing – Original Draft Preparation, Supervision. Elena Scherbakova: Methodology, Validation, Formal Analysis, Writing – Review & Editing. Dmitriy Vorobiev: Conceptualization, Methodology, Validation, Data Curation. Vladimir Safonov: Software, Validation, Investigation, Resources.

ETHICAL APPROVAL

The study was conducted in accordance with the ethical principles approved by the Human Experiments Ethics Committee of Astrakhan State University (Protocol № 4 of 24.03.2020).

REFERENCES

• Belyaev VA (2011). Pharmacotoxicological properties and application of new selenium-based drugs: the case of the North Caucasus region. Kuban State Agrarian University Krasnodar, Kuban. (in Russian)
• Braun U, Forrer R, Furer W, Lutz H (1991). Selenium and vitamin E in blood sera of cows from farm with increased incidence of disease. Braun, U. Selenium and vitamin E in blood sera of cows from farm with increased incidence of disease. Veterin. Rec. 128(23): 543-547. https://doi.org/10.1136/vr.128.23.543
• Britskie ME (1982). Spectrochemical analysis by atomic absorption. Khimiya Publishing House, Moscow. (in Russian)
• Buzlama VS, Titov YuV, Vostroilova GA, Vaschenko YeV (1997). Guidelines for the Rapid Biotest: Biological monitoring of ecological systems. Nauka Publishing, Voronezh. (in Russian).
• Chevari SI, Chaba I, Sikey G (1985). The role of superoxide dismutase in the oxidative processes of the cell and the method for its determination in biological materials. Rus. J. Lab. Analys. 3: 678-681. (in Russian)
• Corrie FE (1996). Some elements of plants and animals. Feitilizer feedings stuff and farm supplies. J. 32(21): 40-48.
• Drinskaya LM, Reshetova LV, Dudin VI (1979). Guidelines for determining fat-soluble vitamins in biological substrates. Nauka Publishing, Borovsk. (in Russian)
• Ermakov VV, Tytikutov SF, Safonov VA (2018). Biochemical identification of microelements. Russian Academy of Sciences, Moscow. (in Russian)
• Ermakov VV, DanilovaVA, Degtyarev AP, Krechetova VE, Safonov AA, Tytikutov SF (2008). Selenium status in Russia and its correction. In: Proceedings of the 6th International Biogeochemical Conference. Astrakhan, Russia, p. 121. (in Russian)
• Fisinin VI (2012). Poultry Industry Performance and...
