AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 2, pp. 386-398
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.52/.58.084:636.085.12

doi: 10.15389/agrobiology.2023.2.386eng
doi: 10.15389/agrobiology.2023.2.386rus

AGE-DEPENDENT ACCUMULATION OF ESSENTIAL AND TOXIC CHEMICAL ELEMENTS IN FEATHER OF ARBOR ACRES BROILERS (*Gallus gallus* L.) REARED IN THE SOUTH URAL BIOGEOCHEMICAL PROVINCE OF RUSSIA

S.V. LEBEDEV, O.A. ZAV'YALOV ✉, A.N. FROLOV, M.YA. KURILKINA, V.V. GRECHKINA

Federal Research Centre of Biological Systems and Agrotechnologies RAS, 29, ul. 9 Yanvarya, Orenburg, 460000 Russia, e-mail lsv74@list.ru, Oleg-Zavyalov83@mail.ru (✉ corresponding author), forleh@mail.ru, k_marina4@mail.ru, viktoria1985too@mail.ru

ORCID:
Lebedev S.V. orcid.org/0000-0001-9485-7010
Zav'yalov O.A. orcid.org/0000-0003-2033-3956
Frolov A.N. orcid.org/0000-0003-4525-2554
Kurilkina M.Ya. orcid.org/0000-0003-0253-7867
Grechkina V.V. orcid.org/0000-0002-1159-0531

The authors declare no conflict of interests
Acknowledgements:
Supported financially from the Russian Science Foundation, project No. 21-16-00009
Final revision received June 20, 2022
Accepted July 22, 2022

Abstract

Microelement imbalances are a serious threat to the health and productive traits of farm animals and poultry, so it is important to control and optimize input of chemical elements with regard to the conditions of a biogeochemical province and anthropogenic pollution. Currently, blood is used as a biosubstrate to determine the elemental status in birds. However, preference is given to feather when it comes to assessing the elemental status over a relatively long period, also because obtaining this biosubstrate is minimally invasive. The amount of available information on macro- and microelements content in the feather of poultry breeds and crosses is limited (especially in terms of age), which makes it difficult to objectively interpret laboratory data. In the presented work, we confirmed the possibility of using feathers as a biomaterial for determining the essential and toxic chemical elements status in Arbor Acres cross broiler chickens in commercial breeding in the conditions of South Ural biogeochemical province with areas of technogenic pollution. In addition, the age-related changes in elements content in chickens' feathers under the specified conditions were characterized for the first time, and the percentile ranking of the obtained indicators was performed according to the method recommended for determining the physiological norm in the sample. The aim of the study was to determine the essential and toxic elements content in Arbor Acres broiler chickens of different ages under the conditions of South Ural biogeochemical province and to rank the obtained indicators by percentiles to estimate the limits of the age norm for the sample. The research was carried out on the territory of South Ural biogeochemical province of Russia on clinically healthy Arbor Acres broiler chickens at the age of 7 days (n = 120), 21 days (n = 120) and 35 days (n = 120). The content of nutrients, macro- and microelements in the diet of the examined birds was within the limits of the requirements for broiler chickens during the corresponding growing periods. Blood and feathers (the flight feathers were plucked out) were used as compared biosubstrates. The proximal part of the feather weighing at least 0.4 g was selected for research. The elemental composition of feathers and blood serum was determined by inductively coupled plasma atomic emission and mass spectrometry (ICP-DRC-MS). The results of the quantitative determination of each element in feather were ranked in ascending gradation in the form of an ordered series according to the recommendations of the American Society for Veterinary Clinical Pathology (2.5 and 97.5 percentile). It was found that the content of P, Cr, I, Se, Li, Si, As, Hg, Sn, Sr in feather of broiler chickens varied significantly depending on age. In this regard, the age factor must be taken into account when developing reference intervals for specific regions. In this study, percentile ranking (2.5 and 97.7 percentiles with a confidence interval CI = 90 %) of data on 25 chemical elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, I, K , Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Hg, Sr, V, Zn) in feather of physiologically healthy Arbor Acres cross broiler chickens at different ages (7; 21; 35 days). The obtained data can be useful in detecting elementoses in Arbor Acres broiler chickens.

Keywords: broiler chickens, Arbor Acres, blood, feather, essential elements, toxic elements, age, percentile ranking

Imbalance of minerals poses a serious threat to the health and productivity of farm animals and poultry [1, 2], and it is important to take into account the intake of both vital and toxic chemical elements into the body [3]. Excess of toxic metals can weaken the immune system by causing oxidative stress [4] which negatively affects biochemical parameters, reproductive function and productivity, increases the incidence of heart disease and leads to various mutations and neoplasms [5].

The South Ural territory is a biogeochemical mosaic with a deficiency of iodine, fluorine, selenium, and the presence of local areas with high levels of zinc, copper, strontium and nickel. Along with natural biogeochemical provinces, technogenic provinces of nickel, manganese, fluorine, and lead are being formed in local territories [6, 7]. Modern poultry farming uses water treatment systems, mixed feed, feed additives and rationing for nutrients and minerals with regard to the age and physiological needs of crosses developed for diferent purposes. This sufficiently standardizes feeding regimes and avoids effects of local conditions on the growth and productivity performance of poultry. However, when developing and adapting such regulations, local biogeochemical factors must be taken into account, including the nature and level of technogenic pollution [3-5].

It is obvious that when monitoring and optimizing the intake of mineral substances, non-invasive methods for assessing metabolic status, including determining the elemental composition in biosubstrates, are most acceptable. Such work was carried out on beef [8] and dairy [9] cattle, in sports horse breeding [10], and in commercial poultry farming [11]. Currently, blood mostly serves as a biosubstrate to analyze the elemental status of poultry [12], but in long and low-sress monitoring feathers are preferable [13]. In addition, it has been proven that the concentration of heavy metals in bird feathers is an informative indicator when assessing the contamination of territories with heavy metals [14]. It has also been found out that the amount of trace elements in feathers can generally characterize the metabolic pool of the main essential and toxic elements in broiler chickens and laying hens [15].

However, the available information on the accumulation of macro-, micro- and toxic elements in poultry (in particular, broiler chickens) during different periods of ontogenesis is limited. Moreover, for most breeds and crosses there are no reference intervals calculated by the recommendations of the American Society of Veterinary Clinical Pathology [16], including those for local breeding conditions. This hinders to objectively interpret and compare results when analyzing the bioelemental status of poultry.

Arbor Acres is a large meat cross with strong bones, high meat yield from the carcass (up to 72%), developed muscles, which reduces the fat content of the product, and shortened fattening periods [17]. According to data on 2016, in Africa, over 10 years, the cross has occupied up to 20% of the market; the cross is popular in Algeria, Egypt, Tunisia, Mozambique, and is raised for meat in the USA and China [18].

Here, we confirm that feathers are suitable to evaluate the essential and toxic elements of Arbor Acres cross broilers in the South Ural biogeochemical province with areas of technogenic pollution. For the first time, we revealed age-related changes in the accumulation of chemical elements in feathers under these conditions. The experimental data was processed according to the unified methodology of the American Society of Veterinary Clinical Pathology that is recommended for assessment of the physiological norm in the sample. The obtained percentile ranking can serve for detection of elementosis in Arbor Acres broilers during growth and for metabolic correction of timely detected disorders using mineral complexes.

Our goal was to determine the concentration of essential and toxic elements in the feathers of Arbor Acres cross broiler chickens of different ages under the conditions of the South Ural biogeochemical province and to rank the obtained indicators by percentile to assess the boundaries of the age norm for the sample.

Materials and methods. Experiments were consistent with the instructions, recommendations, and protocols of the Geneva Convention and the principles of good laboratory practice (Order of the USSR Ministry of Health No. 755 of 08/12/1977 "On measures to further improve work using experimental animals", "National Standard of the Russian Federation GOST R 53434-20092"). All manipulations were performed in accordance with the rules of the Animal Ethics Committee of BST RAS. All efforts were made to minimize animal suffering and reduce the number of samples analyzed.

The poultry was raised at three poultry farms (ZAO Orenburg Poultry Farm, Individual Peasant Farmer T.P. Tuzikov, Peasant Farmer V.A. Malyshev) located in the South Ural biogeochemical province (Orenburg Province) [6, 7].

Clinically healthy Arbor Acres broilers aged 7 days (n = 120), 21 days (n = 120), and 35 days (n = 120) were used. Feeding and housing conditions for all chickens were approximately identical and corresponded to recommendarions for the cross. The birds were kept in metal cages (145×70×250 cm width×depth×height) with mesh floors. Nutrients of the diets corresponded to the requirements of broiler chickens during each growing period [19]. Complete feeds (Russia) PK-0 (0-10 days of life), PK-5 (11-24 days), PK-6 (25 days and older) were added with the premix Koudais MKorma (Russia) (25 kg/t) as a mineral component. The premix comtains mineral components (69.10%), limestone (21.6%), and bran (9.3%).

Feather and blood for analysis were collected from three individuals aged 7, 21 and 35 days in each of 40 randomly selected cages from different sections of workshops. Five fight feathers were plucked from the wing; the proximal part of the feather weighing at least 0.4 g was selected for analysis. Blood (at least 2 ml) from the axillary vein of birds aged 21 and 35 days was sampled in the morning into 7 ml vacuum tubes with a blood clotting activator (Hebei Xing Sky & Co., China). Blood serum was separated by centrifugation for 10 minutes at 1000 rpm. The tubes were cooled to –8 °C and stored until elemental analysis.

The feather was ground to a fraction of no more than 2 mm. The resulting feather samples were washed in acetone (Sigma-Aldrich, Co., USA) to remove external contaminants, then three times in deionized water (18 MOM/cm) and dried at 60 °C to constant weight. Feather samples, 50 mg each, were placed in Teflon tubes with 5 ml of concentrated nitric acid (Sigma-Aldrich, Co., USA) and processed in a microwave system (Bergh of Products + Instruments GmbH, Germany). The resulting solutions were poured into 15 ml polypropylene tubes, diluted with deionized water to a final volume of 15 ml and mixed thoroughly by shaking.

Concentration of essential, conditionally essential (Ca, K, Mg, Na, P, Co, Cr, Cu, Fe, I, Li, Mn, Se, Si, Sr, V, Zn), toxic and potentially toxic (As, B, Cd, Hg, Ni, Pb and Sn) elements were measured (a NexION 300D spectrometer, Perkin Elmer, USA, with an ESI SC-2 DX4 autosampler, Scientific, Inc., USA). For calibration, standard solutions with different concentrations of microelements were used, prepared from Universal Data Acquisition Standards Kits (Perkin Elmer, Inc., USA). Single element purity standard (10 g/) for yttrium and rhodium (Perkin Elmer, Inc., USA) were used for internal online standardization. For laboratory quality control, continuous analysis of a certified reference substrate (the hair GBW09101, Shanghai Institute of Nuclear Research, China) was used. The analysis was carried out before and after each stage of the survey. The degree of extraction of all analyzed trace elements was no less than 88%.

The results of the quantification of each element were reranked in ascending gradation as an ordered series according to the recommendations of the American Society for Veterinary Clinical Pathology [16] with concentration intervals of chemical elements by percentiles, 2.5 and 97.5. The reliability of differences was assessed by Mann-Whitney U-test. The significance level (p) was taken to be less than or equal to 0.05. To calculate the correlation between the assessed parameters, the Spearman rank correlation method was used. The tables show the mean values (*M*) and their standard deviations (±SD). Data processing used the Statistica 10.0 application package (StatSoft, Inc., USA).

Results. In Russia, a significant part of the Arbor Acres cross population is bred in the conditions of the South Ural biogeochemical province. According to data from open sources, at the ZAO Orenburg Poultry Farm, the annual turnover of this cross is 1.8 million chickens, which indicates its prospects. AO Bashkir Broiler (Republic of Bashkortostan) is a second-order reproducer for the production of Arbor Acres hatching eggs for the Republic of Bashkortostan and the Orenburg Province.

The territory of the South Ural biochemical province, along with a deficiency of some elements, is characterized by an uneven distribution of various sources of pollution, e.g., enterprises of the chemical, petrochemical, fuel, mining industries (iron, copper, nickel ores, asbestos, oil) and ferrous and non-ferrous metallurgy (production of nickel, cobalt, copper) [6, 7].

Table 1 submits the concrnetration of chemical elements in the daily diet of broiler chickens at different age periods.

1. Content of chemical elements in the daily diet of the Arbor Acres cross broiler chickens by age periods of reared (Orenburg Province, South Ural biogeochemical province, 2022)

Element	Age, days		
	0-14	15-28	29-42
Macronutrients			
Ca	287.2	821.5	1533.6
K	251.7	339.6	452.4
Mg	60.8	87.9	127.1
Na	41.2	57.1	66.5
P	203.6	589.7	1102.3
Essential elements			
Co	0.007	0.010	0.011
Cr	0.304	1.883	4.526
Cu	1.25	1.55	2.00
Fe	5.69	10.36	15.98
Mn	3.51	6.55	9.38
I	0.027	0.044	0.034
Se	0.006	0.015	0.025
Zn	4.42	8.25	11.55
Conditionally essential elements			
B	0.504	0.695	0.860
Li	0.0021	0.0025	0.0030
Si	1.07	2.54	5.86
Ni	0.042	0.095	0.174
V	0.023	0.167	0.473
Toxic elements			
Al	0.337	0.524	0.800
As	0.001	0.028	0.043
Cd	0.001	0.002	0.003
Hg	0.000	0.000	0.000
Pb	0.002	0.015	0.022
Sn	0.000	0.000	0.000
Sr	0.400	0.754	1.258

In all farms and at all age periods, the chickens were physiologically healthy, developed normally and had relatively high dynamics of live weight and its growth (Table 2).

2. Bodyweight dynamics in the Arbor Acres cross broiler chickens at poultry breeding enterprises (n = 120, M±SD, Orenburg Province, South Ural biogeochemical province, 2022)

Parameter	Enterprise		
	ZAO Orenburg Poultry Farm	Individual Peasant Farmer T.P. Tuzikov	Peasant Farmer V.A. Malyshev
Bodyweight, г:			
7 days	183.2±19.34	179.4±14.48	180.2±18.61
21 days	653.2±84.34	634.3±76.61	652.1±87.64
35 days	1862.4±201.09	1811.6±187.69	1830.3±202.11
Average daily weight gain, g/(head · day)	60.0±21.17	58.3±16.34	58.9±18.64
Absolute weight gain, kg/(head · test)	1.68±0.49	1.63±0.38	16.65±0.43

3. Content (mg/kg) **of macro-, essential and toxic elements in the feathers of Arbor Acres cross broiler chickens of different ages at poultry farms** (n = 120, M±SD, Orenburg Province, South Ural biogeochemical province, 2022)

Element	Age, daysт		
	7	21	35
Macronutrients			
Ca	1496±124.9	1214±144.4	928.1±118.1
K	2429±622.0	2510±155.7	2283±199.3
Mg	314.0±64.41	435.2±51.31	419.3±83.13
Na	1346±311.4	1665±98.15	1801±105.6
P	1060±428.0	1382±102.5	1072±86.28[b]
Essential elements			
Co	0.0741±0.0151	0.0588±0.0075	0.0618±0.0120
Cr	3.02±0.264	4.63±0.421	3.03±0.261[b]
Cu	13.32±2.22	9.60±1.55	10.64±1.78
Fe	89.74±14.38	81.93±16.35	57.23±6.20
I	8.54±2.03	0.838±0.0828[a]	0.477±0.0562[ab]
Mn	10.27±1.86	16.38±3.25	15.66±4.46
Se	1.020±0.1417	0.597±0.0204[a]	0.568±0.0184[a]
Zn	176.0±13.48	314.0±50.54	268.2±42.99
Conditionally essential elements			
B	1.84±0.359	2.65±0.261	2.43±0.354
Li	0.106±0.0236	0.0464±0.0071[a]	0.0467±0.0047[a]
Ni	0.879±0.125	1.38±0.266	1.36±0.342
Si	103.0±12.58	80.55±5.02	39.52±5.52[ab]
V	0.225±0.0247	0.473±0.0604	0.254±0.0370[b]
Toxic elements			
Al	21.74±4.20	13.09±2.09	13.44±2.43
As	0.0672±0.0065	0.0617±0.0047	0.0496±0.0032[b]
Cd	0.0310±0.0091	0.0318±0.0147	0.0142±0.0035
Hg	0.0357±0.0127	0.0094±0.0012[a]	0.0130±0.0016[a]
Pb	1.78±0.335	1.84±0.384	2.44±0.700
Sn	0.234±0.117	1.50±1.34	0.0715±0.0102[a]
Sr	4.57±0.790	2.45±0.348[a]	2.14±0.352[a]

Note. Data are provided for three enterprises (CJSC Orenburg Poultry Farm, Individual Peasant Farmer T.P. Tuzikov, Peasant Farmer V.A. Malyshev).
[a] Dfferences for the values on days 21 and 35 compared to day 7 are statistically significant at p ≤ 0.05.
[b] Dfferences for the values on day 35 compared to day 28 are statistically significant at p ≤ 0,05.

A comparative analysis revealed a significant difference in the content of the main essential and toxic elements in the re-examined birds at different periods of growth and development (Table 3).

In the feather of 7-day-old broiler chickens, we found the highest content of P, I, Se, Li, Si, As, Hg, Sn and Sr. As they grew older, in the period from day 7 to days 21 and 35, the values significantly decreased (see Table 3). It has been previously reported that age may influence micronutrient metabolism in poultry [20]. A possible reason for the decrease in parameters we identified as the bird matures may be that elements such as Ca, P, Zn, I, Cu play an important role as cofactors of enzyme systems associated with bone mineralization, a process subject to age-related changes [21]. Thus, it has been established that during the formation of the organic matrix of bone tissue and its mineralization, two ages stand out as critical, 14 and 35 days, when the mineral saturation of the organic bone matrix decreases, which weakens the bones of the supporting limbs [22]. It is noteworthy that the minimum content of almost all assessed chemical elements in our experiment was noted during the period of the beginning of molting at the age of 35 days. The molting period is usually accompanied by a natural decrease in feed intake and suppression of micronutrient metabolism [23]. When determining the age-related dynamics of the amount of toxic elements, we revealed a significant ($p \leq 0.05$) decrease in the accumulation of As, Hg, Sn, Sr in the feather of broiler chickens at the age of 21 and 35 days compared to 7 days of age. These differences may be associated with the metabolism of metallothionein, a protein that is synthesized in the body of animals in response to the intake of heavy metals from the external environment. Metallothionein levels, in turn, are regulated by sex hormones [24], the concentration of which depends on age [25]. It should be noted, however, that in our other study, toxic elements, on the contrary, accumulated with age in poultry (we believe, due to a longer intake of toxic metals with feed and drinking water) [11].

4. Concentration (mg/l) **of macro-, essential and toxic elements in the blood serum of Arbor Acres cross broiler chickens of different ages at poultry farms** (n = 120, M±SD, Orenburg Province, South Ural biogeochemical province, 2022)

Element	Age, days	
	21	35
Macronutrients		
Ca	0.159±0.0151	0.125±0.0132*
K	0.289±0.0447	0.282±0.0421
Mg	0.0372±0.0014	0.0322±0.0015
Na	3.01±0.482	3.22±0.484
P	0.263±0.0221	0.196±0.0225*
Essential elements		
Co	0.0021±0.0003	0.0036±0.0005**
Cr	0.0112±0.0021	0.0053±0.0022*
Cu	0.131±0.0154	0.178±0.0195*
Fe	1.98±0.182	1.32±0.144**
I	0.0621±0.0061	0.0452±0.0052**
Mn	0.0351±0.0133	0.0231±0.0034
Se	0.212±0.0423	0.154±0.0331
Zn	2.32±0.355	2.22±0.328
Conditionally essential elements		
B	0.714±0.145	0.537±0.115
Li	0.0188±0.0034	0.0247±0.0042
Ni	0.0093±0.0034	0.0082±0.0027
V	0.0052±0.0021	0.0074±0.0025
Toxic elements		
As	0.0032±0.0006	0.0051±0.0007*
Al	0.0633±0.0082	0.0854±0.0076*
Cd	0.0011±0.0002	0.0004±0.0002*
Hg	0.0002±0.0002	0.0007±0.0003
Pb	0.0003±0.0001	0.0003±0.0001
Sn	0.0009±0.0003	0.0007±0.0003
Sr	0.142±0.0264	0.135±0.0277

Note. Data are provided for three enterprises (CJSC Orenburg Poultry Farm, Individual Peasant Farmer T.P. Tuzikov, Peasant Farmer V.A. Malyshev).
*, ** Dfferences from the values on day 21 are statistically significant at $p \leq 0.05$ and $p \leq 0.01$.

To assess the possibility of using bird feathers as a biosubstrate for elemental analysis, we compared the data obtained for feather and for blood samples (Table 4). These data showed that the dynamics of element concentrations in the blood serum generally corresponded to those in feathers of the birds.

Correlation analysis (Table 5) revealed a significant ($p \leq 0.05$) positive relationship between the concentration of bioelements in feathers and blood serum in our study was established for Al, Ca, Cr, Cd, Fe, I, Mg, P, Pb, Se, Zn.

Thus, it can be stated that, in general, the elemental analysis of feathers reflects the concentration of chemical elements in the blood serum, which allows us to recommend flight feathers as a biosubstrate for non-invasive assessing the elemental status of broiler chickens. We note that for As and V there were significant

($p \leq 0.05$) but negative correlations. A possible reason for such differences is that the composition of blood is much more dynamic and reflects changes over a much shorter period of time, and feathers as a biosubstrate make it possible to characterize the elemental status over a long period [26]. In addition, the content of chemical elements in feathers is higher than in blood serum. This increases the analytical sensitivity of the determination and the information content of the assessment of changes in elemental status when using a pen.

5. Spearman correlation coefficients (*r*) between macro-, essential and toxic element content in the feathers and blood serum of Arbor Acres cross broiler chickens at poultry farms (*n* = 360, Orenburg Province, South Ural biogeochemical province, 2022)

Element	Al	As	B	Ca	Cd	Co	Cr	Cu	Fe	Hg	I	K	Li	Mg	Mn	Ni	P	Pb	Se	Sn	Sr	V	Zn
Al	0.8*	-0,4	0,0	-0,2	0,4	0,1	-0,4	0,4	-0,6	0,0	-0,3	-0,2	0,0	-0,3	0,2	0,4	-0,3	-0,2	-0,2	-0,7	-0,3	0,3	0,0
As	0.4	-0,8*	0,4	0,0	0,5	-0,2	-0,6	0,6	-0,5	0,2	-0,2	0,1	0,0	-0,5	0,5	0,5	-0,3	-0,1	-0,1	-0,8	-0,4	0,2	-0,3
B	0.4	-0,3	0,4	0,0	0,5	-0,2	-0,6	0,6	-0,5	0,2	-0,2	0,1	0,0	-0,2	0,5	0,5	-0,3	-0,1	-0,1	-0,8	-0,4	0,2	-0,3
Ca	0.4	-0,4	0,4	0,7*	0,5	-0,2	-0,6	0,6	-0,5	0,2	-0,2	0,1	0,0	-0,2	0,5	0,5	-0,3	-0,1	-0,1	-0,8	-0,4	0,2	-0,3
Cd	0.4	-0.4	0.2	-0.1	0.6*	-0.1	-0.6	0.6	-0.5	0.1	-0.4	0.0	0.1	-0.4	0.4	0.6	-0.2	-0.1	-0.4	-0.8	-0.4	0.3	-0.2
Co	0.4	-0.5	0.4	0.0	0.5	-0.2	-0.6	0.6	-0.5	0.2	-0.2	0.1	0.0	-0.5	0.5	0.5	-0.3	-0.1	-0.4	-0.8	-0.4	0.2	-0.3
Cr	0.5	-0.5	0.5	0.1	0.4	-0.3	-0.6	0.7	-0.4	0.3	-0.2	0.2	-0.1	-0.6	0.5	0.4	-0.3	0.0	-0.3	-0.7	-0.3	0.2	-0.5
Cu	0.2	-0.6	0.0	-0.2	0.4	0.1	-0.4	0.4	-0.6	0.0	-0.3	-0.2	0.0	-0.4	0.2	0.4	-0.3	-0.2	-0.2	-0.7	-0.3	0.3	0.0
Fe	0.4	-0.5	0.4	0.0	0.5	-0.2	-0.6	0.6	0.6*	0.2	-0.2	0.1	0.0	-0.5	0.5	0.5	-0.3	-0.1	-0.4	-0.8	-0.4	0.2	-0.3
Hg	0.0	-0.3	-0.2	-0.3	0.2	0.2	-0.2	0.2	-0.6	-0.2	-0.2	-0.6	0.1	-0.4	0.0	0.3	-0.2	-0.2	-0.2	-0.5	-0.1	0.3	0.2
I	0.5	-0.3	0.5	0.1	0.4	-0.3	-0.6	0.7	-0.4	0.3	0.8*	0.2	-0.1	-0.4	0.5	0.4	-0.3	0.0	-0.2	-0.7	-0.3	0.2	-0.3
K	0.4	-0.4	0.4	0.0	0.5	-0.2	-0.6	0.6	-0.5	0.2	-0.2	0.1	0.0	-0.3	0.5	0.5	-0.3	-0.1	-0.2	-0.8	-0.4	0.2	-0.3
Li	0.1	-0.2	0.0	-0.3	0.2	0.0	-0.3	0.5	-0.6	-0.1	-0.2	-0.3	0.1	-0.4	0.2	0.4	-0.2	-0.2	-0.4	-0.7	-0.3	0.3	0.1
Mg	0.4	-0.3	0.4	0.0	0.5	-0.2	-0.6	0.6	-0.5	0.2	-0.2	0.1	0.0	0.7*	0.5	0.5	-0.3	-0.1	-0.3	-0.8	-0.4	0.2	-0.3
Mn	0.4	-0.4	0.4	0.0	0.5	-0.2	-0.6	0.6	-0.5	0.2	-0.2	0.1	0.0	-0.3	0.5	0.5	-0.3	-0.1	-0.3	-0.8	-0.4	0.2	-0.3
Na	0.2	-0.2	0.0	-0.2	0.4	0.1	-0.4	0.4	-0.6	0.0	-0.3	-0.2	0.0	-0.3	0.2	0.4	-0.3	-0.2	-0.5	-0.7	-0.3	0.3	0.0
Ni	0.4	-0.4	0.4	0.0	0.5	-0.2	-0.6	0.6	-0.5	0.2	-0.2	0.1	0.0	-0.4	0.5	0.5	-0.3	-0.1	-0.3	-0.8	-0.4	0.2	-0.3
P	0.4	-0.4	0.3	0.2	0.3	-0.3	-0.5	0.7	-0.4	0.1	-0.1	0.1	-0.2	-0.4	0.4	0.3	0.8*	0.0	-0.4	-0.7	-0.3	0.2	-0.4
Pb	0.2	-0.2	-0.1	-0.2	0.3	0.2	-0.4	0.3	-0.5	0.0	-0.4	-0.3	0.0	-0.3	0.1	0.4	-0.3	0.7*	-0.4	-0.6	-0.3	0.4	0.1
Se	0.2	-0.3	0.2	-0.1	0.4	-0.1	-0.4	0.5	-0.6	0.1	-0.1	-0.2	0.0	-0.5	0.3	0.3	-0.4	-0.2	0.6*	-0.7	-0.3	0.2	-0.1
Si	0.7	-0.3	0.7	0.3	0.5	-0.4	-0.8	0.6	-0.2	0.4	-0.1	0.5	-0.1	-0.2	0.7	0.5	-0.5	0.2	-0.3	-0.7	-0.2	-0.1	-0.4
Sn	0.2	-0.3	0.0	-0.2	0.4	0.1	-0.4	0.4	-0.6	0.0	-0.3	-0.2	0.0	-0.4	0.2	0.4	-0.3	-0.2	-0.5	-0.7	-0.3	0.3	0.0
Sr	0.3	-0.2	0.3	0.0	0.3	-0.2	-0.5	0.6	-0.5	0.1	-0.2	0.0	0.0	-0.5	0.4	0.4	-0.3	-0.1	-0.6	-0.8	-0.4	0.2	-0.2
V	0.6	-0.4	0.6	0.2	0.4	-0.4	-0.7	0.7	-0.3	0.3	-0.2	0.4	-0.1	-0.2	0.6	0.6	-0.4	0.1	-0.8	-0.8	-0.3	-0.6*	-0.5
Zn	0.3	-0.5	0.2	-0.2	0.5	0.1	-0.5	0.4	-0.5	0.2	-0.4	-0.1	0.1	-0.3	0.3	0.5	-0.3	-0.2	-0.6	-0.7	-0.3	0.3	0.7*

N o t e. Data are provided for three enterprises (ZAO Orenburg Poultry Farm, Individual Peasant Farmer T.P. Tuzikov, Peasant Farmer V.A. Malyshev).
* Correlations are statistically significant at $p \leq 0.05$.

The essence of the percentile method of ranking measurement results [16] that we used is that the series covering the entire range of quantitative fluctuations of the attribute (100%) is divided into 100 intervals and percentile probabilities are established, the intervals between which constitute percentile intervals. In the calculations, we followed the international recommendations for veterinary laboratory standards [16] according to which, after excluding outliers (abnormally high and/or low values of the analyzed indicator), it is proposed to use the interval from 2.5 to 97.5 percentile as a physiological norm in a sample studied. A prerequisite for the use of this method is the calculation of 90% confidence intervals for the upper and lower limits, which make it possible with a known probability to estimate the mathematical expectation of the general population with further expansion of the experimental sample.

The content of chemical elements in the feather of broiler chickens at different periods of growth and development (7, 21, 35 days) differed significantly ($p \leq 0.05$), so we ranked the values of the indicators for each age separately (Table 6).

The largest intervals for almost all studied elements (with the exception of Na, P, Se, Li, Hg) occurred in broiler chickens at the age of 7 days. The widest ranges for K, Mg, Co, Cu, Mn, Se, Zn, B, Ni, Al, Pb, Sr were characteristic of young birds aged 35 days. In general, wider concentration ranges detected in birds at an early age indicate instability of metabolism for the noted elements, which may be associated with the individual characteristics of individuals.

6. Percentile rank intervals for macro-, essential and toxic element content (mg/kg) **in the feathers of Arbor Acres cross broiler chickens at poultry farms** (n = 120, Orenburg Province, South Ural biogeochemical province, 2022)

Element	2.5 (CI = 90 %)	97.5 (CI = 90 %)
	7-day age	
	Macronutrients	
Ca	1222 (794.3-1649)	1821 (1183-2458)
K	1053 (684.4-1421)	3972 (2581-5362)
Mg	177.0 (115.1-238.9)	475.0 (308.7-641.2)
Na	665.2 (432.3-897.7)	2094 (1361-2826)
P	302.3 (196.3-407.7)	2892 (1879-3904)
	Essential elements	
Co	0.0343 (0.0223-0.0465)	0.104 (0.0676-0.140)
Cr	2.63 (1.71-3.55)	3.76 (2.44-5.07)
Cu	7.33 (4.77-9.89)	17.91 (11.64-24.17)
Fe	50.3 (32.7-67.91)	118.0 (76.7-159.3)
I	4.94 (3.21-6.67)	13.49 (8.76-18.21)
Mn	5.34 (3.47-7.21)	13.34 (8.67-18.01)
Se	0.637 (0.414-0.860)	1.29 (0.838-1.74)
Zn	144.2 (93.63-194.4)	210.0 (136.5-283.5)
	Conditionally essential elements	
B	1.24 (0.806-1.67)	2.70 (1.75-3.64)
Li	0.0644 (0.0423-0.0873)	0.174 (0.113-0.234)
Ni	0.552 (0.359-0.745)	2.16 (1.40-2.91)
Si	76.67 (49.83-103.5)	126.0 (81.9-170.1)
V	0.170 (0.111-0.230)	0.290 (0.188-0.391)
	Toxic elements	
Al	10.45 (6.79-14.12)	30.68 (19.94-41.41)
As	0.0481 (0.0313-0.0653)	0.0786 (0.0507-0.1050)
Cd	0.0082 (0.0054-0.0114)	0.0582 (0.0373-0.0776)
Hg	0.0154 (0.0102-0.0203)	0.112 (0.0728-0.151)
Pb	0.848 (0.551-1.14)	2.39 (1.55-3.22)
Sn	0.0974 (0.0634-0.131)	0.584 (0.379-0.788)
Sr	3.03 (1.97-4.09)	6.60 (4.29-8.91)
	21-day age	
	Macronutrients	
Ca	462.0 (300.3-623.7)	3592 (2334-4849)
K	1594 (1036-2151)	4740 (3081-6399)
Mg	209.0 (135.8-282.1)	1298 (843.7-1752)
Na	981.0 (637.6-1324)	3254 (2115-4392)
	Essential elements	
Co	0.0135 (0.0087-0.0173)	0.158 (0.102-0.213)
Cr	1.66 (1.08-2.24)	9.98 (6.48-13.47)
Cu	3.34 (2.17-4.51)	43.04 (27.97-58.10)
Fe	29.13 (18.93-39.33)	427.0 (277.5-576.4)
I	0.230 (0.150-0.311)	1.73 (1.12-2.33)
Mn	1.39 (0.904-1.87)	69.89 (45.42-94.35)
Se	0.441 (0.287-0.595)	0.791 (0.514-1.060)
Zn	136.0 (88.40-183.6)	929.2 (603.8-1254)
	Conditionally essential elements	
B	0.791 (0.514-1.07)	6.43 (4.17-8.68)
Li	0.0093 (0.0062-0.0134)	0.167 (0.108-0.225)
Ni	0.154 (0.100-0.208)	4.62 (3.01-6.23)
Si	42.45 (27.59-57.31)	143.0 (92.95-193.1)
V	0.112 (0.0732-0.151)	1.33 (0.86-1.79)
	Toxic elements	
Al	2.79 (1.81-3.76)	44.12 (28.67-59.56)
As	0.0301 (0.0193-0.0407)	0.124 (0.0806-0.167)
Cd	0.0034 (0.0023-0.0045)	0.372 (0.241-0.502)
Hg	0.0022 (0.0014-0.0025)	0.0284 (0.0184-0.0383)
Pb	0.226 (0.147-0.305)	6.44 (4.18-8.69)
Sn	0.0194 (0.0125-0.0265)	33.60 (21.84-45.36)
Sr	0.595 (0.387-0.803)	7.31 (4.75-9.86)
	35-day age	
	Macronutrients	
Ca	349.0 (226.8-471.1)	3390 (2203-4576)
K	949.0 (616.8-1281)	7422 (4824-10019)
Mg	148.0 (96.20-199.8)	2736 (1778-3693)
Na	876.0 (569.4-1182)	2838 (1844-3831)
P	371.0 (241.1-500.8)	2750 (1787-3712)

	Essential elements	
Co	0.0065 (0.0043-0.0094)	0.277 (0.180-0.373)
Cr	1.47 (0.956-1.98)	7.12 (4.62-9.61)
Cu	4.08 (2.65-5.51)	59.12 (38.42-79.81)
Fe	22.99 (14.94-31.03)	191.0 (124.15-257.8)
I	0.158 (0.103-0.213)	1.84 (1.19-2.48)
Mn	0.894 (0.581-1.20)	133.0 (86.45-179.50)
Se	0.338 (0.220-0.456)	0.933 (0.606-1.250)
Zn	125.0 (81.25-168.7)	1362 (885.3-1838)
	Conditionally essential elements	
B	1.07 (0.696-1.44)	12.25 (7.96-16.53)
Li	0.0089 (0.0062-0.0124)	0.125 (0.0812-0.168)
Ni	0.0957 (0.0623-0.129)	9.52 (6.18-12.85)
Si	10.63 (6.91-14.35)	140.0 (91.21-189.5)
V	0.0473 (0.0315-0.0643)	1.06 (0.689-1.430)
	Toxic elements	
Al	1.87 (1.21-2.52)	75.90 (49.33-102.4)
As	0.0267 (0.0173-0.0365)	0.0934 (0.0607-0.126)
Cd	0.0013 (0.001-0.002)	0.107 (0.0695-0.144)
Hg	0.0018 (0.0012-0.0026)	0.0474 (0.0302-0.0629)
Pb	0.243 (0.158-0.328)	21.87 (14.21-29.52)
Sn	0.0078 (0.0053-0.0113)	0.238 (0.154-0.321)
Sr	0.365 (0.237-0.493)	9.66 (6.27-13.04)

N o t e. Data are provided for three enterprises (ZAO Orenburg Poultry Farm, Individual Peasant Farmer T.P. Tuzikov, Peasant Farmer V.A. Malyshev).

Feeds contain a wide range of microelements, some of which have nutritional value (Fe, Cu, Zn, Mn, Se, Co, Cr), while others have toxic properties (Pb, Cd, Ni) [27]. According to various studies, the content of bioelements is related to the geochemical conditions of the poultry breeding region [6]. Thus, in our experiment, the indicators for Zn were higher than those for chickens (*Gallus gallus domesticus*) bred in environmentally safe regions of Malaysia [28], but almost comparable to those obtained for this cross in Pakistan [29], for Cu and Mn, the values were comparable to the results for Korea [30] and significantly lower than in Belgium [31]. The content of Pb and Cd in the fether of broiler chickens in our study (South Ural biogeochemical province of Russia) was significantly lower than in chickens bred in regions with high technogenic load, in particular in South Korea (cross Ross 308, influencing factor is proximity to a large metropolis) [32], in China (cross is unknown, the influencing factor is mining metallurgy) [33], in the Republic of Kosovo (cross *G. gallus domesticus*, the influencing factor is proximity to a large metropolis) [34], but it differed little from indicators for poultry (*G. gallus domesticus*) raised near the capital of Oyo State in Nigeria [35]. The observed differences may be due to differences in regional and local background toxic metal pollution in the study areas [36]. Cross and species can also have a significant impact on the mineral composition of feathers. Thus, in a comparative assessment of the content of chemical elements in chickens of crosses *G. gallus domesticus* and *Coturnixcoturnix japonica*, bred in the same biochemical province, significant differences were revealed, in *C. japonica* the accumulation of Al, Mn, Co, Cu, Fe and Pb was higher than in the *G. gallus domesticus* cross [28]. In addition, the methodology for selecting, evaluating, and statistically analyzing the mineral composition of biosubstrates can have a significant impact on the results obtained, which makes it difficult to compare the results obtained in different studies [37, 38].

So, the accumulation of P, Cr, I, Se, Li, Si, As, Hg, Sn and Sr in the feather of Arbor Acres broilers raised in the South Ural biogeochemical province (Russia) varied significantly ($p \leq 0.05$) depending on age. In this regard, the age factor must be considered when developing reference intervals for the physiological norm of macro- and microelements and limit values of toxicants in poultry in specific regions. We quantified 25 chemical elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Hg , Sr, V, Zn) in feathers of physiologically healthy Arbor Acres broilers at different ages (7, 21 and 35 days). Following international veterinary standards for calculating percentile intervals to determine the boundaries of the physiological norm in the analyzed sample (2.5 and 97.5 percentiles at CI = 90%), we ranked the obtained data for each age of the bird. These results may serve for identifying elementosis in Arbor Acres cross chickens.

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