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FORTIFICATION OF MEAT PRODUCTS OF GEESE FARMING WITH LITHIUM BY INTRODUCING IT INTO POULTRY MIXED FEED

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ABSTRACT. We studied the possibility of fortification of goslings' products with lithium and peculiarities of its depositing in the organs and tissues of goslings concerning lithium level in the mixed feed. Experimental studies have been conducted on the goose breed Legart. 320 one-day-old goslings were divided on the principle of analogues into four groups, 80 heads each. The goslings of the first control group did not receive the lithium supplement with the feed mix. Experimental groups were fed with the feed where additionally was supplemented with different doses of lithium by the scheme of the experiment. After 70 days of rearing, three birds were randomly selected from each group and control slaughtered. The lithium content in the representative samples of muscle tissue and organs of goslings was determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES). It was established that feeding the growing goslings with mixed feed containing lithium supplements in doses of 0.05, 0.10 and 0.15 mg kg^{-1}, contributed to the increase ($P < 0.001$) of the concentration of this trace element in the muscles of the thigh and drumstick 789.5, 1589.5 and 3447.4%, in the muscles of the breast 1096.8, 2080.6 and 3948.4%, liver are 455.4, 824.6 and 1440.8% respectively, compared to goslings that did not receive lithium supplements. Significant high values of lithium accumulation factors in organs and tissues of gosling (3.21–14.44) indicated that this element has a substantial accumulating capacity. The meat of goslings enriched with lithium can be considered a natural product with bio-corrective action that can be used in human nutrition. These meat products can be particularly useful for people that are living in regions with a low environmental level of lithium.

Introduction

Currently we know more than 50 mineral elements that are constantly present in humans and animals. Recently, it has experimentally proved the vital necessity of several trace elements, which previously were considered conditionally essential. Lithium is one of these biogenic elements. The biochemical mode of action of lithium is diverse and related to the action of hormones, enzymes, vitamins, trace elements and transcription of the genes that regulate cell growth (Mikosha *et al.*, 2017).

The results of numerous scientific studies conducted on different species of animals and poultry demonstrated that lithium has anti-stress, adaptogenic (Miftahutdinov, Terman, 2014; Ostrenko *et al.*, 2017), antiviral (Ren *et al.*, 2011; Chen *et al.*, 2015; Cui *et al.*, 2015; Qian *et al.*, 2018), antibacterial (Khalid *et al.*, 2014; Stachelska, 2015), radioprotective (Antushevich *et al.*, 2013), antitumor (Kaufmann *et al.*, 2011), anti-metastatic (Maeng *et al.*, 2016), antioxidative (Khairova *et al.*, 2012; Plotnikov *et al.*, 2016) and immunomodulatory properties (Rybakowski, 1999; Maddu, Raghavendra, 2015). Experimental data indicates the positive effects of lithium on osteogenesis (Clement-Lacroix *et al.*, 2005; Wang *et al.*, 2015).

Lithium has been successfully used in medicine as an effective remedy for the prevention and treatment of many diseases, including bipolar disorder, manic and depressive phases (Machado-Vieira *et al.*, 2009; Malhi *et al.*, 2017), dementia (Mauer *et al.*, 2014; Gerhard *et al.*, 2015), Alzheimer's disease (Matsunaga *et al.*, 2015;

Nunes *et al.*, 2015), Parkinson's and Huntington's diseases (Lazzara, Kim, 2015), some types of cancers (Li *et al.*, 2015; Berk *et al.*, 2017), osteoporosis (Tang *et al.*, 2015). Low doses of lithium reduce total mortality and promote the prolongation of human life (Zarse *et al.*, 2011).

Considering all the above, the provision of the human body with lithium in optimal quantities is essential. Currently, the majority of the world's population consumes less lithium than required. The exceptions are some geographical regions such as Northern Chile and Northern Argentina (Sobolev *et al.*, 2019; Szklarska, Rzymski, 2019).

The level of lithium consumption (mg day^{-1}) by the population varies from country to country: Belgium – 0.001–0.015; Canada – 0.022; Finland – 0.035; France – 0.048; Turkey – 0.029–0.051; Spain – 0.011–0.105; England – 0.107; Austria – 0.348; Germany – 0.182–0.546; Japan – 0.812; USA – 0.429–0.821; Denmark – 1.009; Sweden – 1.09; Mexico – 1.485; China – 1.560 (Van Cauwenbergh *et al.*, 1999; Schrauzer, 2002; Kalonji *et al.*, 2015).

People with low lithium status are impaired in tissue growth and reproductive function, life expectancy is reduced due to premature ageing, and they exhibit increased aggressiveness and behavioural problems. Using correlation analysis methods, the researchers have established an inverted relationship between the level of lithium in the human body and the level of suicide among the population, as well as the level of violent crimes such as murder, rape, and robbery (Giotakos *et al.*, 2015; Kohno *et al.*, 2020).

There are two main ways of providing the organism with this microelement: 1) taking lithium supplements in form of inorganic or organic compounds and 2) consumption of lithium-enriched food. There are reports of ways to increase the concentration of lithium in vegetables and fruits, as well as products of their technological processing (Pifferi, 2017). However, the number of scientific publications on the possibility and methods of enrichment of poultry products with lithium for human consumption is scarce.

Miftakhutdinova *et al.* (2020) reported that with the introduction of lithium additives in feed for broiler chickens at a dose of 66.0 mg kg^{-1}, its concentration increased in white meat by 211.1%, in red meat by 426.4% and in the liver by 257.6%, compared with the control group, which did not receive lithium with food. In another paper, the authors argued that even with short-term feeding of broiler chickens (five days) feed mixed enriched with lithium at the rate of 47.5 mg kg^{-1}, its concentration in white meat increases by 33.6%, in red at 104.2% (Miftakhutdinov *et al.*, 2021). It should also be noted that recently the technology for the preparation of paste from poultry meat enriched with lithium has been developed (Miftakhutdinova *et al.*, 2021).

Analysis of the results of experimental studies suggests that the amount of lithium deposition in poultry meat depends on its content in the diet, the form of the drug and the duration of introduction into the diet. In addition, a significant role in the accumulation of lithium is played by species and breed characteristics of poultry, which are probably due to genetic and physiological factors. Some researchers have found that the introduction of lithium supplements in poultry diets increases the content of protein and fat in the muscles of the chest and legs, as well as their energy and biological value (Grybanova, Sobolev, 2014), improves organoleptic and technological characteristics of meat (Miftakhutdinova *et al.*, 2020).

Our research aimed to study the possibility of fortification of gosling's meat with lithium and the investigation of peculiarities of lithium deposition in organs and tissues of goslings, depending on the level of this element in the feed mix.

Materials and Methods

Birds and experimental conditions

Experimental studies were conducted on goose breed Legart. Four groups of one-day-old goslings were formed on the principle of analogues. The goslings in the first control group did not receive the lithium supplement with the feed mix. Experimental groups were fed with the feed mixed that additionally was supplemented with different doses of lithium by the scheme of the experiment (Table 1). Each group had 80 birds. The duration of the experiment was 70 days and matched the growth period of goslings for meat. At the end of the rearing period, the average live weight of goslings was 4251.0 g in the control group 1, 4309.5 g in the experimental group 2, 4324.2 g in the experimental group 3 and 4354.7 g in the experimental group 4.

Table 1. The scheme of the feeding of goslings during the experiment

Group	Lithium additive in mixed feed, mg kg^{-1}
1 control	Complete mixed feed (CMF)
2 experimental	CMF + 0.05
3 experimental	CMF + 0.10
4 experimental	CMF +0.15

During the experiment, goslings were fed with the dry mixed feed balanced on the main nutritive and biologically active substances according to the existing norms (Table 2). Feed for the goslings was supplied *ad libitum* throughout the trial period. Lithium in feed for goslings was introduced as part of the mineral premix in the nano-aquachelated form obtained from Nanomaterial and Nanotechnologie Ltd. (Ukraine).

The birds were kept on the floor with free access to food and water. Technological parameters of gosling's keeping in all groups were similar and corresponded to the existing standards, by the national recommendations for young goslings. The density of planting goslings aged 1–3 weeks was eight birds per m^2 and at the age of 4–10 weeks four birds per m^2. The feeding front of goslings at the age of 1–3 weeks was 1.5 cm per bird, and at the age of 4–10 weeks 2.5 cm per bird. The watering front of goslings at the age of 1–3 weeks was 1.5 cm per bird and at the age of 4–10 weeks 2.0 cm per bird.

Table 2. Composition and nutritional value of complete compound feeds for goslings

Component, %	Age of goslings, weeks	
	1–3	4–10
Barley	9.50	12.50
Wheat	64.70	–
Corn	–	56.20
Wheat crops	–	7 00
Sunflower meal	3.00	5.00
Sunflower oil	1.00	–
Meat and bone meal scraps	5.00	5.00
Fish meal	9.50	4.00
Feed yeast	3.00	5.20
Lysine-NSI	0.06	0.20
Methionine	0.16	0.13
Salt	0.32	0.45
Monocalcium phosphate	0.26	0.75
Limestone	2.00	2.07
Vitamin premix	0.50	0.50
Mineral premix	1.00	1.00
Content in 100 g of mixed feed		
exchange energy, kcal	280.1	277.3
crude protein, g	20.0	17.1
crude fibre, g	2.7	3.4
calcium, g	1.4	1.3
phosphorus, g	0.8	0.8
sodium, g	0.3	0.3
lysine, g	1.0	0.9
methionine + cysteine, g	0.8	0.7

Sample collection

At the end of the scientific experience, at the age of 70 days, three birds (1 male and 2 females) were randomly selected as representatives from each group and their control slaughter was carried out by the law requirements (Zakon Ukrai'ny, 2006). After the controlled slaughter of the gosling, complete anatomical disassembly of their carcasses was carried out according to the existing guidelines (Lukashenko, 2013). During anatomical dissection of goslings' carcasses, representative samples of muscles of the thigh, drumstick, breast muscles and liver were collected according to state standards. Each sample was packed in a thick, moisture-proof plastic bag. From the moment of sampling to the beginning of the analysis, samples were stored at a temperature of 0 to 2 °C for no more than 24 hours (GOST 7702.2.0-95, 2009).

Chemical analysis

The chemical analysis of muscle tissue and poultry organs for lithium content was carried out in the certified laboratory of analytical chemistry and monitoring of toxic substances of the State Institution Kundiiev Institute of Occupational Health of The National Academy of Medical Sciences of Ukraine (Kiev).

The lithium content in the muscle tissue and organs of poultry was determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) using Optima 210 DV by Perkin Elmer (USA). The operation of the spectrometer was controlled by WinLab32 software. The results were processed by the device and displayed on the monitor in the required format.

Preparation of samples for analysis was carried out according to the guidelines (Andrusyshyna *et al.*, 2014). Samples were prepared for analysis in two stages. In the first stage, the muscles of the thigh, drumstick, breast and liver were dried to a constant mass in a drying cabinet (Memmert UF55, Germany) at a temperature of 103 ± 2 °C (DSTU ISO 1442:2005, 2008). Moisture content in the muscles of the thigh and drumstick the average for the groups ranged from 71.4 to 74.3% and in the muscles of the breast – from 73.2 to 74.8%. In the second stage, 0.1g of dried muscles of the thigh, drumstick, breast and liver were added to 2.0 ml of concentrated nitric acid (HNO_3) (Merck, Germany) followed by mineralization in the microwave MWS-2 (Berghof, Germany). The resulting mineralized substance was dissolved in deionized water (18 Ω) at a volume of 10 ml and analyzed by the ICP-AES.

The intensity of the biological accumulation of lithium in the muscle tissue and liver of goslings was estimated by the accumulation coefficient (AC), which was calculated by the formula:

$$\mathrm{AC} = \frac{\mathrm{CT}}{\mathrm{D}}, \tag{1}$$

where CT – is the lithium content in the tissue of muscle or liver of goslings mg kg^{-1} of fresh tissue; D – is the dose of the introduction of lithium in the feed mix, mg kg^{-1}.

Statistical analysis

The computer program of statistical processing of Microsoft Excel 2010 was used for the mathematical processing of obtained results. To detect a statistically significant difference between the mean values in the experimental groups' analysis of variance (one-way ANOVA procedure) was used. Differences between average values were considered statistically significant at $P < 0.05$.

Results and Discussion

The obtained data showed that with the increase of lithium levels in compound feeds for goslings, its concentration in the muscle tissue and liver of poultry probably increased (Fig. 1).

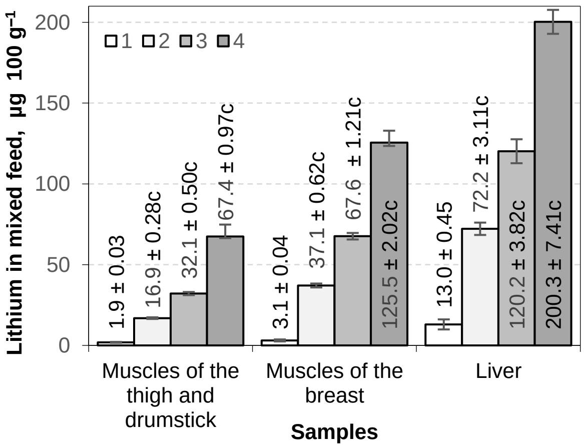

Figure 1. The concentration of lithium in muscle and liver of 70-days-old geese (± sd), µg 100 g^{-1} fresh tissue (experimental groups: 1 – control, 2 – 0.05, 3 – 0.10 and 4 – 0.15 mg kg^{-1} Li content in the mixed feed, c – similar lowercase letter indicate the statistical difference between the control group and all research groups was considered reliable at $P < 0.001$)

Analysis of the samples of muscle tissue of goslings demonstrated that in the muscles of the thigh and drumstick of the second experimental group the concentration of lithium was 16.9 ± 0.28 µg 100 g^{-1} (P <0.001), the third group was 32.1 ± 0.50 µg 100 g^{-1} (P <0.001) and the fourth – 67.4 ± 0.97 µg 100 g^{-1} (P <0.001) while in the control group the concentration of lithium in the same tissue was only 1.9 ± 0.03 µg 100 g^{-1}. Thus, the lithium content in these groups increased by 789.5, 1589.5 and 3447.4% correspondingly.

In the breast muscles of the control group of goslings, the concentration of lithium was 3.1 ± 0.04 µg 100 g^{-1}, while in their peers from the second experimental group it was 37.1 ± 0.62 µg 100 g^{-1} (P <0.001) or 1096.8% higher than in the control group, in the third group it was 67.6 ± 1.21 µg 100 g^{-1} (P <0.001) or 2080.6% of what was in the control and the fourth was 125.5 ± 2.02 µg 100 g^{-1} (P <0.001) or 3948.4% higher than in control.

The liver is commonly considered the main trace elements depot in the animal and poultry bodies (Falandysz, 1991; Suttle, 2010; Counotte *et al.*, 2019). Therefore, expectedly the maximum concentrations of lithium were found in the liver tissues of goslings. Like in the muscle tissue, the concentrations of lithium in the liver also are depended on its content in the given feed mix. In particular, the concentration of lithium in the liver of goslings from the second experimental group was 72.2 µg 100 g^{-1} (P <0.001), the third was 120.2 (P <0.001) and the fourth 203.3 µg 100 g^{-1} (P <0.001) while in the control birds the amount of lithium in the liver was 13.0 ± 0.45 µg 100 g^{-1}. Proportionally the difference in lithium concentration between the control and experimental groups was slightly less significant than in muscle tissue. Thus, in the second, third and fourth groups the liver's lithium was correspondently 455.4, 824.6, 1440.8% higher than in the control group.

The calculations of the coefficients of dose-dependent accumulation of lithium in different tissues revealed that they were not linear *i.e.* the increase in the concentration of lithium in the body of goslings was not proportional to the amount of lithium consumed with the feed mix (Fig. 2).

Thus, when the lithium was supplied at a dose of 0.05 mg per 1 kg of feed mix, the accumulation coefficient in the gosling's muscles of the thigh and drumstick of the second experimental group was 3.38, in the muscles of the breast was 7.42 and, in the liver 14.44. Although the third experimental group was fed with a feed mix enriched with lithium at a dose of 0.10 mg kg^{-1}, the corresponding coefficients were slightly lower compared to the second group and amounted to 3.21, 6.76 and 12.02 respectively. The goslings of the fourth experimental group received a feed mix enriched with lithium at a dose of 0.15 mg kg^{-1}. For them, the coefficients of accumulation of lithium in two groups of muscle and liver were 4.49, 8.37 and 13.35. Thus, the intensity of the biological accumulation of lithium in the muscle tissue and liver of the geese was undulating. High values of lithium accumulation factors in organs and tissues of gosling (3.21–14.44) indicated that this element has a substantial accumulating capacity.

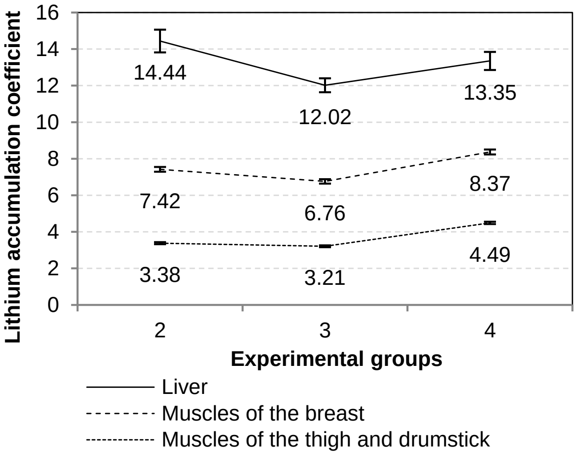

Figure 2. Lithium accumulation coefficient in the muscle tissue and liver (± sd) of the goslings from experimental groups (experimental groups: 2 – 0.05, 3 – 0.10 and 4 – 0.15 mg kg^{-1} Li content in mixed feed)

The obtained results are consistent with the findings of other scientists who in experiments on broiler chickens found that with an increase in the level of lithium in mixed feed its concentration in poultry products increases. However, data on the effect of adding different doses of lithium to mixed feed on its accumulation in the organs and tissues of broiler chickens are contradictory and do not always allow correct comparison. Miftahutdinova *et al.* (2020) reported that the introduction of lithium additives in mixed feed for broiler chickens at the rate of 66.0 mg kg^{-1} helped to increase its concentration in white meat to 4.18 mg kg^{-1}, in red meat increase up to 4.52 mg kg^{-1} and in the liver increase up to 3.40 mg kg^{-1}. The difference compared to the control group was 211.1, 426.4 and 257.6%.

In their later study, Miftakhutdinov *et al.* (2021) obtained results that prove that when feeding broiler chickens five days before slaughter with the mixed feed which was enriched with lithium at the rate of 47.5 mg kg^{-1}, lithium concentration in the white meat was 1.43 mg kg^{-1}, in red meat was 1.54 mg kg^{-1}, which is 33.6 and 104.2% more than in the control group. In broiler chickens of the control group, which were raised with the natural content of lithium in mixed feeds its concentration in white and red meat in the first research accordingly was 1.98 and 1.06 mg kg^{-1} and in the second study was 1.07 and 0.71 mg kg^{-1}.

At the same time, there is evidence in the literature that in the absence of lithium supplements in feed, the average content of lithium in the breast muscles of broiler chickens is 2.581 mg kg^{-1}, and in the leg muscles is 2.130 mg kg^{-1} (González-Weller *et al.*, 2013). Differences in the concentrations of lithium in broiler chicken meat of the control groups can be explained by differences in geochemical zones feeding conditions in which research was conducted and the composition of the diets. In general, this does not contradict previously published data that in poultry meat, lithium levels can range from 0.006 mg kg^{-1} (Leblanc *et al.*, 2005) up to 3.217 mg kg^{-1} (Mueller *et al.*, 2010).

Our experimental data once again confirmed the dependence found previously by other researchers and allows us to state with a high degree of confidence that poultry meat products can be enriched with lithium by introducing it into poultry feed. This approach will also exclude cases of toxicosis in the population, in the case of excessive consumption of individuals enriched with micronutrient meat, due to the buffer effect of animal tissues. It should be noted that the threshold of the toxic dose of lithium ranges from 90 to 200 mg day^{-1} for humans and it depends on the sex, body weight, age and physiological status of a particular individual (Shaposhnikova, Bolgova, 2012).

At present, there are no official recommendations of FAO/WHO experts on dietary standards of lithium consumption for humans (WHO/IAEA/FAO, 1996). At the same time, an adequate and upper acceptable (safe) level of lithium consumption has been established by separate countries for their citizens. For example, in Russia, these limits are set accordingly to 0.1 and 0.3 mg day^{-1} (Tutel'jan *et al.*, 2004). Existing research recommends that the suggested dose of lithium consumption with food and water for an adult weighing 70 kg be set at 1.0 mg day^{-1} (Schrauzer, 2002). Subsequently, the recommended dose of daily lithium consumption is 14.3 µg per 1 kg of human body weight (Aral, Vecchio-Sadus, 2008).

Since muscle tissue, unlike the liver, has a significantly higher portion than the edible parts of the poultry products, it can be considered the main source of lithium for humans. Our calculations show that the consumption of lithium-enriched goslings' meat within the recommended physiological norms in Ukraine (145 g day^{-1} meat and giblets) (Postanova Kabinetu Ministriv ..., 2016) on average can cover from 3.9 to 14.0% of the daily requirement of an adult in this trace element, depending on gosling's diet and hence on the concentration of lithium in gosling's products. Based on the proposed dose of daily lithium consumption at 14.3 µg per 1 kg of body weight, it is possible to estimate the level of physiological needs in this trace element in children and adolescents. Similar calculations can be made for the main social and demographic groups of the population in other countries considering the norms of consumption of meat and meat products operating in these countries.

Our experimental data allow us to assert with a high degree of confidence that gosling's meat products can be enriched with lithium by introducing it into the all mash for poultry. This approach will also exclude cases of toxicosis in the population, in the case of excessive consumption of individuals enriched with micronutrient meat, due to the buffer effect of animal tissues.

Conclusion

The amount of lithium in poultry products for human consumption and particularly in geese products can be efficiently regulated by supplementing feed mixes for birds with lithium additives. Analysis of the collected data allowed us to establish some facts and patterns: (i) lithium was found in all samples that were studied; (ii) with an increase in the level of lithium in the feed mix for geese its concentration in muscle tissue and liver of poultry was increasing correspondently; (iii) the concentration of lithium in tissues and organs increases in the following range: the muscles of the thigh and drumstick < muscles of the breast < liver. From the food hygiene point of view the introduction of lithium in the nano-aquachelated form into the feed mix for goslings at doses of 0.05, 0.10 and 0.15 mg kg^{-1}, provides an increase in the concentration of this trace element in the muscles of the thigh and drumstick respectively at 789.5, 1589.5 and at 3447.4% higher, in the muscles of the breast at 1096.8, 2080.6 and at 3948.4% higher, liver at 455.4, 824.6 and at 1440.8% higher when compared to similar indicators in goslings, which were fed with feed mix without lithium additives. Non-linear increase of lithium accumulation coefficients in the muscle tissue and liver of geese with the dose of lithium in the feed mix may indicate a stepwise biochemical mechanism of lithium metabolism in the organism. Additional research is required for the elucidation of lithium homeostasis.

Our results demonstrate that the meat of young goslings can be significantly enriched with lithium, and consequently can be considered a natural lithium fortified product that may be included in daily the diet of the human. These meat products can be particularly useful for people who are lived in regions with low levels of lithium in the natural environment.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

OS, OOB – study conception and design, drafting of the manuscript;
OS – acquisition of data;
OS, OOB, IR, OK – analysis and interpretation of data;
IR – critical revision and approval of the final manuscript.

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