DOI: https://doi.org/10.21323/2414-438X-2023-8-1-26-33

Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

Received 09.01.2023
Accepted in revised 28.02.2023
Accepted for publication 06.03.2023

EFFECT OF ORGANICALLY BOUND IODINE IN CATTLE FEED ON HEALTH INDICATORS

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Keywords: *organically bound iodine, iodotyrosine, cattle, livestock breeding*

Abstract
Currently, the problem of iodine deficiency is actual in the world, which may cause a large number of diseases and disorders. The problem of iodine deficiency for humans may be partly solved by enriching agricultural products with iodine, i.e. by providing animals with an increased intake of iodine during their growth. Theoretically, the most effective way to use iodine is the form bound to tyrosine, since diiodotyrosine has been proven to be a thyroxine precursor. Taking it into account, a supplement was developed containing iodine organically bound to tyrosine and histidine. In this work, we studied the effect of this supplement introduced into the diets of cattle on biochemical parameters of animal blood. In the test group, which received the supplement with organically bound iodine, an improvement in nitrogen metabolism was noted compared to the control group. This was most clearly demonstrated by the content of urea, since in the test group, its content decreased by ≈15 percentage points, and by the content of creatinine, since its increase in the test group was more than 20 percentage points. Differences in the parameters of carbohydrate and lipid metabolism between treatments were also noted, as in the blood of animals from the test group, the content of cholesterol, triglycerides, phospholipids, glucose and malondialdehyde was lower than in the control group. In mineral metabolism and morphological parameters, there was no significant difference between treatments. Among the indicators of pigment and hormone metabolism, it is important to note the reduced content of cortisol in the blood of animals from the test group. Its level was lower by ≈17.23 percentage points compared to the control group. A decrease in cortisol levels indicated a lower stress load in the test group. In general, studies have shown that the use of a feed supplement containing organically bound iodine has a positive effect on the metabolism of animals.

For citation: Lukin, D.E., Utyanov, D.A., Milushev, R.K., Vostrikova, N.L., Knyazeva, A.S. (2023). Effect of organically bound iodine in cattle feed on health indicators. *Theory and Practice of Meat Processing*, 8(1), 26-33. https://doi.org/10.21323/2414-438X-2023-8-1-26-33

Introduction

To maintain health and immunity, the human body needs a wide range of microelements, macronutrients and vitamins. Currently, the conventional lifestyle does not allow proper monitoring the diet. To a greater extent, this applies to residents of large industrial cities, where it is especially important to maintain immunity due to poor ecological conditions. Many people do not think about microelement and macronutrient deficiencies in their diet until such a deficiency leads to serious illness. It is also worth noting the large-scale nature of commodity production, the main goal of which is not the quality of products, but the profit from their sale. That's why manufacturers reduce the price of production technology.

Iodine is one of the microelements essential for humans. In the body of animals and humans, iodine is part of the thyroid hormones produced by the thyroid gland, i.e. thyroxine and triiodothyronine. These hormones play an important role in the development, growth and metabolism of the body [1]. According to WHO, the average daily intake of iodine for the humans is 120 to 150 micrograms. At the same time, it should be noted that natural analogues of the studied supplement have not been studied and there are no statistical data on their reference intake in both humans and animals.

Lack of iodine leads to a large number of diseases, including cretinism, thyroid disease, hypothyroidism. Even a small deficiency of iodine leads to fatigue, laziness, headaches, depressed mood, causes nervousness and grumpiness, reduces intelligence and weakens memory. One of the most important tasks that must be solved with the elimination of iodine deficiency is a healthy generation of people and procreation. In general, iodine deficiency diseases are one of the most common non-communicable diseases. According to WHO, more than 2 billion people are iodine deficient [2].

Often, the problem of iodine deficiency is tried to be solved with dietary supplements and iodized salt. But iodine in the inorganic form is more difficult to absorb by the body than iodine in the organic form (for example, associated with the amino acid tyrosine in the form of mono- and diiodotyrosines). Therefore, the most relevant direction for enriching the diet of people with iodine is the enrichment of food with the organic form of iodine, i.e. iodine covalently bound to the amino acid.

The best options in this case are either consumption of products with already high content of bound iodine, or production of plants and animals using iodine, so it is consumed by humans in its natural form.

More and more works are currently focused on this approach. Enrichment of feed with iodine during animal maintenance increases the amount of iodine in the final raw materials, which improves product quality and increases attractiveness to consumers. Moreover, it has a positive effect on the health of animals themselves due to the same need for iodine like in humans.

For example, lettuce was grown with the addition of iodine to the soil in an inorganic form of potassium iodate and in an organic form bound to salicylic acid [3]. As a result, it was found that in the leaves of lettuce grown with the addition of iodine to the soil, it accumulated more than in the control samples without soil enrichment with iodine. It has also been found that organic iodine accumulates better than inorganic iodine.

Significantly increased iodine amount in the leaves and fruits of tomato was demonstrated [4]. Similarly to the above work [3], the enrichment was carried out with potassium iodate. In addition to potassium iodate, potassium iodide was used, as well as their mixtures with salicylic acid. The results showed that the use of potassium iodate had the best effect on the nutritional value of tomatoes. It is not recommended to use these clathrates directly in human nutrition due to their toxicity and negative effects on mucous membranes.

Animal breeders are also engaged in similar work. The most suitable direction in enrichment of products with an organic form of iodine through feeding them to animals is the dairy industry.

Milk itself is a rich source of iodine [5]. This is also why it is important to add iodine to the diet of dairy cows in order to supply them with iodine that goes into milk. But before considering the issue of cattle diet enrichment with iodine, it should be considered in other areas of livestock breeding.

Works focused on animal diet enrichment with iodine have been carried out for a relatively long time. So, the effect of iodine on the health of goats, both castrated and uncastrated, was studied [6]. In addition to a positive effect on health, iodine supplementation has been shown to improve seminal properties, i.e. sperm/ejaculate volume, motility, concentration and count.

Russian scientists are also working to enrich the diet of small cattle, in particular sheep, with essential elements. According to published studies, in test groups of buck lambs that received additional iodine, selenium and silicon in their diet, higher parameters of live weight gain were noted. In one of the test groups, greater chest girth and greater height were noted. An 18.8% higher content of free amino acids compared to the control group was noted. The meat contained more protein and less fat [7].

The results of the above work [7] showed the effect of enriched diets in the production of small cattle on the fatty acid composition, activity of lipolytic enzymes, and lipid oxidation process. A significant dependence of the fatty acid composition of lamb meat on the content of microelements in the supplement (in particular, iodine and selenium) was shown. In test samples, a more balanced fatty acid composition was noted with near optimal ω6/ω3 ratios.

Further studies in this direction [7] showed that the use of supplements containing iodine in a natural bound form led to an increased iodine content in the thyroid gland of test groups by 5–6 times compared to the control group. In this case, iodine accumulated in the thyroid gland in an organic form of iodotyrosine. This clearly shows the effectiveness of supplements containing iodine in organically bound form.

In addition to the described benefits of enriching the diet of buck lambs with essential elements, the meat obtained from such animals contains more iodine, which makes it a functional product with more beneficial properties for humans. According to the above work [7], iodine content varied in the range of 54.6 to 61.5 μg/kg.

When enriching the diet of cattle with microelements, in addition to the benefits described, milk enriched with iodine obtained from lactating cows is of great interest. This is confirmed by the results of work, where iodine in the form of iodate and iodide was added to the diet of cows [8]. But, when enriching the diet of animals with iodine, especially in inorganic form, it is important not to exceed the reference levels required for the body [9]. Concerning the milk enriched in this way, the products of its processing will also have a high content of iodine. What was shown in the relevant work [10].

In terms of biology, the enrichment of animal diet with an organic form of iodine will be more favorable for them. As such sources, either complex supplements containing iodine in the form of mono- and diiodotyrosines, or natural sources of iodine in organic form, may be used.

In addition to obtaining better quality raw materials as a result of enriching the diet of cattle with essential elements, it is necessary during lactation. A well-known scientific fact: if a heifer does not meet daily weight gain parameters, then high production characteristics cannot be expected from her. High-yielding cows with a daily milk yield of 30–35 kg excretes from the body about 1000 g of protein, 1500 g of lactose, 1000 to 1200 g of fat per day, which is ensured by a high-intensity metabolism. The realization of such a genetically defined productivity potential is determined by the full-value feeding and depends 50% on metabolizable energy, 25% on protein and 25% on minerals and vitamins. Moreover, in the nutrition of such cows, a catalyst for such high metabolic processes should be presented, which is the basis of the hormonal background of the animal, i.e. covalently bound iodine [11].

The high milk yield leads to a weakening of natural defense mechanisms, immunity, failures in metabolism mechanisms and, as a result, reproductive system diseases, udder diseases, musculoskeletal system disorders and a long recovery period after calving (open period). It is no coincidence that 25% of culls occur at the peak of milk production in the first 60 days of lactation. Often the situation is aggravated by violations in feeding organization, especially during the critical period, i.e. three weeks before calving and in the first three weeks after it, which leads to a negative balance of energy in the body. In the last decade, the average tenure of cows in the herd has decreased to 2.9 to 3.7 lactations, and the payback period for one animal is about 4 to 4.5 lactations. For this reason, annual losses amount to hundreds billion rubles. So, for example, in the USA, 25% to 40% of cows are culled annually; the world average is 35%. Reasons for culling cows in the US are: reproductive dysfunction (27%), mastitis and other udder diseases (27%), lameness/limb injuries (16%), low productivity (19%), aggressiveness (1%), and other reasons (10%). According to foreign data, the total culling of cows should not exceed 25% to 28% [12]. In Russia, due to the lack of clear up-to-date statistics, this parameter is about 32% to 37%, and there is also no well-defined state policy aimed at preserving the dairy herd and its production characteristics.

Unbalanced diets and poor-quality feed are the main causes of metabolic disorders. Its consequences are an increase in barrenness, birth of a weak offspring, decrease in resistance to diseases, decrease in live weight and milk production, deterioration in milk quality, which ultimately leads to premature culling of animals.

With respect to lactating cow diet enrichment with selenium and iodine, Russian scientists received the RU2769978 C1 patent. As a result of patented supplement using, it was possible to increase milk yield and improve biochemical parameters of lactating cows' blood [13].

Another Russian preparation is based on iodine bond to the complex protein, i.e. casein. It was shown that the use of Iodocasein in the diet of cows with hypothyroidism completely restores the concentration of thyroid hormones [14]. The results of the studies showed that the use of Iodocasein in test groups helped to achieve a decrease in milk production by only 2.3%, while in the control group this parameter ranged from 3.3% to 11.8% [15]. Moreover, Iodocasein helped to increase the fat content in milk.

In the mineral supply of high-yielding cows, iodine is of particular importance. With its deficiency in cows, the estrus cycle is disturbed, impregnation capacity and fertility decrease, fetal resorption, miscarriages in the early stages of pregnancy, abortions, retention of the placenta are observed. The birth of dead or non-viable offspring with a goiter (thick neck) is possible. Milk productivity and fat content are reduced. Weight gain in young animals is reduced. This may mainly be due to the content of the inorganic form of iodine in the blood and milk. With an excess of the inorganic form of iodine, which is primarily due to the use of premixes and various supplements to eliminate IDD in calves, there is a decrease in live weight gain and milk production, an increase in feed costs per production unit. Moreover, the existing supplements based on inorganic forms of iodine (iodides, iodates, clathrates and others) are known not to produce the desired result, and negative dynamics are increasingly observed.

Depending on the species, age and physiological state of the animals, the need for iodine ranges from 0.2 to 1.4 mg per 1 kg of feed dry matter. The need for iodine in non-milking cow is 5.4 to 10.7 mg/animal/day depending on the live weight and planned milk yield. The need for iodine in lactating cows with a live weight of 600 kg is 8.9 to 27.7 mg/animal/day depending on the daily milk yield (16 to 40 kg). According to the nutrient reference intake for cows (USA), the need for iodine per 1 kg of dry matter in non-milking cows is 0.33 mg, and per 1 kg of dry matter in lactating cows it is 0.45 mg. It is necessary to point out the fact that these reference values in scientific work were based on the use of inorganic matter, and did not imply other forms similar to natural ones, while the covalently bound form of iodine is much more effective and biologically available for a living organism.

With an iodine content in milk of 30 μg/L, a high-yielding cow excretes about 1 mg of iodine daily. Therefore, when consuming 18 kg of feed dry matter with apparent absorption of iodine of 80% and iodine concentration in feed of 0.1 mg, a cow receives ≈1.4 mg of iodine per day per 1 kg of feed dry matter. However, this amount of iodine does not guarantee that the lower limit of requirements is met, and therefore it is still recommended to give lactating animals 0.4 mg of iodine per 1 kg of feed dry matter. When feeding cruciferous plants, this dose should be increased to 0.8 mg per 1 kg of feed dry matter.

Due to the fact that iodine deficiency is most often detected mineral deficiency, it is recommended to increase its reference intake to 1.3 mg/kg [16]. At the same time, its toxicity and effect on the general condition of the animal's body are not taken into account at all.

In summer period, high-yielding cows eat with feed 28% to 41% of recommended iodine amount. The physiological role of iodine is associated with its participation in the formation of the thyroid hormone thyroxine. Deficiency of correct bound iodine leads to reduced fertility of cows, poor digestibility of dietary nutrients, low milk production and reduced fat content in milk. This is due to several factors, such as a decrease in the total amount of iodine in soils, a decrease in the quality of bacteria organizing iodine on plant roots, a lack of proper education for people who make decisions about how and what to feed, which leads to the absence of covalently bound iodine in feed and premixes.

Recently, interest in iodine-containing supplements has increased significantly, which is associated with an increase in the need for iodine by animals in intensive livestock production, as well as with the deterioration in the general condition of animals throughout the country.

Therefore, it is necessary to reconsider not only the sources of iodine intake in the nutrition of high-yielding cows, but also develop its bioavailable chemical compound.

Such a compound in the present study is the Carbon-Iodine (C–I) formed by a covalently polar bond. To designate the compound, the introduction of the term CI is proposed, and the method of enrichment with this form of bound iodine is CI technology. To solve the problem, a group of Russian specialists with the support of the Research and Testing Center of the Federal State Budgetary Scientific Institution "V. M. Gorbatov Federal Research Center for Food Systems" of the Russian Academy of Sciences developed a supplement based on a covalently bound form of iodine (containing the CI component in its structure) in a protein matrix, and produced on the basis of iodized milk protein, which is a mixture of whey proteins containing 2.5% of iodine atoms covalently bound to them. Iodine is in the tyrosine or histidine amino acid molecule and has a positive valence, due to which it is bioavailable. Due to the covalent bond between iodine and proteins, the supplement has a high stability when heated up to 300 °C, resistance to light and heat during long-term storage, which guarantees iodine content in the production of various premixes and starter feeds for all types of animals. It is especially important in the early stages of animal growth, when the main physical and chemical indicators of future performance are formed.

Based on the above, the aim of this work was to study the effect of adding organically bound iodine to the diet of lactating cows on biochemical parameters of their blood.

Objects and methods

The experiment was carried out at the Zybino farm, Federal State Unitary Enterprise Research Farm "Klenovo-Chegodaevo" of the Federal State Budgetary Scientific Institution "All-Russian Research Institute of Animal Husbandry named after L. K. Ernst", with 2 groups of white-and-black cattle (10 animals in each) during the summer pasture period. The experiment was carried out only on lactating cows. In one group of cows, the supplement was added to the main diet (test group). 100 g of supplement contains: 25 g of crude protein (protein and non-protein nitrogenous substances), 65 g of glucose, 0.7 g of covalently bound iodine, ash elements (≈2%) and moisture (≈7.3%).

The cows that participated in the experiment arrived at the Zybino farm from the Dubrovitsy farm in March. As a result, they suffered transport stress and, before the start of the experiment, were in a state of adaptation to new technological, hygienic and zootechnical conditions, in particular, to the maintenance conditions (shortened stalls), the change from triple to double milking, the new composition and nutritional value of the diet, as well as grazing together with the Zybino cow herd (social stress). The calculation of cows' need for iodine was made on the basis of recommendations by the All-Russian Research Institute of Animal Husbandry [11] and for lactating cows was 0.45 mg of iodine per 1 kg of diet dry matter. Thus, the daily requirement of a lactating cow is approximately 8 to 10 mg of iodine. For feeding test and control animals, the same diet based was used [11]. The control group received iodine in the form of potassium iodide, and the test group received iodine in the form of a developed supplement. Based on the needs of cows for iodine and its content in the supplement, cows in the test group were given 1.3 g of the developed supplement mixed with bran per animal per day. The supplement was added manually to the feed of each cow individually during their feeding. Thus, one animal received ≈9.1 mg of iodine per day. Cows in the control group were given 2 tablets each containing 6 mg of potassium iodide. The tablets were pre-crushed, after which they were added manually to the feed also in a mixture with bran to each cow individually during their feeding. Thus, one animal received ≈9.0 mg of iodine per day.

The experiment lasted for 100 days, except for the period of adaptation to new maintenance conditions. At the end of the experiment, material for determining the following indicators was taken from the animals:

- morpho-hematological blood parameters: (leukocytes, erythrocytes, hemoglobin, hematocrit), color index on the ABC VET analyzer (Horiba, ABZ, France);
- biochemical parameters of serum (plasma): (total protein, albumin, globulin, urea, uric acid, creatinine, malondialdehyde, cholesterol, phospholipids, triglycerides, NEFA, glucose, total bilirubin, ALT, AST, phosphorus, calcium, iron, magnesium, chlorides, alkaline phosphatase) on the Chem Well automatic biochemistry analyzer (Awareness Technology, USA);
- iodine by volt-amperometry method according to GOST R52689–2006[1];
- thyroxine by enzyme immunoassay;
- cortisol by enzyme immunoassay.

Statistical data processing was carried out using the Statistica software ver. 10.0.1011 (StatSoft). For statistical processing, Mann-Whitney U-test was used. At $p \leq 0.01$, the results were considered statistically significant; at $p \geq 0.05$, the results were considered statistically insignificant.

Results and discussion

When assessing the state of nitrogenous substances metabolism in the body of cows, great importance is attached to the content of total protein in the serum, as well as albumins, globulins, their ratio, urea and creatinine.

In cows fed a diet containing CI supplement, the content of total protein ($p \leq 0.01$) in serum was higher by 3.9% with a lower (by 3.4%) content of albumin ($p \leq 0.01$) and higher (by 7.9%) content of globulin ($p \leq 0.01$). As a result, their protein index was lower and amounted to 0.46 versus 0.52 in the control group. This ratio characterizes the level of protein metabolism and its direction associated with the increased need of the body for amino acids and energy. These parameters did not go beyond the physiological reference values, i.e. the content of total protein in serum

[1] GOST R52689–2006 "Foods. Anodic stripping voltammetric method of iodine mass concentration determination". Retrieved https://docs.cntd.ru/document/1200051514/titles Accessed December 27, 2022. (In Russian)

of 72.00 to 86.00 g/L, and albumin content in serum of 27.00 to 43.00 g/L [17–19].

Table 1. Parameters of nitrogen metabolism in the blood of cows, (n = 3)

Parameters	Treatments		Test to control ratio	Test vs. control difference
	Control group	Test group	%	±
Total protein, g/L	85.64 ± 2.50	89.0 ± 2.99	103.9	+3.36
Albumin, g/L	29.2 ± 0.78	28.2 ± 1.33	96.6	–1.0
Globulin, g/L	56.4 ± 2.03	60.8 ± 2.39	107.9	+4.4
A/G	0.52 ± 0.02	0.46 ± 0.03	—	–0.06
Urea, mmol/L	4.81 ± 0.29	4.12 ± 0.34	85.6	-0.69
ALT, IU/L	22.79 ± 2.15	17.67 ± 1.87	77.53	–5.12
AST, IU/L	78.6 ± 0.19	68.99 ± 4.45	87.77	–9.61
De Ritis ratio	3.83 ± 0.43	4.08 ± 0.44	106.53	0.25
Uric acid, mmol/L	123.4 ± 15.79	98.2 ± 5.76	79.5	–25.2
Creatinine, μmol/L	87.8 ± 13.13	106.3 ± 6.98	121.2	+18.6

Urea is formed in the liver during the neutralization of ammonia. It quite accurately reflects the concentration of ammonia in the rumen, the level and quality of dietary protein. In these studies, the level of urea in the serum of the test group was lower by 14.4% ($p \le 0.01$), which was ensured by a well-balanced diet in terms of nutrients and, accordingly, good work of the rumen microbiome during the breakdown of protein and a decrease in ammonia transfer into the blood. The level of urea in the blood of animals that received the experimental supplement as a part of the diet also indicated normal liver function. Other researchers also report this [20, 21].

According to the researches, to assess the functional state of the liver in cows, it is recommended to determine the activity of AST and ALT in the blood, and for accurate differentiation, it is recommended to calculate their ratio (De Ritis ratio) [22, 23]. In the blood of cows from both groups, a high activity of transamination enzymes was noted, i.e. aspartate aminotransferase (AST, EC2.6.1.1) and alanine aminotransferase (ALT, EC2.6.1.2). Parameters of ALT ($p \le 0.01$) and AST ($p \le 0.01$) activity in the blood of animals from the test group decreased by ≈22.47 and ≈12.23 percentage points, respectively. The increase in the De Ritis ratio in the test group compared to the control group is insignificant due to the decrease in ALT and AST in the test group. These values were above the reference limits. In this case, the De Ritis ratio in animals from the control group was 3.83, and in the test group it was 4.08. Beliaeva et al. [23] report the values of this ratio at the level of 2.58 to 3.08. Miller et al. [24] gives the De Ritis ratio for healthy cows as 3.56. The values of this parameter obtained in this work are comparable with the data of these researchers and, in this case, indicate a high metabolic rate to ensure lactation.

Creatinine is a product of protein metabolism and its level in the blood depends both on the intensity of metabolism and on its excretion by the kidneys. In these studies, creatinine content ($p \le 0.01$) in the serum of cows receiving the new supplement was higher by 18.6 μmol/L than in the control group. This may be due to the intensive protein and energy metabolism necessary for the intensive synthesis of milk components. In general, this indicator was within the physiological reference values, i.e. 55.8 to 176.8 μmol/L [25].

The content of creatinine in the serum of test and control cows is consistent with the content of uric acid, a product of the metabolism of nucleic acid purine bases, which reflects the degree of body cells decay. In cows from the test group, the content of uric acid ($p \le 0.01$) was lower by 20.5% and amounted to 98.2 mmol/L compared to 123.4 mmol/L in the control group.

The use of supplement in the diet of animals in a state of homeostasis led to micro-shifts in it. In nitrogen metabolism, these micro-shifts were manifested by an increase in the amount of total protein, globulins, and creatinine concentration, a decrease in the amount of urea and uric acid, reduced activity of AST, ALT, and a higher AST/ALT ratio. The amount of total protein, albumin, uric acid, urea, and creatinine in animals from both groups did not exceed normal values [21]. The values of biochemical parameters obtained as a result of this work are consistent with those given in previous studies [19, 26, 27].

In addition to indicators of nitrogen metabolism, blood obtained from test and control cows was examined for parameters of carbohydrate and lipid metabolism. The results are shown in Table 2.

Table 2. Parameters of carbohydrate and lipid metabolism in the blood of cows, (n = 3)

Parameters	Treatments	
	Control group	Test group
Triglycerides*, mmol/L	0.24 ± 0.03	0.22 ± 0.018
Cholesterol, mmol/L	5.44 ± 0.46	4.25 ± 0.39
NEFA*, mmol/L	0.41 ± 0.06	0.44 ± 0.05
Cholesterol/NEFA ratio	0.09 ± 0.03	0.13 ± 0.02
Phospholipids, mmol/L	3.12 ± 0.38	2.57 ± 0.17
Glucose, mmol/L	4.46 ± 0.14	3.49 ± 0.20
Malondialdehyde, μmol/L	1.98 ± 0.24	1.60 ± 0.14

* $p \ge 0.05$

Of the studied metabolism indicators, the most striking changes were noted in the phospholipids ($p \le 0.01$), i.e. their decrease in the blood of animals from the test group relative to the control group was ≈1.2 times. There was also a decrease in the amount of cholesterol ($p \le 0.01$) in their blood by ≈1.3 times relative to the control animals. In these cows, the glucose content decreased ($p \le 0.01$) relative to the control group and amounted to ≈21.75 percentage points. Cholesterol/NEFA ratio in the test group increased by ≈42.20 percentage points. The amount of malondialdehyde decreased ($p \le 0.01$) by ≈1.18 times in the test group.

The decreased cholesterol level in the blood in animals from the test group was due to its use in the synthesis of milk components, and decrease in glucose was because it provides the body with energy. This is also evidenced by a higher cholesterol/NEFA ratio and a lower level of phospholipids.

Lactation is the most difficult period for cows. During it, the likelihood of developing oxidative stress in these animals is high. It may cause metabolic shifts [28, 29]. However, a decrease in the amount of malondialdehyde in the blood of cows that received supplement indicated that the processes of lipid peroxidation were occurring normally.

Studies on the content of microelements, macronutrients, and alkaline phosphatase in serum showed the following results (Table 3).

Table 3. Parameters of mineral metabolism in the blood of cows, (n = 3)

Parameters	Treatments	
	Control group	Test group
Calcium*, mmol/L	2.68 ± 0.05	2.59 ± 0.08
Phosphorus**, mmol/L	2.43 ± 0.08	2.41 ± 0.16
Alkaline phosphatase activity*, IU/L	108.65 ± 23.80	91.60 ± 6.14
Phosphorus/calcium ratio	0.90 ± 0.02	0.93 ± 0.04
Iron*, μmol/L	17.65 ± 0.78	17.82 ± 0.85
Magnesium*, mmol/L	0.79 ± 0.06	0.99 ± 0.13
Chlorides*, mmol/L	94.12 ± 1.12	94.60 ± 2.15
Iodine*, μmol/L*	0.123 ± 0.010	0.156 ± 0.0025

* $p \leq 0.01$, ** $p \geq 0.05$

For most indicators of mineral metabolism in the blood obtained from animals in both groups, no difference was found. All of them were within the normal values. The greatest differences between the test and control groups were found in the content of magnesium. In animals from the test group, its amount increased by ≈25.32 percentage points relative to control. This fact should be regarded as positive one. This is due to the fact that this microelement plays a significant role in protein, mineral and fat metabolism, digestion in rumen, as well as acts as an activator for protein synthesis, promotes the formation of enzymes and maintains acid-base balance, and has a positive effect on milk production and milk quality [30–33].

It was found that iodine content was higher in the blood of cows from the test group. The increase was ≈26.83 percentage points relative to control. This difference was significant ($p < 0.05$, $n = 3$), indicating a positive effect of the diet containing the studied supplement.

The parameters characterizing the functional state of the liver and endocrine glands were also studied in the blood. The results are shown in Table 4.

Table 4. Parameters of pigment and hormone metabolism in the blood of cows, (n = 3)

Parameters	Treatments	
	Control group	Test group
Total bilirubin, μmol/L	7.65 ± 0.95	7.61 ± 0.48
Cortisol, nmol/L	15.44 ± 5.50	12.78 ± 3.64
Thyroxine, nmol/L	5.04 ± 1.71	6.99 ± 1.69
Thyroxine/cortisol ratio	0.32	0.54

To analyze the excretory capacity and the pigment metabolism by the liver, the amount of total bilirubin in the blood of cows was determined ($p \leq 0.01$). Its level in the blood of cows from both groups was the same. According to Korochkina et al. [22], determination of the bilirubin amount in the blood is necessary to assess the liver function and the intensity of hemolytic processes in the body. Total bilirubin is the end product of hemoglobin breakdown. Its concentration may be increased in the blood when liver is damaged. According to the latest scientific data, normal total bilirubin is 3.4 to 17.1 μmol/L [21]. However, higher values of this indicator for cows were also established, i.e. 8.6 to 18.2 μmol/L [34]. In some works, reference value for this metabolite is stated at the level of 0.5 to 10 μmol/L [24, 28]. Bilirubin values in the blood obtained in this work are comparable with the data of these studies, and correspond to the reference, thus indicating normal liver function. It was found that in the blood of animals from the test group, the level of cortisol ($p \leq 0.01$) decreased by ≈17.23 percentage points, which indicates that the animals in the test group experienced less stress. Therefore, the raw materials obtained from them will have the best organoleptic properties. In the test group, the content of thyroxine ($p \leq 0.01$) increased by ≈38.69 percentage points. The main function of this hormone is the activation of metabolic processes, which is carried out through the stimulation of protein synthesis. Therefore, its increased amount in the blood of animals treated with an iodine supplement and characterized by an intensive protein metabolism is quite reasonable. The values of thyroxin established in the experiment are comparable with those given by other researchers [26, 35]. A decrease in cortisol and an increase in thyroxine led to a decrease in the thyroxine/cortisol ratio.

Blood tests for morphological parameters did not show a big difference between the treatments (Table 5).

Table 5. Morphological parameters in the blood of cows, (n = 3)

Parameters	Treatments	
	Control group	Test group
Leukocytes*, 10^9/L	8.46 ± 0.66	9.03 ± 1.11
Erythrocytes*, 10^{12}/L	8.36 ± 0.23	8.00 ± 0.35
Hemoglobin*, g/L	100.9 ± 2.83	96.3 ± 2.03
Hematocrit*,%	40.5 ± 1.13	38.5 ± 0.95
Color indicator**	0.37 ± 0.01	0.36 ± 0.01

* $p \leq 0.01$, ** $p \geq 0.05$

In addition to the positive effect on health parameters, feeding cows with a diet containing the supplement with CI component had a positive effect on the composition of milk and some of its technological properties, such as milk fat content, lactose content, and dry matter content. The use of the supplement also had a positive effect on the content of somatic cells in milk, and on its thermal stability.

Conclusion

In the studies conducted, it was found that the diet containing organic or covalently bound iodine (CI component) had a positive effect on the homeostasis of cows, which was manifested by an intensive metabolism of nitrogenous substances, i.e. an increase in the amount of total protein, concentration of globulins, creatinine, a decrease in the amount of urea, uric acid, reduced activity of AST, ALT and a higher AST/ALT ratio. In carbohydrate and lipid metabolism, a decrease in blood cholesterol levels was noted in animals from the test group, due to its use in the synthesis of milk components and glucose to provide the body with energy. The intensity of this metabolism was also evidenced by an increased cholesterol/NEFA ratio and normal processes of lipid peroxidation. The established parameters of mineral metabolism in cows from the test group as expressed in increased content of magnesium in the blood and significantly increased iodine level in the blood and milk, confirmed the effectiveness of the diet with the new supplement. The relationship of the above facts allows to conclude that it had an anabolic effect on the metabolism in the body in general, and hormones, liver pigment in particular. All these facts ultimately contributed to an increased milk productivity in animals.

REFERENCES

1. Ryabukha, O., Greguš, M. (2019). Correlation analysis as a thyroid gland, adrenal glands, and liver relationship tool for correcting hypothyroidism with organic and inorganic iodine. *Procedia Computer Science,* 160, 598–603. https://doi.org/10.1016/j.procs.2019.11.041
2. Editorial. (2008). Iodine deficiency — way to go yet. (2008). *The Lancet*, 372(9633), 88. https://doi.org/10.1016/S0140-6736(08)61009-0
3. 3. Smoleń, S., Kowalska, I., Skoczylas, L., Tabaszewska, M., Pitala, J., Mrożek, J. et al. (2022). Effectiveness of enriching lettuce with iodine using 5-iodosalicylic and 3,5-diiodosalicylic acids and the chemical composition of plants depending on the type of soil in a pot experiment. *Food Chemistry*, 382, Article 132347. https://doi.org/10.1016/j.foodchem.2022.132347
4. Smoleń, S., Wierzbińska, J., Sady, W., Kołton, A., Wiszniewska, A., Liszka-Skoczylas, M. (2015). Iodine biofortification with additional application of salicylic acid affects yield and selected parameters of chemical composition of tomato fruits (Solanum lycopersicum L.). *Scientia Horticulturae,* 188, 89–96. https://doi.org/10.1016/j.scienta.2015.03.023
5. Coneyworth, L.J., Coulthard, L.C.H.A., Bailey, E.H., Young, S.D., Stubberfield, J., Parsons, L. et al. (2020). Geographical and seasonal variation in iodine content of cow's milk in the UK and consequences for the consumer´s supply. *Journal of Trace Elements in Medicine and Biology,* 59, Article 126453. https://doi.org/10.1016/j.jtemb.2020.126453
6. Pattanaik, A.K., Khan, S.A., Mohanty, D.N., Varshney, V.P. (2004). Nutritional performance, clinical chemistry and semen characteristics of goats fed a mustard (Brassica juncea) cake-based supplement with or without iodine. *Small Ruminant Research,* 54(3), 173–182. https://doi.org/10.1016/j.smallrumres.2003.10.014
7. Giro, T.M., Kulikovsky, A.V., Knyazeva, A.S., Domnitsky, I. Yu., Giro, A.V. (2020). Biochemical and microstructural profile of the thyroid gland from lambs raised on test diets. *Food Processing: Techniques and Technology,* 50(4), 670–680. https://doi.org/10.21603/2074-9414-2020-4-670-680 (In Russian)
8. Franke, K., Meyer, U., Wagner, H., Hoppen, H.O., Flachowsky, G. (2009). Effect of various iodine supplementations, rapeseed meal application and two different iodine species on the iodine status and iodine excretion of dairy cows. *Livestock Science,* 125(2–3), 223–231. https://doi.org/10.1016/j.livsci.2009.04.012
9. Borucki Castro, S.I., Berthiaume, R., Robichaud, A., Lacasse, P. (2012). Effects of iodine intake and teat-dipping practices on milk iodine concentrations in dairy cows. *Journal of Dairy Science,* 95(1), 213–220. https://doi.org/10.3168/jds.2011-4679
10. Moschini, M., Battaglia. M., Beone, G.M.., Piva, G., Masoero, F. (2010). Iodine and selenium carry over in milk and cheese in dairy cows: effect of diet supplementation and milk yield. *Animal,* 4(1), 147–155. https://doi.org/10.1017/S175173110999098X
11. Nekrasov, R.V., Golovin, A.V., Makhaev, E.A., Anikin, A.S., Pervov, N.G., Strekozov, N.I. et al. (2018). Nutrient requirements for dairy cattle and pigs. Moscow, Russian Academy of Sciences, 2018. (In Russian)
12. PennState Extension. (2020). Cull Rates: How is Your Farm Doing? Retrieved from https://extension.psu.edu/cull-rates-how-is-your-farm-doing Accessed December 2022.
13. Milushev, R., Zharikov, V., Shulaev, G., Dorovskih, V. (2021). Effects of a diet containing flax seed concentrate on indicators of nitrogen exchange in the blood of cows. *Science in the Central Russia*, 3(51), 119–128. https://doi.org/10.35887/2305-2538-2021-3-119-128 (In Russian)
14. Babkina, T.N., Lenkova, N.V., Zavodova, A.A., Solokhina, E.D. (2022). Influence of yodkazein on biochemical indicators with hypothyroidism in cows. *Technologies of the Food and Processing Industry AIC — Healthy Food Products,* 2, 184–190. (In Russian) 10.24412/2311-6447-2022-2-184-190
15. Karabaeva, M.E., Kolotova, N.A., Grinyaeva, Yu, G. (2017). Milk production and milk fat in lactating cows using iodocasein. *Agricultural conferences,* 1, 8–10. (In Russian)
16. Volgin, V.I., Romanenko, L.V., Prokhorenko, P.N., Fedorova, Z.L., Korochkina, E.A. (2018). Complete feeding of dairy cattle is the basis for realizing the genetic potential of productivity. Moscow: Russian Academy of Sciences, 2018. (In Russian)
17. Gromuiko, E.V. (2005). Appreciation of cows' organism state by biochemical methods. *The North Caucasus Ecological Herald*, 1(2), 80–94. (In Russian)
18. Gusev, I.V., Rykov, R.A. (2018). Reference intervals of biochemical blood parameters for control of usefulness of dairy cattle feeding. *Journal of Dairy and Beef Cattle Breeding*, 6, 22–25. (In Russian)
19. Kaneko, J.J., Harvey, J.W., Bruss, M.L. (2008). Clinical Biochemistry of Domestic Animals. Academic Press, 2008. https://doi.org/10.1016/B978-0-12-370491-7.X0001-3
20. Kulikova, N.A. (2017). Study of the content of bilirubin in the blood of cattle. *International Student Research Bulletin*, 4–5, 616–618. (In Russian)
21. Khvostova, O.V. (2004). Biochemical indicators of blood in various functional states of the cattle liver. *Vestnik Vitebskogo Gosudarstvennogo Medicinskogo Universiteta*, 3(3), 23–28. (In Russian)
22. Korochkina, E.A., Nikitin, V.V. (April 14, 2022). *Biochemical markers' analysis of liver damage in dairy cows during the transit period.* Agrarian science in the context of modernization and digital development of the agro-industrial complex of Russia International scientific and practical conference, Kurgan, Russian Federation. (In Russian)
23. Beliaeva, N. Iu., Ashenbrenner, A.I., Chekunkova, Iu.A., Khaperskiy Iu.A. (2019). The study of cows' metabolic status when applying prevention specimens. *Vestnik NGAU (Novosibirsk State Agrarian University)*, 3(52), 74–81. https://doi.org/10.31677/2072-6724-2019-52-3-74-81 (In Russian)
24. Miller, T.V., Konoplev, V.A. (May 25, 2018). *De Ritis ratio of dairy cows.* Nauka agrarnomu proizvodstvu: aktual'nost' i sovremennost'. Belgorod, Russian Federation. (In Russian)
25. Romenskaya, N.V. (2007). Blood disorders in liver dysfunction in cattle. Author's abstract of the dissertation for the scientific degree of Candidate of veterinary sciences Belgorod: Belgorod State Agricultural Academy, 2007. (In Russian)
26. Vasileva, S.V., Konopatov, Yu. V. (2017). Clinical biochemistry of cattle. Saint-Petersburg: Lan, 2017. (In Russian)
27. Abramov, S.S., Goridoves, E.V. (2011). Features of metabolism in highly productive cows in different physiological periods with biochemical changes characterizing polymorbid pathology. *Transactions of the educational establishment "Vitebsk the Order of "the Badge of Honor" State Academy of Veterinary Medicine*, 47(1), 141–143. (In Russian)
28. Walter, L.L., Gärtner, T., Gernand, E., Wehrend, A., Donat, K. (2022). Effects of parity and stage of lactation on trend and vari-

ability of metabolic markers in dairy cows. *Animals*, 12(8), Article 1008. https://doi.org/10.3390/ani12081008
29. Kyreev, I.V., Orobec, V.A., Choricshko, P.A. (2022). Prevention of oxidative stress in cows in the first month of lactation. *Herald of the Kursk State Agricultural Academy*, 6, 91–96. (In Russian)
30. Varakina, E.A. (2008). Increasing dairy cows milk productivity and milk quality by using in rations stern additives, containing magnesium and sulphur. *Proceedings of Lower Volga Agro-University Complex: Science and Higher Education*, 3(11), 70–75. (In Russian)
31. Galert, N.A. (2004). The influence of magnesite on the productivity of dairy cows, the composition and quality of dairy products. Author's abstract of the dissertation for the scientific degree of Candidate of agricultural sciences. Troitsk: Ural State Academy of Veterinary Medicine, 2004. (In Russian)
32. Editorial. (2022). A natural source of magnesium in the diets of cattle, pigs and laying hens. *Combicorma*, 5, 36–37. (In Russian)
33. Ermolova, E.K. (2005). Influence of magnesite on metabolic processes in the body of dairy cows and their productivity. Author's abstract of the dissertation for the scientific degree of Candidate of agricultural sciences. Troick: Ural State Academy of Veterinary Medicine, 2005. (In Russian)
34. Kovtunenko, A. Yu. (2012). Biochemical parameters of blood of cows during adaptation to subzero temperatures. *Modern Problems of Science and Education*, 6, Article 568. (In Russian)
35. Zhukov, A.P., Seryayev, V.L. (2006). The presence of thyroid hormones in dairy cows of the biogeochemical province of East Orenburg areas and beyond its boundaries. *Izvestia Orenburg State Agrarian University,* 1(9), 80–82. (In Russian)

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The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

The authors declare no conflict of interest.