Functional Activity and Morphological Structure of Endocrine Glands at Different Level of Mineral Consumption

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Abstract--- *The article describes the results of the functional morphology of the thyroid gland in heifers grown on diets with macro and micro elements deficiency and with the optimal level of mineral consumption. The aim of the study was to evaluate the morphology of the thyroid gland in heifers grown on diets with macro and micro elements deficiency and with optimal level of mineral consumption. Methods of the study: histochemical, biochemical, laboratory, zootechnic, and statistical. The performed study showed that the optimization of macro and micro elements diet of the replacement heifers led to moderate activation of the function of the thyroid gland, which provided a more intensive growth of the body and organs and optimal time of reproductive and physiological maturation of animals.*

Keywords--- *Activity, Endocrine Glands, Histochemistry, Macro-Microelements, Microscopy, Reproduction.*

I. INTRODUCTION

The increase in the productivity of animals and improvement of their reproductive capacity can be provided only on the condition of a balanced diet composed according to the requirements of the organism in all the nutrient elements, including minerals (Abramkova, 2002; Vasina et al., 2007; Gamko and Samuseva, 2016; Menkova and Andreev, 2016; Andreev et al., 2012; Shulekina et al., 2013). Mineral elements play a significant role in all the physiological processes of the synthesis and degradation, absorption and excretion of substances. They provide favorable conditions for normal activity of the enzymes, hormones, and vitamins, maintain osmotic pressure and pH balance, take part in the digestive, respiratory and hematopoietic processes, protective and reproductive functions of animals, and finally, contribute to the improvement of health status and reproductively (Andreev, 1998; Andreev, 2002; Elovikov and Menkova, 2006; Menkova, 2002; Shevelev, 1996). A deficit or disbalance of macro and microelements is associated with deep morphofunctional changes in cells, tissues, and a decrease in the natural resistance and immune biological response of the organism (Gamko and Pilyaugaitsev, 2018; Mamaev et al., 2012).

Significant changes in the heifers' organism are observed during the period of reproductive maturation (Krisanov, 1988; Mustafin and Skovorodin, 2005). The revealed influence of the conditions of feeding on the

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development of endocrine glands in heifers aged 6-18 months old provided rationale for the studies on morphofunctional status of heifers organism during the period of sexual maturation for the creation of optimum conditions for the growth, development and formation of the reproductive system by means of optimization of diet nutritional content, including microelements (Menkova and Andreev, 2017; Menkova, 2002; Skovorodin and Grebenkova, 2009; Skovorodin et al., 2007).

II. Materials and Methods

The studies were performed on heifers of black and white breed aged 6 – 18 months old that were kept in the facilities of «Dyat'kovo» (Bryansk Oblast, Russia). The feeding plans of animals were balanced by all the nutrients according to the detailed norms of Russian Academy of Agricultural Sciences (2003) based on their age, live weight, productivity, and chemical content of local feed.

Two analog groups of heifers were formed. The groups included 30 heifers. Heifers from Group I (control) received feed according to the in-house plan that was deficient by some macro and micro elements. Heifers from Group II (test) were fed according to the feeding plan with the optimum level of minerals.

The main feeding plans in the summer period included green mass of perennial grass and oat chop, in the winter period – hay, silage, feeding beet, barley chop. Heifers from Group I (15 heads) were fed according to the in-house feeding plan that was deficient by several macro and micro elements. The feeding plan of heifers from Group II (15 heads) contained optimum level of minerals. The main feeding plans of heifers from the control group contained a deficit of mineral elements according to the detailed norms: calcium – 23%, phosphorus – 19%, sodium – 27%, magnesium – 18%, copper – 35%, zinc – 29%, manganese – 17%. The optimization of macroelements in the feeding plan of heifers from the test group was performed by adding disodium phosphate, lime, magnesium oxide, and sodium chloride, and microelements – by adding their sulfates. The animals were fed two times per day according to the in-house schedule, and the housing of animals was free. Daily doses of mineral supplements were mixed with the concentrates, which provided their palatability. The accounting of the feed was performed every decade.

Control slaughtering of heifers (three heads of each age) was performed at the age of 6, 9, 12, 15 and 18 months by the generally accepted method proposed by the All-Russian Institute of Animal Breeding. The authors measured the size, weight of organs and tissues and took samples for further studies.

The material fixed in the Carnoy's fluid or in 10% solution of neutral formalin was dehydrated and waxed by the method of Merculova G.A.

Rotor microtome was used to make sections 5-6 µm thick. After the dewaxing, they were hematoxylin and eosin stained by Pappenheim panoptic and Romanowsky-Giemsa methods.

Collagen fibers were identified by van Gieson's stain and Mallory methods, deoxyribonucleoproteids (DNP) and ribonucleoproteins (RNP) – by Einarson method, glycogen and neutral glicosaminoglicans – by Shabadash method, acidic glycosaminoglyforicans – by Steedman method.

The material fixed in formalin was used to obtain sections by a freezing microtome, which were stained with black sudan by Lizosone method, with Sudan Sh – by Daddy, and impregnated with silver salts by Bilypovsky-Gros.

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The pituitary gland was dissected, weighted and cut in two halves. One part was fixed in sulema, the other – in Bouin's fluid (picric acid). The parts were fixed in paraffin and stained by Galmi by a modified method of staining with alcian blue – acid-Schiff reaction – orange G (pH = 0.2). Besides, the authors used other stains (erythrosine, etc) that allowed them to differentiate the cell types of adenocytes in other animal species. It should be noted that the later do not provide proper differentiation of endocrinocytes in bovine cattle.

Electron microscopy was performed by a generally accepted method. The material was fixed in a cooled 2.5% gluteraldehyde on a phosphate buffer with pH 7.3 with further additional fixation in 1% of osmic acid on a phosphate buffer. The pieces were placed in epon-araldit mixture. After the staining, the blocks were used to obtain ultrathin sections by ultratome Ultrakut, mounted on the supportive nets and blends, contrasted with lead citrate by the method of E. Reynolds. The samples were investigated using electron microscope EMF-100I and EM-125 at the magnification x6000-22000.

The obtained data were processed with a software package for a PC using Student's t-test. Along with the descriptive statistics, the authors calculated mean arithmetic and its error, geometric, square root and cubic mean, variation coefficient, the accuracy of the arithmetic mean, asymmetry, accession, confidence interval. The authors performed correlation, regression, and two-way analysis of variance of the obtained data (Eduvie et al., 1984).

Microimaging was performed with a microscope MBI-15-2 and MBI-6. The film used was photo-32, "Micrat" with green light filter, "ORVOCHROM-UT-18", "Kodak-200" with a blue filter, "ORVOCHROM-UT-17" with mat light filter. Electronograms were obtained on photographic film. The images were printed on photographic paper "Unibrom" with an image magnifier "Belarus" or after the scanning on a laser or ink jet printer and Corel Draw 7.0 software.

III. RESULTS AND DISCUSSION

The weighing of the thyroid glands obtained from heifers of different age showed that there was a significant increase in the glands' mass by the 9th and 12th month of age. It was proved by the calculation of relative organ's weight increase by the method of Brodi (Kostomakhin et al., 2006; Sadirova, 1994; Skovorodin and Menkova, 2002; Skovorodin, 2000). By the 9th month of age, this parameter reached 33.4 – 35.8%, by the 12th month, 24.3 – 24.5% in groups I and II, respectively. Further, it significantly decreased.

A close positive correlation was observed between the increase in the gland mass and body weight. Two-way analysis of variance showed that the influence of the factor B (optimization of the diet) on the thyroid gland mass reached 5.09%.

Thus, at the macroscopic level, the influence of the optimization of the diet on the thyroid gland mass was proved.

With the method of points calculation in the sections stained with hematoxylin and eosin, the authors identified the relative number of points per colloid, epithelium, and stroma of the organ. It was taken for 100% and defined relative volumes of these structures (Table 1).

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Table 1: Relative volumes of colloid, epithelium, and stroma in the thyroid gland of heifers (%)

Age, months	6		9		12		15		18	
Groups	1	2	1	2	1	2	1	2	1	2
Colloid	57±3	56±2	59±3	57±4	56±2	52±4	53±3	48±4	49±3	45±4
Epithelium	36±2	38±3	33±1	34±2	36±3	41±2	40±2	45±3	45±4	48±2
Stroma	7±1	6±2	8±3	9±2	8±3	7±1	7±2	7±1	6±3	7±2

It was established that the relative volume of the colloid varied with age. When the reproductive cycles develop normally, this parameter decreases, which indicates the enhancement of the endocrine function of the thyroid gland. Primarily, this is observed in heifers from Group II. The opposite consistency was observed for the epithelium of the thyroid gland. The relative volume of the organ's stroma remained stable and changed insignificantly.

These consistencies reflected the index of activity that we calculated by the division of the relative volume of epithelium by the relative volume of colloid (Table 2).

Table 2: Index of activity of the thyroid gland

Age, months	6	9	12	15	18
Group 1	0.6	0.6	0.6	0.8	0.9
Group 2	0.7	0.6	0.8	0.9	1.1

It should be noted that heifers from Group I had this parameter stable until the age of 15 months, and it began to increase only during the establishment of proper reproductive cycles. At the same time, heifers from Group II had this parameter slightly decreased by the age of 9 months, and by the age of 12 months and further, a significant increase in the index of activity. This consistency is well seen in Figure 1.

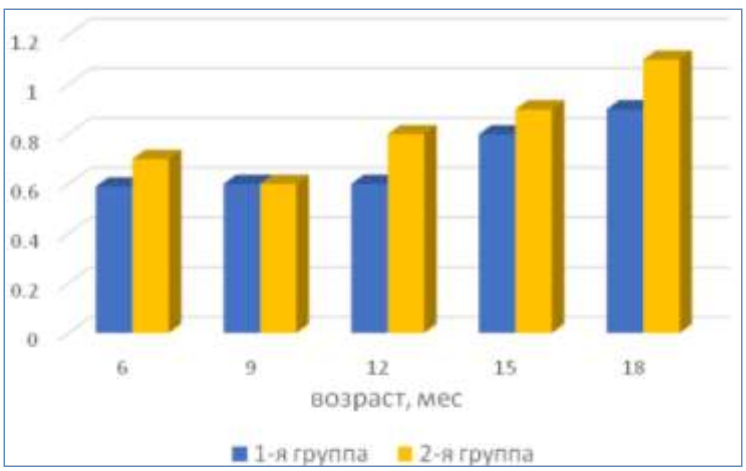

Figure 1: The increase in the index of activity of the thyroid gland in heifers during their sexual maturation

In the central regions of the thyroid glands, follicles of average size prevailed (Figure 2). The follicular epithelium was cubic or cylindrical. Apical ends of the cells were vacuolated. The thyrocytes nuclei were large. Deoxyribonucleoproteids structurally look like small lumps located on the periphery of the karyoplasm. Ribonucleoproteins in the cytoplasm of thyrocytes are spread evenly and their number is moderate. Glycogen was revealed only in seldom cells. Vacuoles of resorption were expressed in the colloid. In general, it was oxyphilic and expressed intensive acid-Schiff reaction (ASR). In larger vacuoles in the center, the colloid was basophilic. Connective tissue was moderately developed. There were not many granulated forms of mast cells.

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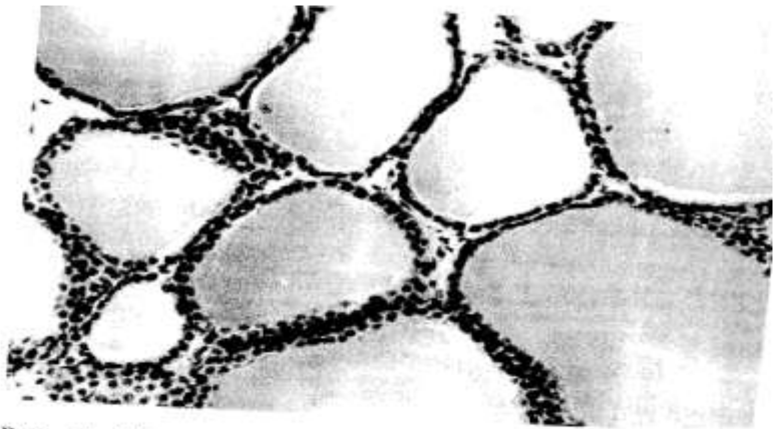

Figure 2: Histologic structure of the thyroid gland, 18-month old heifers from Group 2 with euthyroidism

Heifers from Group I had a larger size of follicles in comparison with heifers from group II. The follicular epithelium was cubic or even flat. Thyrocyte nuclei were more hyperchromatic, and flat epitheliocytes nuclei were flattened. Some follicles contained desquamated epithelium in the state of dystrophy and necrosis. The content of ribonucleoproteins and ASR-positive substances in the thyrocyte cytoplasm was visually lower. The colloid was homogenous with expressed eosinophilic properties and did not contain vacuoles of resorption.

Mallory staining of the colloid gave blue color. 9 and 12-month old heifers had single cyst-like expanded follicles formed due to the fusion and colloid leakages to the surrounding connective tissue. The content of granulated forms of mast cells was increased.

The authors performed a radioisotope study of thyroxin and triiodothyronine that focused on the binding of erythrocytes with exogenous hormones with Iodine-125. Heifers from Group II at the age of 12, 15 and 18 months had the concentration of thyroxin and triiodothyronine lower by 4% and 2% in comparison with animals from Group I ($P<0.05$). These parameters indicate the increased concentration of endogenous hormones, which was also confirmed by morphological studies (Menkova et al., 2014; Menkova and Andreev, 2010; Ball and Peters, 2004; Norris and Lopez, 2010).

Macroscopic study of the pituitary gland in heifers showed that the organ consisted of two main parts: anterior pituitary and posterior pituitary. Anterior pituitary consists of three lobes: anterior (distal), posterior and intermediate. Posterior pituitary consists of the median eminence, gray tuber, pituitary stalk, and posterior lobe. The posterior lobe of the anterior pituitary surrounds infundibular stalk as a cuff, goes up and under the median eminence. Intermediate part lies ventrally to the posterior pituitary and surrounds infundibular stalk. The authors revealed small cone on the intermediate part described by A. Trenkle (Trenkle, 1970).

The distal part of the anterior pituitary gland lies close to the areas described above. On the sagittal section, it had an oval shape and was surrounded by a part of posterior pituitary that had a scyphoid shape of the ventral surface. The distal part was separated from the intermediate one by an intraglandular cleft. The distal part had a protrusive tuberal zone. It has a cranioventral position and is well-fed with vessels, connective tissue, and glandular cells.

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The sagittal section in the median plane in the distal part of the anterior pituitary showed well seen central and peripheral zones. Acidophilic cells prevailed on the periphery. In the center, the trabecules were rich in basophilic cells.

Aldehyde-fuchsin staining in the anterior pituitary of heifers revealed zones that did not have expressed borders but were characterized with certain peculiarities of blood supply and position of cellular elements. The ventral zone was characterized with a presence of large trabecules that had a majority of degranulated and poorly granulated acidophilic cells and single granulated acidophiles and basophils.

In the dorsal zone, the trabecules were small, contained acidophiles and basophils with single chromophobes that contained some orange-positive granules. Acidophiles were large with small evenly spread granules in the cytoplasm, which created its dustiness. The nucleus in such cells was large and close to the size of a nucleus in slightly granulated cells. There were some giant acidophiles.

In the central zone, small trabecules prevailed, which nearly completely consisted of basophiles and intermediate slightly granulated forms. These cells were small, but some had the size close to acidophiles and chromophobes. Their shape was round and somewhat angular. Granules did not fill the cytoplasm completely but were evenly spread in it. Their size varied. The nucleus was large and light with well-stained orange G nucleole. Basophils were located near capillaries.

In the cranial zone, the trabecules were small with the prevalence of basophil cells or consisting of acidophiles filled with granules. The cytoplasm was dark and the granules were large. In the cranial zone, some animals had follicle-like structures with colloid in the center and several layers of cells around the border. Colloid was intensively stained with aldehyde-fuchsin. There were some basophils on the periphery of pseudofollicles. These were small cells with rare granules. The nuclei were small and slightly wrinkled.

In the caudal zone, the trabecules were large, but they contained two-three times more acidophiles than the trabecules of the ventral zone. The heifers at the age of 6 and 9 months old were characterized by the presence of endocrinocyte mitoses. According to the anatomic nomenclature, the following cells are identified: 1) somatotropocytes or STH-cells; 2) prolactinocytes or mammotropocytes or LTH-cells; 3) gonadotropocytes or GTH-cells; 4) thyrotropocytes or TSH-cells; 5) corticotropocytes or ACTH-cells; 6) melanotropocytes or MTH-cells. This classification agrees with the hypothesis “one cell – one hormone”, although it is not confirmed in case of gonadotropocytes, i.e. the possibility of the synthesis of both hormones of one cell was proved by means of immunocytochemical methods.

For the identification of these cell types, the authors performed Halmi aldehyde-fuchsin staining and a modified method of staining with alcian blue-ASR-orange G (pH = 0.2). Besides, the authors used other stains (erythrosine, etc) that allowed them to differentiate cellular types of adenocytes in other animal species. It should be mentioned that they do not provide effective differentiation of endocrinocytes in the bovine cattle, while the mentioned above method of staining with alcian blue-ASR-orange G (pH = 0.2) provides proper differentiation and shows the topography and distribution of the cells in the organ.

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Acidophilic cells (SRH - and LTH-cells). In the studied animals, acidophils were spread primarily on the periphery of the distal lobe of the anterior pituitary, in the center there were small groups of these cells. The authors tried to differentiate two types of acidophils with a combination of orange G and erythrosine. The cells were stained evenly, which made it impossible to differentiate them. Still, Dubois (1968) in his study evidently showed that STH-cells had a regular oval shape and were spread primarily in the central region of the distal lobe of the anterior pituitary. On the contrary, LTH-cells primarily spread in the lateral regions and were rarely met in the central and cranial regions of the distal lobe of anterior pituitary. These facts were taken into account during the cytological studies of these cells.

Somatotropocytes (STH-cells) were observed in the central medial region of the distal lobe of the anterior pituitary. In 6-month old heifers, they have signs of secretory activity. Further, their number increases and they are well-differentiated. They have a round or slightly oval shape and their average diameter was 12 microns. The cytoplasm was thick and contained large orange G-positive granules. Large light nucleus was located eccentrically and contained well visible nucleoli. These parameters do not change in heifers with age.

Prolactinocytes (LTH-cells) were observed in the peripheral regions of the distal lobe of the anterior pituitary as follicle-like groups. In heifers, the number of these cells was high. LTH-cells had a round shape, while STH-cells were primarily oval. They spread in groups and were separated from each other only by poorly visible cellular membranes. The cytoplasm of these cells contained similar large-grained secretory granules that were well-stained in animals with orange G and were ASR-negative. Eccentrically located light nucleus contained one or two well-visible nucleoli. Sometimes, there was a granule-free Golgi area around the nucleus. The number of LTH-cells slightly decreased in heifers with age. It was especially evident in animals with established reproductive cycles and slaughtered at the stage of establishment of the reproductive cycles.

Basophil cells (GTH-, TTH-, ACTH-cells) were observed primarily in the central region as well as in the cranial and caudal ones. They had small diameter of secretory granules, so their cytoplasm looked homogenous during a light-optical study. These cells were differentiated by the tinctorial properties, cytomorphology and their spread in the distal lobe of the anterior pituitary.

Gonadotropocytes (GTH-cells) had various shapes (oval or even polygonal) and lied close to blood vessels. On the periphery of the cells, small granules were localized that were stained blue or cyan with alcian blue (pH = 0.2)-ASR-orange G. Such cells in heifers were relatively evenly spread with some prevalence in the central zone. They contained eccentrically located vesicular nucleus poor in heterochromatin with clearly visible nucleoli, especially in granulated cells. In cells with expressed granulation, the nucleus was pyknotized. In large cells, the Golgi complex was well visible near the nucleus and had a ring-like shape. Smaller cells usually contained tightly granulated cytoplasm and pyknotized nucleus. With age, the number of granulated forms of cells decreased in heifers, especially in those that had growing ovarian follicles.

Thyreotropocytes (TTH-cells) had polygonal shape due to close contact with the surrounding cells. Their size reached 12-14 microns, sometimes – up to 30 microns. There were two types of granules in these cells: large, located on the periphery, and small, evenly spread around the nucleus. This type of cells is relatively rare and they

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are not connected with any blood vessels. It is difficult to count and define their relative number. Although, it should be noted that with age, the number of granulated cells slightly decreased.

Corticotropocytes (ACTH-cells) are large, relatively rare cells. They had an oval shape, contained irregular nuclei, and were connected with blood vessels. Their cytoplasm was purple-red after staining with alcian blue (pH = 0.2)-ASR-orange G. The cells were primarily observed in the cranial and central zone of the anterior lobe of the anterior pituitary. When other methods of staining were used, these cells contained poorly stained small granules and they could have been classified as chromophobes. The authors did not reveal any significant age-related differences in the cytomorphology and topography of these cells in the studied animals.

Thus, the authors established that with age, the cytomorphology and the relative number of anterior pituitary cells changed. The age-related increase in the cellular and nuclear diameters of adenotropocytes was relatively even and synchronous, so nuclear-cytoplasmic ratio hardly changed during the studied period. It should be noted that 6 months old heifers had a relatively higher content of STH-cells in the anterior lobe of the anterior pituitary in comparison with other endocrinocytes. The later had an oval shape, clear cellular borders, and closely grouped orange G-positive granules. LTH-cells prevailed in the anterior pituitary of 18 months old heifers. They also had an increasing number of GTH-cells. Heifers from Group II, that had a feeding plan enriched with mineral elements, had the morphology of the pituitary gland different from the structure of this organ in animals from Group I, which was defined by a more intensive growth and early establishment of reproductive cycles.

Zonality of the distribution of the cells and their ratio change. In heifers from Group II, the number of chromophobes slightly increases, which can be explained by the degranulation of functionally active cells. The number of adipocytes is lower than basophils. The relative volume of the organ’s stroma is also smaller than the parenchyma. The zonality of the distribution of cell types in heifers from Group II remained and, by the 18th month of age, became more expressed. It should be noted that in heifers from Group II, polychromatophilic colloid in the central zone of the pituitary was observed significantly rarer.

Cytological study of the distal part of the anterior pituitary in heifers from Group II in comparison with Group I showed a higher activity of STH-cells. They were poorly granulated, contained larger and less stained nucleus and large nucleole. LTH-cells had the signs of expressed activation. They were larger, granulation reduced, and the nucleus was lighter. Still, some cells had the signs of a decrease in the activity. Their size decreased, their granules become rougher, and the nuclei contained more heterochromatin.

The changes in the gonadotropocytes as well as in LTH-cells were characterized by the appearance of the features of functional activity but not as expressed as in the later. TTH-cells and ACTH-cells insignificantly changed in animals from Group II as compared to Group I. Still, they expressed certain signs of the increase in the secretory activity.

The results of the weighing of the adrenal glands are presented. They indicate that these organs increase in volume with age by 1.7 and 1.6 times, respectively. According to Brodi’s definition of the relative mass gain, their growth was most intensive by the age of 12 months (30.7 – 32.2%) in both groups. Although there was no significant difference between the groups, relative mass gain by Brodi in Group II was higher by the age of 9 and 12

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months, and further, this parameter increased in animals from Group I, which indicated the delay in the growth of this organ. By the relation of the adrenal glands to the body weight, the adrenal glands were heavier by the age of 12 months and, further, this parameter became similar.

The nuclear-cytoplasmic ratio was calculated by the method of points calculation. At the magnification of 900 times, under the immersion, an ocular net was placed on the sections of the suprarenal cortex. The authors calculated the number of points per nuclei and cytoplasm. The relation of these amounts expressed nuclear-cytoplasmic ratio (NCR). The increase in the NCR indicates the increase in the relative volume of the nucleus and, consequently, the increase in its functional activity (Table 3).

Table 3: Nuclear-cytoplasmic ratio in the zona fasciculata of the adrenal cortex in heifers

Age, months	6	9	12	15	18
Group 1	0.41+0.04	0.42+0.04	0.44+0.05	0.51+0.05	0.50+0.05
Group 2	0.40+0.03	0.46+0.04	0.52+0.05	0.48+0.04	0.49+0.04

It is known that zona fasciculata occupies more than 75% of the volume of supraadrenal cortex. Glucocorticoids are synthesized in it. It was established that NCR of the cells in the zona fasciculate in heifers increases with age, but in heifers from Group II, this process started earlier, which was probably associated with more intensive growth and earlier establishment of sexual and physiological maturation.

Histological and histochemical studies of the adrenal glands in heifers from Group II showed that the organ capsule was well developed. Its connective tissue fibers penetrate the parenchyma. The cells of the zona glomerulosa that synthesize mineralocorticoids are round shaped, and of average size in comparison with the cells of other zones. Their cytoplasm is dark, grained or reticular. There are more ribonucleoproteins in the cells of these zones than in other zones of the supraadrenal cortex. The nuclei are large with a reticular structure of heterochromatin.

Zona fasciculata is well visualized. It is four times thicker than zona glomerulosa and two times thicker than the reticular one. The fascicles consist of large polygonal cells with a round light nucleus. The cytoplasm is grainy with a high content of ribonucleoproteins. Phospholipids are evenly spread around the zones of the cells. There are few neutral fats contained.

In the reticular zone, small cells with dark angular nuclei prevailed. There were some well-preserved cells with a minor content of fatty substances in the cytoplasm.

The medullary area contains primarily A-cells that were spread around wide venous sinuses. The nuclei are vesicular, eccentrically located. H-cells were polygonal and located as groups separated by the layers of connective tissue. There was no significant difference in the content of nucleotroteids in A- and N-cells revealed. Ribonucleoproteins in the cytoplasm of A-cells had only perinuclear localization.

Heifers from Group I that did not have diet with balance content of minerals had well-an expressed layering of the capsule. Its outer layer consists of collagen fibers and the inner layer consists of loose connective tissue rich in poorly differentiated cells. Zona glomerulosa is not vast. The cytoplasm contains significantly more neutral fats as average drops. The glomeruli are formed by angular cells with a dark nucleus and eosinophil cytoplasm with a low

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level of ribonucleoproteins.

Zona fasciculata is thinner and its border with zona glomerulosa is well-expressed (Figure 3).

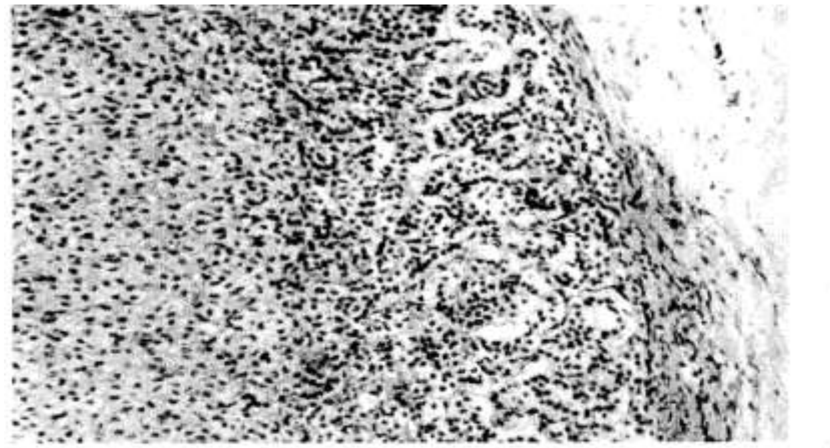

Рис.10 Надпочечник 15-месячной телки второй группы. Четко выраженная граница клубочковой и пучковой зон. Окраска гематоксилином и эозином. Ув. x 100

Figure 3: Adrenal gland of a 15-month old heifer from Group 2. Clearly expressed border of the zona glomerulosa and zona fasciculate. Hematoxylin and eosin staining. Magnification X 100.

The cells were vacuolated and contained the following types of nuclei: wrinkled with imprints and increased concentration of deoxyribonucleoproteids; dark hyperchrome and pyknotized; round large with grains of chromatin evenly distributed in the karyoplasm. The ratio of these types of nuclei was 1:1:2, respectively. There were some ribonucleoproteins in the cytoplasm of zona fasciculate. There is a higher content of neutral fats.

In the reticular zone, the cells are spread close to each other and the vessels are compressed. The cytoplasm was vacuolated, there were more dark nuclei. There was a moderate amount of neutral fats. In the medullary area, moderate degranulation and decompensation of A- and H-cells were observed. The content of ribonucleoproteins in their cytoplasm was lower and some of them were vacuolated. There were giant nuclei in A-cells. In some animals, focal lymphoid infiltration of medullary substance was observed.

One of the main ways to improve the utilization of heifers is an intensive type of growing and timely insemination of the replacement heifers (Shipilov, 1977; Mingxun et al., 2019; Thuróczy et al., 2017), which is possible only in the conditions of balanced feeding plan, obligatory biological control of the feeding, state of animals' health, and level of metabolism (Rodríguez-Sánchez et al., 2018).

Thus, the aim of the study was to evaluate the reproductive function in replacement heifers and to provide morphological characteristics of the reproductive organs during the reproductive maturation.

During the study, the authors evaluated the clinical condition of the animals. Clinical gynecological examination of heifers was performed for the identification of the time of reproductive maturation and organism maturation, and establishment of reproductive functions depending on the feeding and handling of the animals. Animals' corpses and organs were examined after the slaughter. Morphological and morphometrical studies of the reproductive organs

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were performed.

The study results showed that the clinical condition of the experimental animals was within the physiological norm. Reproductive maturation in heifers was observed at the age of 9-12 months, and organism maturation – at the age of 15 months at 300-330 kg. Reproductive cycles repeated every 17-20 days. The stage of initiation of the reproductive cycle was characterized by the following features: edematose vulva, hyperemic mucosa, open uterine cervix, viscous mucus going out of interlabial space. The heifers were exited, lifted their tails. The mating call continued for 24-48 hours. Clinical gynecological observations were confirmed by the results of the morphological study.

Macroscopic study of the ovaries in 6-months old heifers revealed the lack of ovulatory function. At the same time, major follicles develop in the organs followed by the second wave of smaller follicles. Major cavitary follicles obliterate and the development of the next wave of follicles are observed. Thus, the function of the organ is being established.

In 9-month heifers, the follicle-stimulating function gets activated. Along with obliteration, there are some luteinized ovarian follicles, which indicates the activation of the pituitary-hypothalamic-ovarian system and an increase in the level of gonadotropic hormones, including FSH and LH.

The examination of 12-months old heifers showed that they had all the structures typical to ovarian glands in adult animals.

The peculiarity of the morphology of reproductive organs in 15-month old heifers is a larger size of corpus luteum (up to 23 mm) and follicles that exceed the size of 20 mm before the ovulation. The structure of the reproductive organs in 18-month old heifers is similar to 15-month heifers, which indicates the stabilization of the growth and establishment of generative and endocrine function of the organs.

Thus, by the 12th month, replacement heifers had established reproductive cycles, which was characterized by the presence of corpus luteum and developing follicles in the ovaries and the alternation of proliferative and secretory processes in the uterus. Until this age, anovulatory cycles were observed (Rodríguez-Sánchez et al., 2018). Further, the reproductive function stabilizes and enhanced.

Besides, in heifers from Group II at the age of 12, 15 and 18 months, the activity of alkaline phosphatase in the endometrial epithelium was significantly ($P<0.05$) higher (Table 4), which indicated an earlier establishment of morphofunctional maturity of the organ and capability of these structures to implantation and pregnancy.

Table 4: Histochemical activity of alkaline phosphatase in the uterus and ovaries of heifers (CU)

Parameters	Group	Age, months				
		6	9	12	15	18
Endometrium epithelium	1	53.9±1.4	71.2 ±2.8	83.6±1.9	80.2±2.4	84.0±2.6
	2	51.8±1.9	82.1±2.7	93.4±1.1	96.3±1.4	95.7±1.5
Inner sheath of ovarian follicles	1	76.9±2.1	81.4±1.6	93.5±2.6	101.6±3.2	111.9±3.4
	2	75.8±2.2	88.1±0.8	108.1±2.3	114.9±1.3	126.8±2.1
Ateric body of ovaries	1	38.2±1.9	46.9±1.2	51.6±1.6	60.9±2.6	62.2±2.4
	2	39.8±2.3	52.8±0.8	58.9±1.0	70.8±0.7	73.9±1.7

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The histochemical activity of alkaline phosphatase in the ovarian structures in heifers from Group II was significant ($P<0.05$) starting from the 9^{th} month of age. This directly indicated a high level of hormonal activity of ovarian glands in these animals.

IV. CONCLUSION

The study results showed that the optimization of the diet of replacement heifers on macro and micro elements led to moderate activation of the function of the thyroid gland, which provided more intensive growth of the body and organs and optimal time of reproductive and physical maturation of animals. Balanced diet positively influences on the functional activity of the distal part of anterior pituitary. Somatotropocites (STH-cells), prolactinocytes (LTH-cells), and gonadotropocytes (GTH-cells) exert expressed signs of morphofunctional activity. This explains the expressed influence of a balanced diet on the growth of animals and their reproductive maturation. Macro and microscopic picture, histochemical activity of adrenal cells indicate that the animals grown on diets with insufficient content of minerals have hypofunction of adrenal cortex and medulla. Mineral supplements moderately stimulate adrenal glands, which is reflected by morphological parameters of the organ.

Thus, diets with optimum macro and micro elements content moderately stimulate the establishment of the reproductive organs in heifers, which is reflected by an earlier reproductive maturation, morphofunctional maturation of the reproductive organs, expressed generative and endocrine activity of the ovaries, and readiness of the oviducts and uterus for pregnancy.

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REFERENCES

[1] Abramkova, N.V. 2002. Mineral metabolism in young stock of white-and-black Holsteinized cattle depending on the age and diet. Dissertation; Orel.

[2] Andreev, A.I. 1998. Norm setting of mineral elements for heifers that are grown on green feed. Zootechnics. 7:20-22.

[3] Andreev, A.I. 2002. Norm setting of zinc in the diet of replacements heifers. Issues of Russian Academy of Agricultural Sciences. 6:68-71.

[4] Vasina, S.K., N.A. Luybin, L.B. Kokova. 2007. Influence of mineral supplementary feeding on pregnant sows and their offsprings. Veterinary of agricultural animals. 8:62.

[5] Gamko, L.N., D.A. Pilyugaitsev. 2018. Increase and consumption of energy in young bovine stock who are given mineral supplements. Agrarian science. 1:33-35.

[6] Gamko, L.N., N.A. Samuseva. 2016. Complex mineral supplement in the diet of highly reproductive cows. Agrarian science. 11:16-17.

[7] Elovikov, S.B., A.A. Menkova. 2006. Metabolism of nitrogen-containing substances in lactating cows under the influence of protein-vitamin-mineral supplements. Agricultural biology. 41(6):101-104.

[8] Krisanov, A.F. 1988. Norm setting of microelements during the feeding of cattle with silage. Zootechny. 1:39-41.

[9] Mamaev, A.V., B.L. Belkin, A.A. Menkova. 2012. Physiological evaluation of the productive potential of young bovine stock. Issues of Kursk State Agricultural Academy. 8:68-70.

[10] Menkova, A.A., A.I. Andreev. 2017.Macroanatomic characteristics and activity of the enzymes in the reproductive organs of heifers with different levels of mineral consumption. Scientific notes of Vitebsk State Academy of Veterinary. 53(1):101-103.

DOI: 10.37200/IJPR/V24I3/PR2020321
Received: 20 Feb 2020 | Revised: 28 Feb 2020 | Accepted: 14 Mar 2020

[11] Menkova, A.A., A.I. Andreev. 2016. Growth, development and formation of the reproductive system of heifers with different levels of mineral consumption. Food supply in the conditions of the region's import products replacement: issues of theory and practice: collection of scientific articles.
[12] Menkova, A.A., A.I. Andreev, V.I. Chirkunov. 2014. The influence of different levels of mineral consumption on the morphology of adrenal glands in replacement heifers. Issues of Ulyanovsk State Agricultural Academy. 2(26):114-118.
[13] Menkova, A.A., A.I. Andreev. 2010. Histochemical activity of the reproductive enzymes in heifers with different levels of mineral consumption. "Lapshinskie Readings". Resource saving ecologically safe technology of agricultural production. Saransk.
[14] Menkova, A.A. 2003. Mineral consumption in replacement heifers. Agricultural biology.38(4):93-95.
[15] Menkova, A.A. 2002.The weight of body and internal organs depending on the level of mineral consumption. *Agricultural biology.* 37(6):96-100.
[16] Mustafin, R.H., E.N. Skovorodin. 2005. Functional morphology of the glands of internal secretion in heifers. Actual issues of reproduction and mammary glands in animals: materials of international scientific-practical conference dedicated to 35th anniversary of the All-Russian NIVI of pathology, pharmacology and therapy.
[17] Andreev, A.I., A.A. Menkova, V.I. Chikunova, V.N. Pronin. 2012. Peculiarities of mineral metabolism in heifers during reproductive maturation. Issues of Orlov State Agrarian University. 6(39):72-73.
[18] Kostomakhin, N.M., V.P. Potokin, E.K. Kirillova, I.N. Shaidukin, B.F. Bessarabov. 2006. Breeding and issues of zoology. SPb, Lan'.
[19] Sadirova, U.A. 1994. Age-related morphology of reproductive system of heifers depending on different degree of physical activity. Dissertation. M..
[20] Skovorodin, E.N., N.V. Grebenkova. 2009. Morphology of the reproductive organs of heifers with ovarian diseases. Zootechny. 7:32.
[21] Skovorodin, E.N., A.V. Maltsev, E.G. Vokhnovskaya. 2007. Functional morphology of the reproductive organs in new-born calves. Agricultural biology. 42(2):65-72.
[22] Skovorodin, E.N., A.A. Menkova. 2002. Age-related morphology of the reproductive organs in female bovine cattle: monography. Bryansk.
[23] Skovorodin, E.N. 2000. Development of ovaries in bovine cattle during the ontogenesis. Morphology. 3:110-111.
[24] Shevelev, N.S. 1996. Peculiarities of metabolism and utilization of microelements in heifers older than 6 months old. Issues of Timiryazev Agricultural Academy. 1:170-183.
[25] Shipilov, V.S. 1977. Physiological grounds for the prevention of infertility in cows. M..
[26] Shlenkina, T.M., I.I. Stetsenko, N.A. Lyubin. 2013. Peculiarities of the age-related changes in the mineral profile of blood under the influence of different supplements. *Issues of Ulianovsk State Agricultural Academy.* 3(23):72-79.
[27] Ball, P.J.H., A.R. Peters. 2004. Reproduction in cattle. Third edition. Blackwell.
[28] Eduvie, L.O., C.O. Njoku, P.B. Addo, E.C.I. Molokwu, D.I.K. Osori. 1984. Gross and histological changes in the reproductive tract of suckled and nonsuckled postpartum cows. J. Anim. Prod. Res. 4(JVsl):339-345.
[29] Norris, D.O., K.H. Lopez. 2010. Hormones and reproduction of vertebrates. Volume 5 *«Mammals». Academic press.*
[30] Trenkle, A. 1970. Plasma levels of growth hormone, insulin and plasma proteinbound iodine in finishing cattle. *J. Anim. Sci.* 31:389-393.
[31] Summers, A.F., S.P. Weber, H.A. Lardner, R.N. Funston. 2014. Effect of beef heifer development system on average daily gain, reproduction, and adaptation to corn residue during first pregnancy. 92(6)1:2620–2629.
[32] Marks, M.L., M.K. Stanford, L.A. Kriese-Anderson. 2016. Sand Mountain Elite Heifer Development Program III: *Heifer Reproductive Performance and Program Costs.* 95(1):07.
[33] Summers, A.F., T.L. Meyer, R.N. Funston. 2015. Impact of supplemental protein source offered to primiparous heifers during gestation on I. Average daily gain, feed intake, calf birth body weight, and rebreeding in pregnant beef heifers. 93(4):1865–1870.

DOI: 10.37200/IJPR/V24I3/PR2020321
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