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MORPHOMETRIC OF PIG LIVERS UNDER DIFFERENT DOSES OF MINERALS IN FEED ALLOWANCE

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ABSTRACT

It is recognized that microelements' compounds in the inorganic form are difficult to absorb by the organism, and increasing their dose to enhance assimilation's level in animals, as a rule, promotes the development of toxicoses. Therefore, the object of this work is to study the influence of dissimilar doses of microelements' complex in organic form (based on an L-Aspartic acid) on the morphometric of the pig liver. The research and production experiment on White Large pigs living in a large pig-breeding complex was made to achieve this goal. To date, the subject has not been studied very much in literature. According to information received, the average distinction between radii from the central vein to the hepatic lobe in 7-month old pigs from different groups is 4-7 μm. Thus, animals from the control group have 30.3±0.17 μm radius, animals which were given to their ration 7.5% of mineral additive from normal range have 32.5±0.41 μm ($p \leq 0.001$) radius, the 2nd control group (10% of mineral additive) have 34.4±0.29 μm ($p \leq 0.001$) radius and the 3rd control group (12.5% of mineral additive) have 50.2±1.03 μm ($p \leq 0.001$) radius. We found that the addition of a 10% chelate complex of microelements (iron, zinc, manganese, copper, and cobalt) increases the activity of metabolic processes in animal organisms from control groups and causes the most expressive beneficial effect of absorbency.

Disciplinary: Veterinary Medicine, Animal Science, Biology, Biotechnology.

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1 INTRODUCTION

Many organic micronutrient element complexes are more accessible than their inorganic counterparts. According to recent studies, the addition of micronutrient element chelates to feeding pigs' diet significantly increases Cu, Mn, and Zn content in their blood serum (Iliev and Georgieva, 2016; Michiels, 2013; Rekiel and Wiecek, 2005).

Mineral compounds including micronutrient element compounds play an important role in providing animals with fully nutritional mixed feed. Many foreign studies (Iliev, Georgieva, 2016; Povetkin, 2012) point out that the addition of micronutrient element salts (oxalates, citrates, phytates, phosphates, etc.) to farm animals diet to improve its nutritional balance leads to a significant increase in livestock yield by improving metabolic processes in the animal body without causing any adverse effect to the quality of livestock goods (Ao, Pierce, 2013).

According to a study performed (Ao et al., 2010; Povetkin, 2012; Salautin and Ziruk, 2008), major (Ca, P, Mg, K, Na) and micronutrient elements (Fe, Mn, Cu, Co, Zn, I) are mostly present in animal diet as metal inorganic salts as they are the cheapest raw materials. These salts usually dissolve into free ions in the digestive tract and these ions interact with each other and other primary diet constituents, thus, significantly impeding their accessibility during absorption. These salts can also mix with vitamins and risk destroying them in the process.

Using various micronutrient element complexes (milk powders and concentrated soy protein, i.e. Soycoil-R) in animal diet positively affects the functional capacity of many internal organs including the liver by improving the activity of cell synthesis and regeneration processes (Douglas et al., 2013; Katkov et al., 2012; Ziruk, Salautin, 2013).

The liver is the largest accessory digestive gland that performs a multitude of various physiological functions in the animal body and its activity is inextricably bound with high and low tension blood circulation systems (Ao and Pierce, 2013; Lee and Kim, 2004).

Recently, various feed additives containing mineral compounds have found widespread application among animal farms and veterinary clinics. In our opinion, one of the most prospective additives is micronutrient elements complex with L-aspartic acid. 1 kg of such complex contains 10.0 mg of Zn, 10.0 mg of Fe, 2 mg of Cu, 4 mg of Mn, and 0.08 mg of Co.

2 MATERIALS AND METHODS

2.1 ANIMAL MATERIALS AND STUDY DESIGN

There are very few literary data describing mineral compounds impact on pig liver morphology, thus, we have decided to study the impact of various doses of micronutrient elements complex with L-aspartic acid on morphometric parameters of pig liver.

Large White pigs bred in a large pig-breeding complex were chosen as test subjects for our experiment. We have divided test animals into 4 groups consisting of 15 pigs each in accordance with the analogies principle. Animals from the reference group were fed in accordance with the primary diet established by the pig-breeding complex. The daily diet of 1st, 2nd, and 3rd test groups animals also included micronutrient element chelate complex additive with consumption rate equal to 7.5, 10, and 12.5%, respectively, out of the total amount of feed consumed by the control group (as established by the pig-breeding complex).

2.2 COLLECTION OF SAMPLES

Three pigs from each group were slaughtered when they were 4 months old and three more were slaughtered when they were 7 months old.

We took liver specimens from each slaughtered animal, bound them, and made paraffin tissue blocks via standard procedure (using Histomix mounting medium). Histological sections were prepared using MICROM HM 450 sledge microtome and REICHERT freezing microtome and subsequently colored with hematoxylin and eosin. Colored histological sections were examined by Biomed S-1 biological microscope with 10x ocular magnification and 4×, 10×, 40×, and 100× lenses magnification. Radius between central veins of liver lobules and their walls were measured by the ocular ruler (60 units within the field of view) and helical ocular of MOR micrometer with a magnification of 1×15×. Microphotography of tissue specimens was performed by CANON PowerShot A 460 IS camera. Micromorphometric study of hepatic cells was performed with the help of VideoTesT – Morphologiya 5.2 software.

2.3 STATISTICAL ANALYSIS

Statistical analysis of obtained digital data including Student's t-test was performed via standard PC variation statistics software – Microsoft Excel.

3 RESULTS

At the start of the test period, we did not find any significant difference between the general morphologic structure of pig liver specimens from various groups. At the same time, by the end of the test period, the morphological structure of digestive glands varied significantly between all test groups. Pigs from the 1st, 2nd, and 3rd test groups had well-developed hepatic parenchyma consisting of clear lobules delimited by moderately grown interlobular connective tissue. Hepatic cords radiated from lobule walls to central veins; hepatic cells in these cords had polyhedral or cubic shape with well defined tinctorial properties. Central veins at the center of lobules were directed lengthwise, their boundaries were clear and lobule wall integrity was undisturbed. Interlobular triads were clearly defined without any visible disruptions of their structure (Figures 1, 2).

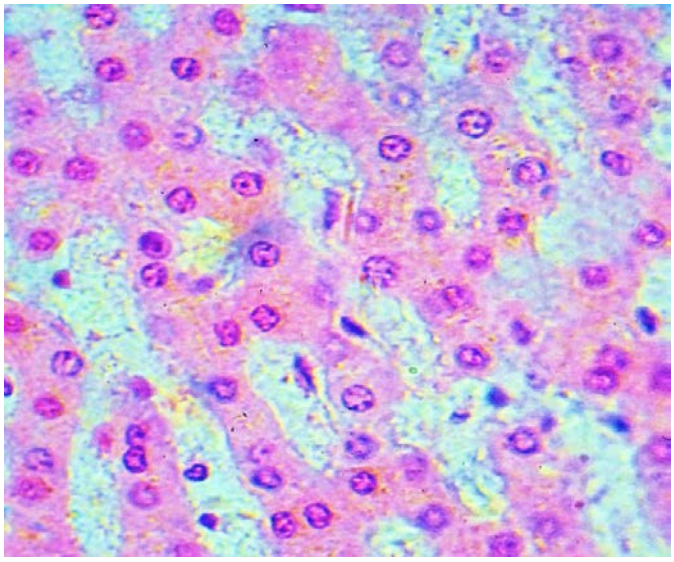

Figure 1: Liver. Experimental group pigs. Hepatocytes are multifaceted, their contours are clear, the nuclei are structured. Coloring hematoxylin-eosin Uv. * 1000

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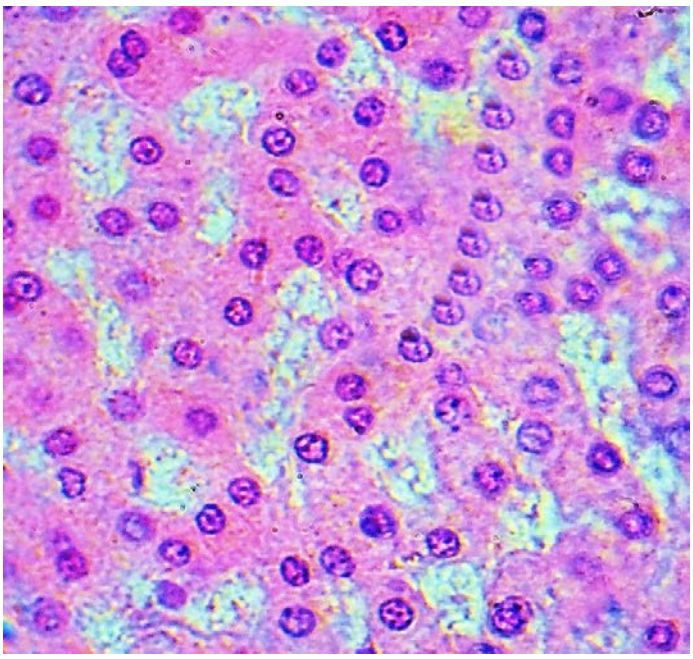

Figure 2: Liver of the control group pigs. Hepatocytes are multifaceted, located in clusters, the nuclei are round - oval in shape. Coloring hematoxylin-eosin Uv.*1000

There were no significant variations in morphometric parameters of pig liver specimens obtained both from test and reference group animals throughout the whole test period. Obtained results are provided in Table 1.

Table 1, at the start of the test period (4 months old pigs) average radius between the central vein and hepatic lobule wall equaled 24.8±0.23 μm for reference group pigs, 28.2±0.24 μm for 1st test group, 27.3±0.24 μm for 2nd test group ($p \leq 0.01$) and 26.10±0.94 μm for 3rd test group.

Table 1: Radius between the central vein and hepatic lobule wall in pig liver specimens, μm

Group	Reference group	1st test group	2nd test group	3rd test group
Start of test period	22.8±1.01	28.20±0.41*	27.30±0.67	26.10±0.94
End of test period	41.0±0.61	42.8±0.72*	51.0±1.02*	50.2±1.03*

Note: n=3; M±m; * $p \leq 0.001$

By the end of the test period, the radius slightly increased for all studied animal groups; the average radius increase varied from 4-7 μm. For example, for reference group animals (whose primary diet was established by pig-breeding complex standards), the average radius between the central vein and hepatic lobule wall equaled 30.3±0.17 μm. The same parameter for animals from the 1st group, who were also fed with micronutrient element complex additive in an amount equal to 7,5% of the total primary diet, equaled 32.5±0.41 μm ($p \leq 0.001$). For 2nd test group (for whom the amount of complex additive equaled 10% of the total amount of feed) the radius equaled 34.4±0.29 μm ($p \leq 0.001$) and for 3rd group (additive equaled 12.5% of the total amount of feed) – 50.2±1,03 μm ($p \leq 0.001$).

Morphometric analysis of hepatic cells has shown that these cells had a relatively similar size both for test and reference groups specimens. As we can see from Table 2, the hepatic cell area for all studied group specimens remained stable and equaled 0.0001 mm^2. Hepatic cell perimeter for 2nd test group pigs (who received an additional 10% of micronutrient complex relative to the total amount of feed consumed) was greater than that of reference group animals by 0.013 mm and that of 1st and

2nd group animals by 0.009 mm. The length and width of hepatic cells for 2nd test group animals were also slightly greater than similar parameters of reference, 1st, and 3rd test groups. The average hepatic cell size did not vary greatly and equaled 0.022 mm both for reference and test group specimens.

Table 2: Morphometric parameters of hepatic cells.

Group	Area, mm^2	Perimeter, mm	Length, mm	Width, mm	Average size, mm	Orientation, degree
Reference group	0.0001±0.0003	0.063±0.031	0.023±0.0011	0.023±0.0012	0.023±0.0009	51.77±2.23
1st test group	0.0001±0.0003	0.067±0.032**	0.024±0.0011	0.017±0.0013**	0.021±0.0009**	53.05±2.22*
2nd test group	0.0001±0.0002**	0.076±0.032**	0.026±0.0010**	0.022±0.0013**	0.024±0.0009**	60.79±2.21*
3rd test group	0.0001±0.0002**	0.067±0.031	0.020±0.0010**	0.020±0.0012	0.020±0.0009**	59.28±2.23*

Note: n=3; M±m; * $p \leq 0.005$; ** $p \leq 0.001$

Cell orientation equaled 51.77±2.23 degrees for reference group animals, 53.05±2.22 degrees for 1st group animals, 59.28±2.23 degrees for third group animals and slightly eclipsed the similar parameter for 2nd test group animals (who received an additional 10% of micronutrient complex relative to the total amount of feed consumed) equaling 60.79±2.21 degrees.

4 DISCUSSION

Minerals hold only a small percentage in animals' rations, but play a major role in the organism: they take part in metabolism and body balance control. A minimal imbalance of one or several minerals between themselves is dangerous for the organism as well as a mineral deficiency (Vasilyeva et al., 2010). The chelate complex of microelements for Large White piglets, using and studying by us, benefits the histologic pattern of a liver. Usually, microelements are included in animals' ration as different inorganic compounds such as oxides, iron carbonates, and salt. Such a choice growers explain by the low price of these additives. In a pigs' digestive tract such compounds decompose on simple ions, beginning to make contacts not only among themselves but with other substances and cause more difficult fixation both organisms and vitamins and minerals (Underwood et al., 1999). Test group pigs that were fed with micronutrient element complex with L-aspartic acid in addition to normal feed had clearly defined vein boundaries with said vein moderately filled with blood. Minimal red blood cell content in some veins is indicative of intensive blood circulation in test group animals livers compared to animals from the reference group. The use of a chelate complex of microelements based on L-Carnitine in bird feeding benefits the structure of a liver, especially improve the anatomical condition of hepatocyte (Shchitkovskaya et al., 2012).

According to morphometric analysis data, by the end of the test period, hepatic cells in all studied animal group specimens had clearly defined boundaries. Specimens taken from animals from the 1st and 2nd test group showed that most hepatic cells had a polyhedral shape with their nuclei located at the centre of the cell. Specimens taken from reference and 3rd test group animals contained a minimal amount of nuclei localized close to cell walls. Hepatic cell nuclei had a round-oval shape. The most well-structured boundaries of hepatic cells were observed in 2nd test group specimens that also contained condensed chromatin bodies and granules. We observed saturated dark blue chromatin color in all tested animal group specimens. Each nucleus had no more than 3-4 nucleoli on average. Reference group pigs had poorly developed extracellular matrix and perisinusoidal space. General cell and extracellular bodies structure in test group pig specimens is intact with hepatic cells bearing against each other. Periosinusoidal space is clearly defined.

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According to our histological and morphological analysis of hepatic cells of all studied pig groups, the microscopic structure of pig liver is intact for all specimens and such specimens contain no visible structural or pathological changes. Increase in studied radius between the central vein and hepatic lobule wall for 2nd test group animals by 10, 8.2, and 0.08 μn compared to the reference, the 1st and 3rd test group animals, respectively, indicate of higher metabolic activity for pigs who were fed chelate complex as a feed additive (in an amount equal of 10% to the total amount of feed).

5 CONCLUSION

In view of the foregoing, we can conclude that adding micronutrient (iron, zinc, manganese, copper, and cobalt) chelate complex with L-aspartic acid to a standard diet (amounting to 10% of total feed amount) with micronutrient doses being 10.02 mg/kg for Zn, 10.02 mg/kg for Fe, 2.01 mg/kg for Cu, 4.01 mg/kg for Mn and 0.1 mg/kg for Co, significantly increases the rate of metabolic processes in pig organisms, thus, maximizing the positive effect of such complex application.

6 AVAILABILITY OF DATA AND MATERIAL

Information can be made available by contacting the corresponding author.

7 CONFLICT OF INTEREST

The authors declare no conflict of interest.

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