Morphological and functional changes of laboratory animals after feeding with cavitation-treated feed

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Abstract. At present, the priority areas of agrarian science development and scientific support of the agricultural sector of Russia until 2025 are connected with the development of high-tech technologies aimed at finding fundamentally new, environmentally safe and effective methods of intensifying technological processes, improving them, and creating a system of resource-saving technological processes and machines. Current trends in science and technology, from the standpoint of healthy nutrition state policy and implementation of anti-crisis measures in the Russian agro-industrial complex should be focused on developing competitive new generation food products that are promising in terms of import substitution and increasing domestic demand.

Introduction. In our work, a significant increase in MEL was revealed in the intestinal mucosa in poultry, when cavitation-treated products were added to their diet. At the same time, the most pronounced increase was in the experimental group using zeolitized tuff, in comparison with group that received cavitation-treated wheat bran.

Thus, this section of the study confirmed that long-term use of such products significantly improves the morphofunctional activity of liver and small intestinal mucosa, it is greater when cavitationally processed products are introduced into the diet, especially when zeolitized tuff is added.

1. Introduction

At present, the priority areas of agrarian science development and scientific support of the agricultural sector of Russia until 2025 are connected with the development of high-tech technologies aimed at finding fundamentally new, environmentally safe and effective methods of intensifying technological processes, improving them, and creating a system of resource-saving technological processes and machines. Current trends in science and technology, from the standpoint of healthy nutrition state policy and implementation of anti-crisis measures in the Russian agro-industrial complex should be focused on developing competitive new generation food products that are promising in terms of import substitution and increasing domestic demand [1, 2, 3, 5].

Highly efficient production processes with due account to the requirements of modern ecology and environmental rehabilitation, should be based on safe acoustic, physical and chemical, electro-physical and mechanical methods of processing agricultural raw materials, including using nanotechnologies, allowing non-reagent regulation of its functional and technological properties. [4, 6]

Among these, the most promising and modern methods of intensifying technological processes in food production, is the use of liquid media activated by various means in combination with rational
hydromechanical effects. At present, activated liquid media with technologically significant functional properties are obtained by both electrochemical treatment and cavitation disintegration. [9-11].

Restraining the pace of introduction and use of acoustic and electrochemical methods of processing raw materials or waste in feed production technology is associated with insufficient information about the mechanisms of influence on the state of internal organs of farm animals.

2. Materials and Methods

As a basic equipment for obtaining cavitation-treated products, a magnetostrictive transducer with a power of 100 W, an amplitude of 50 \( \mu \)m and a frequency of 27 kHz was used. [8] For stronger effect of ultrasound on cellulosic waste, we have proposed the use of zeolitized tuff (clinoptilolite) powder with a particle size of less than 1 mm.

To determine the effectiveness of use of the obtained product for animal feeding, experiments were conducted on broiler hens of Smena 7 crosses. By method of pair analogues, four groups were formed (n = 30), during the preparatory period (14 days) they were under the same feeding and housing conditions. Then, animals of group I (control) received a starter mash first then a grower mash, for group II birds, 10% of grain part was replaced by cavitation-treated wheat bran, for group III, 10% of grain part was replaced by cavitation-treated wheat bran with the addition of zeolitized tuff powder (clinoptilolite) in the amount of 1% with a particle size of less than 1 mm. The experiment lasted 42 days.

Sampling, preparation and processing of materials was carried out in accordance with the “Guidelines for laboratory studies on the pathological anatomy of animals”. Samples of the analyzed organs of birds from the control and experimental groups were taken by section for microscopic examinations. For a general histology of organs, liver and fragments of small intestine were fixed in 10% buffered formalin and embedded in paraffin. Using sliding microtome, sections were prepared with a thickness of 6 \( \mu \)m, which were stained by the standard technique with hematoxylin and eosin. [7].

3. Result an Discussion

According to the results of the research, the histological tissue of liver in the control group has a clear structure (Figure 1, 2). Hepatic lobules are monomorphic, of the same size, separated by thin layers of connective tissue, where interlobular arterial capillary, vein and bile duct are located. The interlobular hepatic beams have a predominantly radial location. The interbalance capillaries are well defined, rather narrow, on the endothelial surface of which star-shaped Kupffer cells are determined. Central vein is with moderate plethora. Hepatocytes are uniform, with the same elongated nuclei, containing nucleoli. Amylase-resistant neutral mucopolysaccharides were found in a small amount at acid-Schiff reaction. Acid mucopolysaccharides were defined as traces.

In addition to liver, samples of small intestine (jejenum) was taken for research. High villi, deep crypts, lined with a cylindrical epithelium with a basally located nucleus were histologically well defined. The epithelium lining the intestinal mucosa is a single layer, prismatic with a high content of goblet cells, mainly in crypts. The cytoplasm of enterocytes, especially crypts, contains a large amount of Schiff + substances and acid mucopolysaccharides. Own layer of mucous membrane contains blood capillary, moderate cell infiltrate with a predominance of cells of the lymphoid series and macrophages.

In the experiment with the replacement of 10% grain part with cavitation-treated wheat bran, the histological structure of organs studied - liver and small intestine - changed slightly, compared with the control group of animals.

In liver, changes were expressed mainly in the form of an increase in plethora, distention and plethora of central veins, inter-beam capillaries, while maintaining a clear beam structure of lobule. The hepatocytes somewhat increased in size, were rounded, and the size of nucleus increased. A feature of the interbalance capillaries in this experimental group was an increase in the number of Kupffer cells, which were determined in large numbers in the central part of the hepatic lobule.
A clear histological structure of body with lobular structure. Hepatic beams of radial location are formed by monomorphic hepatocytes. Moderate liver tissue plethora, especially the central parts of lobules. Stained with hematoxylin and eosin.

A clear beam structure of liver. Plethorical central vein, uniform interlobular capillaries. Stained with hematoxylin and eosin.

Visually, the content of Schiff’s positive substances and acid mucopolysaccharides in hepatocytes, in comparison with the control group, did not change. However, they showed a decrease in amylo-resistant polysaccharides.

In the mucosa of small intestine in experimental conditions using cavitation-treated wheat bran, morphofunctional state of mucous membranes significantly changed in comparison with the control group of animals. This related to the condition of epithelial cells of the mucous membrane, number and composition of glycosaminoglycans in them, the content of cellular composition of its own crypt and villi. According to the study, the increase in height and width of villi in comparison with the control group of animals was insignificant; nevertheless, the depth of crypts and number of villi in the area being determined increased crypts depth - 86 ± 33; number of villi - 2.3 ± 0.9. At that time, their number in the control group was: the depth of crypts - 70 ± 43; the number of villi is 1.6 ± 0.8.

In the mucous membrane of small intestine, under the test conditions using soybean bifidum, the morphofunctional state of mucosa changed significantly, all their morphofunctional parameters increased. The depth of crypts and number of villi on the determined area significantly increased, making: depth of crypts - 100 ± 23; number of villi is 2.85 ± 0.3 (p ≤ 0.05). The number of goblet cells noticeably increased, mainly in crypt mucosa - 18 ± 2 per villus.

Changes in the morphometric parameters of intestinal enterocytes were accompanied by changes in the quantitative and qualitative composition of glycosaminoglycans. It was manifested by a pronounced accumulation of acid-Schiff (PAS)-positive substances in enterocytes, especially crypt mucosa (Figure 3). Schiff’s substances were detected in villi in the form of large droplets in a part of enterocytes (Figure 4). The content of these mucopolysaccharides significantly reduced after treatment of histological sections with amylase. The accumulation of MPS in the enterocytes increased, especially in the foci of their proliferation. Weakly acid sulfated glycosaminoglycans were a significant part of them.

An increase in diffuse cellular infiltration occurred in deep mucosa, especially in the inter-cryptal zone. In the cell composition, lymphoid cells prevailed, with a small number of macrophages and plasma cells. An increase in intraepithelial lymphocytes in the mucous membrane of villi and crypts is also characteristic. Their number is significantly higher than in control mucosa of the control group.

In the experiments with the use of zeolitized tuff (clinoptilolite) in the diet in an amount of 1%, the morphological changes in histological structures of liver and the mucous membrane of jejunum were significantly different from those in the control and group I.

Lobular structure of liver maintained. The liver tissue was characterized by increased plethora with the expansion of the interlobular vessels, especially arteries, an increase of the intergranular capillaries and the central veins of the lobules. The size of hepatocytes increased, which led to a loss of clarity and monomorphism in the construction of hepatic beams. Another peculiarity of liver structure in this experimental group was the accumulation of small vacuoles in hepatocytes, mainly the central parts of lobules.
A large number of goblet cells in mucous membrane of crypts and villi with the accumulation of Schiff-positive substances in cytoplasm. Enhanced round cell infiltrate in mucosa lamina propria. Acid Schiff-positive substances in the form of large droplets in the cytoplasm of enterocytes.

Acid-Schiff (PAS)-positive substances in the cytoplasm of hepatocytes significantly increased, most of them were destroyed after treatment of histological sections with amylase. MPS in hepatocytes were found in small amount. Their high content was found in interlobular tissue, and predominantly they were weakly acid sulfated MPS such as hyaluronic acid and sialoproteins.

Morphofunctional changes of jejunum were observed in all studied histological structures of the mucous membrane. Despite the fact that the height of villi and the depth of crypts did not change significantly, the width of villi and crypts significantly increased. The sizes of enterocytes increased, their proliferative activity increased in crypts.

The next feature of the morphofunctional state of the intestinal mucosa was a sharp increase in cell infiltrate in the villus own plate and the inter-cryptal zone of the mucous membrane. Lymphoid and plasma cells, macrophages prevailed in the cellular infiltrate. Leukocytes were found in a significant number.

The next peculiarity of morphofunctional state of intestinal mucosa was a sharp increase of cell infiltrate in villi lamina propria and inter-cryptal area of mucous membrane. Lymphoid and plasma cells, macrophages prevailed in the cellular infiltrate. Leukocytes were found in a significant number.

The content of both neutral and acid mucopolysaccharides increased significantly. This was especially true for Schiff’s positive polysaccharides. They accumulated in the cytoplasm of crypt enterocytes and, unlike the previous groups, in villus enterocyte. Most of these polysaccharides were destroyed by amylase. MPS were accumulated in cytoplasm of crypt and villus enterocytes. In terms of qualitative composition, both weak acid and high acid mucopolysaccharides were among them.

In the morphological section of this experimental work, changes in terms of using cavitation-treated products, histological structures of liver and small intestine, which are responsible for the processes of digestion and metabolism in body.

An increase in the functional activity of liver was expressed in increase of blood circulation with distention of central veins, diameter of interbeam capillaries, the interlobular artery, size of hepatocytes and their nuclei. They were the most pronounced in the experimental group of animals fed with 1% zeolitized tuff (clinoptilolite) in diet together with cavitation-treated wheat bran, in comparison with group receiving only treated bran as a replacement.

The manifestation of increased functional activity of hepatocytes was expressed not only in an increase in the morphometric parameters of hepatocytes and circulatory system, but also in the accumulation of protein granules in their cytoplasm, mainly in the cells of the central zone of the hepatic lobe, which is the most functionally active. The accumulation of Kupffer cells indicating the protective properties of liver tissue was also registered.

Reactive changes in small intestine of experimental groups were of a polymorphic nature reflecting an increase in the functional activity of the intestines and led to changes in the microrelief of mucous membrane and the state of epithelial cells.

Using cavitation-treated products, especially with the addition of zeolitized tuff (clinoptilolite), there was an increase in the size of villi and crypts, the number of epithelial cells with changes in their morphofunctional state. The linear dimensions of enterocytes and their nuclei increased. At the same time, the number of goblet cells increased, and the accumulation of Schiff-positive substances in cytoplasm increased.

Figure 3. Small intestine, group I

Figure 4. Small intestine, group I
time, secretion of epitheliocytes increased together with the accumulation of Schiff positive substances and acid mucopolysaccharides. This was particularly evident in the reaction of goblet cells. The goblet cells are the main source of mucus, which, having bactericidal properties, protects the intestines from pathogenic factors and is involved in the formation of chyme.

This study also revealed a quantitative increase in enterocytes, including goblet cells, but also changes in the qualitative composition of glucosaminoglycans. If amylase-resistant MPS prevail among acid-Schiff positive substances in the control group and high-acidic substances - among the MPS, the addition of cavitation-treated bran to the diet, will significantly increase the amount of amylase soluble (like glycogen) and low-acid (such as hyaluronic acid) polysaccharides, especially with addition of zeolitized tuff.

In addition, the significant effect of cavitation-treated products was manifested in the quantitative and qualitative composition of cellular infiltrate of the intestinal mucosa. The use of cavitation-treated products, especially with the addition of zeolitized tuff, led to an increase in the content of immunocompetent cells (lymphocytes, plasma cells, macrophages, neutrophils - to a lesser extent). At the same time, the total cellular infiltration and its qualitative composition varied significantly depending on the type of the product used.

In comparison with the control group, the use of cavitation-treated wheat bran in the diet resulted in an insignificant increase in total cellular infiltration in lamina propria of inter-cryptal area and villi. Lymphoid cells, macrophages, fibroblasts with a small number of leukocytes prevailed here. A significant jump in the quantitative and qualitative content of cellular infiltrate manifested itself when cavitation-treated products with zeolitized tuff were used in the diet. At the same time, the total pool of cellular infiltrate has grown in comparison with the group of animals that received cavitation-treated wheat bran. Most of it was composed of lymphocytes, plasma cells and macrophages, the number of leukocytes increased, being mast cell antagonists, they increase the resistance of cells to various pathogenic agents.

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