BLOOD CHEMISTRY VALUES AND HISTOLOGICAL FEATURES OF THE GASTROINTESTINAL TRACT IN YOUNG RABBITS WHEN USING PROBIOTIC AGENTS IN FEEDING DIETS

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Abstract

Probiotic complexes that produce a stimulating effect on an animal’s body, normalize intestinal microbiocenosis and improve resistance of an agricultural animal’s body are widely applied to increase meat production of animals.

The purpose of the studies was to examine blood chemistry values of fattening rabbits with inclusion of Vetom 3.0 probiotic supplement into the diet and effect on histological features of the gastrointestinal tract of young rabbits. The effect of Vetom 3.0 biologically active supplement that contains Bacillus amyloliquefaciens strain VKPM B-10642 produced on the basic blood chemistry values and histological structure of the stomach and liver in rabbits was examined. 2 (experimental and control) groups were formed with 10 chinchilla rabbits aged 60 days in each group. Experimental animals were given Vetom 3.0 probiotic supplement once a day at the dose of 75 mg/kg live weight for 60 days.

Study of blood composition evaluates an animal’s condition and gives a general idea of environmental adjustment. It also allows us to observe different changes that take place in an animal’s body when it is fed and managed evaluating its total physiological condition. The greatest increase of total protein, albumin and globulin content was noted in an experimental group following 60 days, increasing the non-specific resistance and activating mineral exchange. According to histological studies, the use of Vetom 3.0 probiotic in young rabbits produces a
positive effect on the structural organization of the stomach and liver and prevents dystrophic processes.

**Keywords:** Probiotic agent, blood chemistry values, protein exchange, gastrointestinal tract structural organization.

I. Introduction

One of the most important tasks of the Russian agricultural economic sector is to ensure food safety of the country. When solving the problem, a special attention is given to those agricultural branches and foods that can be produced by domestic farmers and compete with import-substituting products.

Meat and meat products belong to one of the main sources of native protein necessary for the normal functioning of a human body. In its deficiency metabolism is disturbed, many diseases are developed and life expectancy is reduced. Currently, particular attention is given to rabbit breeding as a supplier of dietary meat by-products and waste. Rabbit meat contains all essential acids. It’s fine fibrous and highly digestible. A balanced diet containing all nutrients must be supplied to obtain higher values of rabbit meat production [V, VII, XI, XV].

Biologically active preparations, vitamins, mineral substances, antioxidants, immuno modulating agents and various probiotic supplements that increase meat productivity [II, III], produce a stimulating effect on an animal body and normalize intestinal microbiocenos is are widely applied in cattle-breeding.

Supplements based on Bacillus-strain bacteria including Vetom 3.0 series are of interest among the variety of probiotics [I, II, V, VI, XVIII]. Vetom 3.0 is fine white odorless powder, soluble in water with some residue. It contains dried culture of probiotic microorganisms *Bacillus subtilis* recombinant strain VKPM B-10642 (DSM 24614), and excipients such as sucrose (powdered sugar) and potato starch. Vetom 3 stimulates cellular and humoral immunity factors. 1 g of the drug contains \(1 \times 10^6\) CFU (Colony Forming Units) of living microbial cells of *Bacillus amyloliquefaciens* recombinant strain VKPM B-10642 (DSM 24614).

This probiotic agent is used to prevent and treat infectious diseases and disbacteriosis, activate immunity, increase livestock integrity, improve average live weight gain and meat quality [V]. *Bacillus amyloliquefaciens* recombinant strain VKPM B-10642 (DSM 24614) included into the agent composition is highly resistant to digestive fluids, gastrointestinal enzymes and is capable of its fast colonization. Having entered the intestine of an animal, bacterial spores are transformed into vegetative forms. They secrete substances, enzymes, and other biologically active substances similar to antibiotics, that normalize intestinal biocenosis, digestion, absorption, metabolism of iron, calcium, fats, proteins, carbohydrates, triglycerides, amino acid, dipeptides, sucrose and biliary salts.
Examination of blood chemistry values as a factor reflecting peculiar functioning of the body under the new feeding conditions and management of animals that undergo many stresses is of a great importance.

Blood is vital in life-sustaining activity [XVI]. Blood composition is a system that reflects oxidative, restoration and metabolic processes and characterizes normal and pathological processes in an animal’s body [IV, XIII, XVII, XVIII], it ensures interaction between body parts and organs, participates in the delivery of nutrients and oxygen to the cells. Feeding, age, sex, breed, animal welfare, season, etc. produce a great influence on blood composition. Blood composition characteristics evaluate an animal’s condition and give a general idea of environmental adaptation. It also enables control of different changes affected by feeding and maintenance of an animal [I, III, V, VIII, XIII, XVIII]. Blood contains plasma and weighted enzymatic elements mainly consisting of red blood cells [VII] and account for 95% of the total number of blood cells. Normal red blood count in rabbits is 5.0 to 5.8% per 1 mm$^3$ of blood. The value is much lower during the first life month but it becomes normal by the 4th or 5th month.

Thus, the study of blood biochemical characteristics provides for a complete picture of metabolic processes when using probiotic feed additives of different types. The problem is pressing and is of a certain scientific interest for rabbit breeding resources to be developed and stabilized.

**Purpose** of the studies is to examine blood chemistry values of rabbits during feeding when Vetom 3.0 probiotic supplement is included into the diet and to find its influence on the gastrointestinal histological features in young rabbits.

### II. Materials and Methods

20 male Californian rabbits were selected to conduct an experimental part of a scientific procedure. The rabbits aged 60 days were divided into two groups with 10 rabbits in each following the principle of analogous groups. All the rabbits were maintained in similar conditions and got the same basic diet and water ad lib. The first (control) group obtained the basic diet only (PK-90 diet); Vetom 3.0 probiotic agent was added to the basic diet at a dose of 75 mg/kg live weight daily during 60 days for the second group. Blood was taken from the marginal auricular vein in the morning before feeding with compliance of any necessary aseptic and antiseptic procedures to determine the chemistry values.

White and red blood cell count and hemoglobin were determined. WBCs were counted with electronic meters, RBCs were determined using a colorimeter and hemoglobin was estimated by Sahli's haemoglobinometer [XIX]. Concentrations of total proteins, albumins and globulins, AST and ALT activities in the blood plasma were determined. Refractometric determination of total protein in serum and nephelometric determination of protein fractions according to solution thickness established with a photoelectric colorimeter were used.
During a histological study tissue samples were fixed in 10% neutral-buffered formalin. After washing with running water the fixed samples underwent dehydration when the examined material was placed into alcohol with increasing concentration and put into paraffin following a standard method. Histological transverse 4-5 µm sections were stained with hematoxylin-eosin. Microscopy was done using Biomed-5 light microscope (Russia) [XII].

III. Results and Discussion

Nowadays, probiotics that significantly increase the effective restoration of digestive functions and total condition of agricultural animals are used more and more frequently. Probiotics are biologically safe and type dependent.

When the technology of maintenance and feeding is violated, rabbits have lower natural resistance to unfavorable environment. In this case, studying the effect of probiotics on the physiological condition of animals is both of theoretical and practical value [III, IV, V, VIII, XVII, XVIII, XIX].

In spite of the accumulated vast experience on the functional role of intestinal microbiota in animals, many issues regarding structural and functional rearrangements of the digestive system in particular, affected by various changes in the intestinal microbiota are still discussed. The issue of changes in a healthy intestine under the action of probiotics is rather significant. The study of the issue regarding membrane digestion is especially important as this type of digestion, being the final stage of nutritional digestion, significantly determines the total metabolism and its homeostasis.

Clinical medical values, blood chemistry values and histological gastrointestinal structure of rabbits were examined to estimate the physiological condition of animals.

Table 1: Presents clinical medical values in rabbits. Table 1 – clinical medical values in rabbits (X±Sx; n=10)

| Group       | t,°С     | Pulse, beats per minute | Respiratory rate per minute |
|-------------|----------|-------------------------|-----------------------------|
| Age - 60 days |          |                         |                             |
| 1 (control) | 38.60 ± 0.07 | 146.4 ± 2.48            | 53.8 ± 2.2                  |
| 2 (experiment) | 38.57 ± 0.9 | 148.5 ± 3.67            | 54.5 ± 2.4                  |
| Age - 120 days |         |                         |                             |
| 1 (control) | 38.6 ± 0.05 | 149.6 ± 3.1             | 54.5 ± 2.5                  |
| 2 (experiment) | 38.5 ± 0.04 | 149.1 ± 3.8             | 54.7 ± 1.8                  |
A slight increase of heart and respiration rate was noted in experimental rabbits (table 1) that supplemented the basic diet with Vetom 3.0 at the dose of 75 mg/kg. Increased pulse and respiration rate was possibly associated with the maximum arrival of biologically active substances into the rabbits’ bodies. It must be noted that the value variation was within the physiological range.

Probiotics optimize metabolic processes, improve nutrient digest ability and activate body defenses. A comparative analysis and correlation of the most important blood chemistry values are necessary to obtain a complete view of processes that occur when probiotics are taken by animals. Inclusion of Vetom 3.0 probiotic in the diet of rabbits enables to determine variations in blood chemistry values. They are presented in table 2.

**Table 2: Blood chemistry values of rabbits (X±Sx; n=10)**

| Value           | Group                  |
|-----------------|------------------------|
|                 | 1 (control)            | 2 (experiment)        |
|                 | 60 days | 120 days | 60 days | 120 days |
| Hb, g/l         | 94.0±1.8     | 117.2±2.3      | 98.2±2.1 | 120.7±2.0 |
| RBCs, 10¹²/l    | 3.28±0.02     | 4.95±0.03      | 3.29±0.03 | 5.61±0.02 |
| WBCs, 10⁹/l    | 7.86±0.13     | 8.40±0.12      | 7.90±0.08 | 8.80±0.15 |
| Total protein, g/l | 71.84±1.8 | 73.58±1.6  | 73.00±1.5 | 78.12±2.1 |
| Glucose, mmol/l | 7.68±0.14     | 9.23±0.22      | 8.06±0.12 | 12.00±0.20 |

For the beginning of the experiment, the studied blood values in the experimental group varied insignificantly and were within the physiological limits. According to the study results, inclusion of Vetom 3.0 probiotic into the diet significantly affected the hematology values.

Table 2 shows that long-term use of Vetom 3.0 improves Hb and RBC counts. However, their variation is within the physiological norm. In the experimental group, RBC count is 5.61 10¹²/l, exceeding the same value in the control group by 13.33%, displaying a positive effect of Vetom 3.0 on homopoietic organs and revealing improved metabolism.

Blood serum total protein content characterizes the effect produced by nutrition on a body’s condition. At the end of the experiment, increased content of total protein in control rabbits was 1.74 g/l (2.42%); the same value in the experimental group was 8.12 g/l (11.12%). When the value is improved, the exchange processes are activated and the greatest average live weight gain can be obtained.
At the start of the experiment, the variations regarding serum albumin content were insignificant; at 120 days the control animals had worse values of serum albumin as compared to the experimental group such as 42.79 g/l vs 45.74 g/l, amounting to 6.89%. At the end of the experiment, serum globulin content varied within 28.32-33.64 g/l (control) and 29.72-38.86 g/l (experiment).

Alanin-aminotransferase (ALT) and aspartate-aminotransferase (AST) are important in protein synthesis. The selected biochemistry values display the hepatic function under different physiological and pathological conditions. Disturbed function of the liver is usually accompanied with significant improved aminotransferase activity due to the effect produced by toxic products on the liver.

Variations in the activity of alanin-aminotransferase and aspartate-aminotransferase are displayed in table 3.

| Group  | AST, U/l | ALT, U/l |
|--------|----------|----------|
|        | 60 days  | 120 days | 60 days | 120 days |
| 1 (control) | 93.6±0.005 | 122.0±0.006 | 81.2±0.001 | 124.7±0.002 |
| 2 (experiment) | 96.9±0.003 | 128.0±0.002 | 96.8±0.021 | 100.9±0.015 |

During the experiment it was found out that the AST activity was insignificantly increased from 96.9 to 128.0 U/l and ALT activity was increased from 96.8 to 100.9 U/l in rabbits from the 2nd group. This trend points at more intense protein exchange in the experimental group.

The stomach and liver of rabbits served as material for a histological study. For an experimental group of rabbits, in the histological structure of the stomach the glandular section has mucous, submucous, muscular and serous membranes. It is erosion free. The gastric mucosa is covered with a single-layer columnar epithelium along the entire surface including fossae. The mucosa had numerous folds and was lined with a single-layer cylindrical epithelium.

A moderate amount of mucus produced by the cylindrical epithelium was found in a histological study of the gastric mucosa. The cells of the glandular body and bottom are stained more intense basophilically than the excretory glands. The glandular cells are placed as continuous strands and adhere closely. Nuclei are centrally located in the cells. The nuclei were spherical (fig. 1).
In the control group, architechtionics of the stomach had a proper histological structure. However, there was edema with partial desquamation of villus epithelium in some parts. Round voids were found in cells. Solitary cells had signs of vacuolization and dystrophy. Mucus reduced significantly as compared with the experimental group. In some places cells of the glandular epithelium were located out of center.

In the experimental group, radial arrangement of tubules was found during a morphological study of the liver. Hepatocytes form strands that are closely adjusted. The nucleus apparatus of hepatic cells is pronounced without dystrophic changes. The nuclei stain basophilically. Binuclear hepatocytes were found. Vessels were blood filled to some extent (fig. 3).
Fig. 2: Architechtconics of the stomach in rabbits (control group).

_Hematoxylin and eosin stain. About 10 × rev. 10(a), × rev. 40(b, c)._ 

Fig. 3: Histological structure of the liver in rabbits (experimental group).

_Hematoxylin and eosin stain. About 10 × rev. 10(a), × rev. 40(b)._

Architehtonics of the liver was preserved for the control group (fig. 4). The beam structure was not disturbed; it was diverged from the central vein in a radiated manner. Dystrophic changes were found in hepatocytes of the cytoplasm and nuclei apparatus. The cytoplasm had both large and small voids.

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They fuse into one large vacuole occasionally. Nucleus dissolution is observed in the cells. Small drops move nuclei to different poles of the cells. Oxyphilic fine grains are present in hepatocytes under the organ capsule. In their turn, they produce a negative effect on the nuclear apparatus of cells.

IV. Conclusion

Blood serum total protein content is one of the most important values that describe the effect produced by nutrition on a body’s condition. Average live weight gain can be achieved due to the increased total protein content in the serum. It is established that Vetom 3.0 produces a positive effect on the morphological composition of blood and protein exchange. All qualitative blood variations occurred due to the used probiotic supplement. Blood serum total protein content was lower in the beginning than at the end of the experiment.

Long-term use of Vetom 3.0 probiotic agent at the dose of 75 mg/kg live weight produced no toxic effect, activated erythropoiesis and stimulated exchange processes. At the end of the experiment, experimental rabbits had higher RBCs, WBCs and Hb content showing an improved metabolism, live weight gain and better resistance.

The conducted experimental studies showed that the use of Vetom 3.0 probiotic agent in rabbits produced a positive effect on the structural organization of the stomach and liver. However, control animals had hydropic and cloudy degeneration that caused formation of vacuole in the stomach and hepatic cells. The agent produced a favorable effect on the metabolic products and prevented the development of dystrophic processes.
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