Influence of biocidal preparations on the intestinal microflora of broiler chickens

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Abstract. One of the most important factors affecting poultry productivity is the quality of drinking water. Modern preparations of foreign production that allow to remove biofilm from the water supply system along with all organic and inorganic pollutants are expensive, as well as, as a rule, negatively affect the body of poultry when drinking. The article considers the effect of water solutions of neutral anolyte "ANOLIT ANK SUPER", as well as a solution of the preparation "DUTRION" on the intestinal microflora of poultry. As a result of the experiment, it was found that the use of 0.25% and 10% solutions of neutral anolyte, as well as the solution of the preparation "DUTRION" has a multidirectional effect on the level of bifidobacteria and lactobacilli in the intestine, but at the same time reduce the level of pathogenic and conditionally pathogenic microflora.

1. Introduction

The productivity of poultry depends on many factors: conditions of keeping, genetic potential, food supply, veterinary support. But a very important and often overlooked factor is the availability of quality drinking water. Water quality is one of the most important criteria for poultry health, which in turn leads to profitability of production, since only a healthy bird is able to fully develop its genetic potential and be highly productive.

Drinking water containing microflora is epizootic dangerous, especially for young poultry. In Russia, about 68% of registered diagnoses are for diseases of bacterial etiology [1].

In modern conditions of poultry farming, it is not possible to assess the extent of morbidity and death of animals and poultry caused by conditionally pathogenic microflora, since this group of infectious diseases is not subject to mandatory registration [2]. The ability of conditionally pathogenic microflora to adapt to various abiogenic and biotic influences, their ubiquity and constant circulation in environmental objects, in particular in water sources, creates conditions for their constant contact with the animal body [3].

There are two main approaches to regulating bacterial growth in the water supply system. The first creates a high level of biocidal agent in the water. For example, a large amount of chlorine (in the form of sodium hypochlorite) or chloramines, formalin, iodine-containing compounds and others are added to the source water to ensure their residual content in the water at all points of the system, including the drinkers. However, in this case, the biofilm and salt deposits remain in the system. The second approach...
is to periodically clean and disinfect the system. Biofilm, organic and inorganic contaminants are removed along with microorganisms, and the source of their growth renewal is eliminated [4].

Modern preparations of foreign production allow specialists of poultry enterprises to follow the second way, although at present they are expensive, and, consequently, significantly increase the cost of finished products. One of these effective biocides is the preparation "DUTRION", which includes chlorine dioxide, which is a very strong biocide (kills all bacteria, viruses, spores, fungi, mold, algae, etc.). This preparation effectively eliminates not only pathogenic microflora, but also completely removes biofilm. The dosage during the sanitary break is 1 l of solution per 100 l of water (=10 l / m3), and the exposure time is 12 hours.

In our country since 1974, Professor V. M. Bakhir has been developing equipment (reactors) for the production of electrochemically activated solutions. In 2011, significant progress has been made in the development of the latest preparation generation STEL ANK SUPER (only 3 generations), which allows obtaining a neutral anolyte with properties as close to ideal as possible, and further improvement of which is not currently possible due to the limitations caused by the physical nature of metastable compounds of active substances.

This neutral anolyte, called by the authors "ANOLIT ANK SUPER" is the most perfect product of this type, since the content of ballast ions of sodium chloride in it is much less than the concentration of oxidants or completely absent. The concentration of oxidants in terms of active chlorine is not less than 0.5 g/l (0.05 %) with a total content of solutes (mineralization) of no more than 0.9 g/l and a pH of 5.0-6.5. The metastable mixture of oxidants is represented by chloro-oxygen and hydroperoxide compounds: hypochlorous acid (50-95%), chlorine dioxide (1-7%), hydrogen peroxide (3-8 %), other peroxide and superoxide compounds (1-5 %).

"ANOLIT ANK SUPER" after use completely decomposes into the initial substances (water and salt), does not accumulate in the external environment, does not create films on surfaces, has antimicrobial activity against gram-negative and gram-positive bacteria, viruses, pathogenic fungi, does not require washing off surfaces or decontamination after use. By contacting a microbial cell, a neutral anolyte causes its death by violating the integrity of its single-layer cell wall, leaking intracellular components, disrupting the ribosomal apparatus, coagulating the cytoplasm, and so on [5].

As is known, the normal microflora of the gastrointestinal tract is a set of many biocenoses, each of which includes characteristic constantly occurring additional and random types of microorganisms [6].

Taking into account the extremely important role of the normal intestinal biocenosis of bacteria for the preservation of poultry health, it is necessary to fundamentally review the strategy and tactics of selecting and rational use of modern biocides for therapeutic and preventive purposes, in order to minimize their negative impact on the host's autochthonous microflora, to provide reliable methods of monitoring the intestinal microflora, as well as effective ways and means of correcting the normal microflora. The applied preparations should not have an overwhelming effect on the main types of indigenous flora, or their influence should not exceed the threshold of compensatory capabilities of the intestinal microecological system [7].

So, in order to improve the quality of drinking water and reduce the impact of pathogenic bacteria on the bird's body, it is necessary to use biocidal agents as correctly as possible during the growing period, with mandatory monitoring of the results of growing poultry and evaluating the safety of the bird's body, including determining the content of microflora in the bird's intestines (in the blind processes). Therefore, the topic of this article is relevant.

The purpose of this work was to evaluate the impact of modern biocidal agents used in drinking broiler chickens on the quantitative and qualitative composition of the intestinal microbiota.

The methods used in this article are the graphical method, methods of analysis and synthesis of available data.

2. Materials and methods
The research was carried out in department of production technology of poultry products in the laboratory of poultry meat production technology by Federal Scientific Centre "All-Russian Research
and Technological Poultry Institute" of Russian Academy of Sciences (FSC “ARRTPI” RAS), breeding
and genetic center "Zagorsko experimental breeding farm" (the branch of FSC “ARRTPI” RAS) and
in Federal State Budgetary Educational Institution of Higher Education “Moscow State Academy of
Veterinary Medicine and Biotechnology - MVA named after K.I. Skryabin”.

The research was carried out on cross broiler chickens "Ross 308". The technological parameters for
raising chickens were the same for all groups, with the exception of the studied factor, and corresponded
to the recommendations of FSC "ARRTPI" RAS [8].

Feeding of poultry during the growing period was carried out with compound feeds in accordance
with the recommendations of FSC “ARRTPI” RAS on normalized feeding of poultry [9].

“ANOLYTE ANK SUPER” was obtained by electrolysis of tap water in a flow-through installation
STEL ANK SUPER-100.

Four groups of similar chickens were formed, with 35 heads each. The bird was raised in the R-15
cell batteries. Chickens of the control group 1 received normal tap water. In experimental group 2,
“ANOLIT ANK SUPER” was added to the water to obtain a 0.25% solution of neutral anolyte. In the
experimental group 3 chickens with water received a 10% solution of neutral anolyte. In the drinking
water of the experimental group 4, the preparation "DUTRION" was added on the recommendation of
the manufacturer (first, the mother solution was prepared, one tablet was dissolved in one liter of water),
and then 0.75 ml of the solution per one liter of water. The scheme of experience is presented in table 1.

| group | number of chickens | drinking features               |
|-------|--------------------|---------------------------------|
| 1(κ)  | 35                 | tap water                       |
| 2     | 35                 | 0.25 % neutral anolyte solution |
| 3     | 35                 | 10 % neutral anolyte solution   |
| 4     | 35                 | preparation «DUTRION»           |

Studies of intestinal microflora were conducted in the International laboratory of molecular genetics
and genomics of poultry by Federal State Budgetary Educational Institution of Higher Education "
Moscow State Academy of Veterinary Medicine and Biotechnology - MVA named after K. I. Skryabin"
(Moscow). For research, the contents of blind appendages from healthy birds were obtained (3 samples
from each group). At the time of sampling, the birds were 36 days old.

To assess the microbial profile of the contents of blind appendages, microbial DNA from chyme was
isolated using the QIAampPowerFecalDNAKit (Qiagen, USA). The DNA purity was evaluated by
classical electrophoresis in 1% agarose gel with the addition of ethidium bromide (5-3 µl) in a Mini-
SubCell GT chamber (Bio-Rad, USA) with a TAE buffer (ThermoFisher, USA) and a molecular weight
marker-bromophenol blue for DNA (ThermoFisher, USA) in a 1:1 ratio with the sample in the 220 V
mode for 25 minutes. We also determined the amount of isolated DNA on the Qubit 3.0 fluorometer.

The total microbial number was determined by performing Q-PCR-RT using the SIBR GREEN
fluorescent dye. The reaction was performed on a LightCycler®96 System amplifier (Roche,
Switzerland). The number of microorganisms was determined by comparison with known
concentrations of E. coli in three dilutions. After setting Q-PCR-RT to the total microbial number, the
isolated DNA was prepared for loading into the chip for NGS sequencing. Sample preparation includes
many stages of the Protocol. the library was created using the 16s Metagenomik Kit and Ion 520™ &Ion
530™ Kit-OT2. Sequencing was performed on the Ion Game StudioS 5 System – Thermo Fisher
Scientific device. The results were processed using Ion Report, a network software product designed to analyze data on sequencing results and determine microbial composition.

### 3. Research results

To determine the effect of the use of biocides in broiler chickens on the intestinal microflora, the total microbial number and composition of the microbiome were determined using NGS sequencing (table 2).

**Table 2.** Total microbial number and microbial profile.

| Indicator                              | 1(%)     | Group | difference to the control, % |
|----------------------------------------|----------|-------|-----------------------------|
| Total microbial number                 | 2.7*10^9 | 2     | 5.14*10^8                   | 8.7*10^8 | 8.7*10^8 | -81.0 | -67.8 | -67.8 |
| Actinobacteria phylum                  | 1.09     | 3     | 0.33                        | 0.29     | 0.88     | -69.7 | -73.4 | -19.3 |
| Bifidobacteriales genus                | 0.63     | 4     | 0.04                        | 0.04     | 0.18     | -93.6 | -93.6 | -69.8 |
| Bacteroidetes phylum                   | 47.47    | 2     | 38.49                       | 42.18    | 45.16    | -18.9 | -11.1 | -4.9  |
| Porphyromonadaceae family              | 28.6     | 3     | 21.3                        | 19.12    | 22.68    | -25.5 | -33.2 | -20.7 |
| Firmicutes phylum, incl.: Lactobacillaceae family | 42.28   | 4     | 46.97                       | 48.67    | 45.44    | +11.1 | +15.1 | +7.5  |
| Clostridiales family                   | 1.77     | 2     | 2.28                        | 3.56     | 3.28     | +28.8 | +101.1| +85.3 |
| Ruminococcaceae family                 | 5.85     | 3     | 12.55                       | 9.89     | 9.47     | +114.5| +69.1 | +64.8 |
| Selenomonadales genus                  | 14.08    | 4     | 12.2                        | 9.93     | 12.62    | -13.4 | -29.5 | -10.4 |
| Proteobacteria phylum                  | 4.07     | 2     | 0.09                        | 0.17     | 0.1      | -97.8 | -95.8 | -97.5 |
| Synergistetes phylum                   | 7.31     | 3     | 12.85                       | 15.71    | 6.67     | +75.8 | +114.9| -8.8  |
| Tenericutes phylum                     | 0.1      | 4     | 0.03                        | 0.03     | 0.1      | -200  | -200  | 0     |
| Mycoplasmatale genus                   | 1.79     | 1     | 1.29                        | 1.08     | 1.72     | -27.9 | -39.7 | -3.9  |
|                                        | 0.8      | 2     | 0.43                        | 0.42     | 0.45     | -46.2 | -47.5 | -43.8 |

The results showed that the blind appendages of broiler chickens control group were a major phylum _Bacteroidetes_ 47.47% in relative numbers of microorganisms, whereas in chickens of experimental groups the main phylum _Firmicutes_, in the experimental group 2 46.97%, higher compared to the control by 11.1%, in the experimental group 3 – 48.67% (15.1% higher compared to control) in the experimental group 4 – 45.44% (7.5% higher compared with the control group). This change was due to a decrease in the _Porphyromonadaceae_ family, which is potentially a factor of intestinal wall damage [10].

The number of _Actinobacteria_ phylum bacteria decreased by 69.7%; 73.4%; 19.3%, including the _Bifidobacteriales_ bacteria by 18.9%; 11.1%; 4.9% in chickens that received biocidal preparations with water in experimental groups 2, 3 and 4. In the experimental groups, the increase in the phylum _Firmicutes_ was due to an increase in lactobacilli, which are usually considered a sign of a healthy intestine - by 28.8% in the experimental group 2, by 101.1% - in the experimental group 3 and by 85.3% in the experimental group 4 compared to the control group.

It is also necessary to note a decrease in pathogenic bacteria such as phylum _Synergistetes_ - in the second and third experimental groups, these microorganisms decreased by 3 times, and in the experimental group 4 - the total number of these bacteria remained at the control level. There was also a decrease in pathogenic bacteria of the _Tenericutes_ phylum in the experimental group 2 – by 27.9% compared to the control group, in the experimental group 3 – by 39.7%, and in the experimental group 4 – by 3.9%.
The level of the *Mycoplasmatale* bacteria, which are also pathogenic, in the experimental groups was lower than the level of the control group – in the experimental group 2-by 46.2%, in the experimental group 3-by 47.5%, in the experimental group 4-by 43.8%.

There was also a significant decrease in the bacteria of the *Selenomonadales* genus, which are conditionally pathogenic. The decrease compared to the level of the control group was: in the experimental group 2 – 97.8%, in group 3 – 95.8%, in group 4 – 97.5%.

As follows from the above data, the use of neutral anolyte solutions (0.25% and 10%) and an aqueous solution of the preparation "DUTRION" in drinking water led to a certain decrease in the total number of microorganisms. Thus, the total microbial number in the control group was $2.7 \times 10^9$, while in the experimental group 2; 3 and 4 – $5.14 \times 10^8$; $8.7 \times 10^8$ and $8.7 \times 10^8$, respectively.

Thus, from the results of the research, it is necessary to draw a conclusion about the multidirectional effect of the studied biocidal agents when drinking broiler chickens on the level of bifidobacteria, lactobacilli and other bacteria. At the same time, the use of biocidal preparations "ANOLIT ANK SUPER" and "DUTRION" contributes to the overall reduction of pathogenic and undesirable microflora in the poultry intestines.

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