Microspectral method for assessing the functional state of chickens’ hepatocytes under intestinal infectious diseases

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Abstract. Evaluation of the functional state of hepatocytes of intact chickens and chickens under experimental klebsiellosis, escherichiosis and salmonellosis is given using luminescence spectral analysis with fluorescent dye 5-(4.6-Dichlorotriazin-2-yl)amino) fluorescein hydrochloride. A gradual increase in the proteins number in the cells was recorded from 1th to 30th days in intact chickens, which indicated an increase in the metabolism level in chicken hepatocytes. The reverse trend was observed in chickens with experimental intestinal infectious diseases.

1. Introduction

Currently various biological markers are proposed for monitoring the functional state of poultry gastrointestinal tract [1, 2]. Among them signs of mucosal barrier damage and electrolytes leakage, inflammatory and hypersensitivity markers and other, which can be evaluated in different types of samples, such as blood, serum, liver and mucosal biopsy materials, intestinal content or faeces [3]. They have advantages and certain disadvantages. In particular, some of them do not allow us to detect biochemical changes at the cellular level, which are early precursors of pathological conditions and appear before the clinical signs appearance. In addition, not all biomarkers have been tested in poultry.

Micro-spectral analysis is used to study cellular interactions [4], to monitor the distribution of biochemical cells components, utilizing inherent spectral markers [5]. The method of luminescence spectral analysis can be used to detect changes in biochemical processes at the cellular level. The method has already demonstrated its effectiveness in assessing the functional state of different cell types by determining the amount of organic substances, such as proteins and nucleic acids [6 - 9].

The aim of our research was to study the functional state of hepatocytes of intact chickens and chickens under experimental klebsiellosis, escherichiosis and salmonellosis using the luminescence spectral assay.
2. Materials and methods
The active procyon fluorescent dye 5-[(4.6-Dichlorotrizin-2-yl)amino] fluorescein hydrochloride (DTAF) was used to determine proteins, containing amino, imino, and hydroxyl groups. A single-wave method of luminescence spectral assay had been devoted to detect proteins in chicken hepatocytes.

Highsex brown chickens (cockerels) were used as an experimental model. In accordance with the principle of analogues, the chickens were divided into 4 groups: 3 experimental (n=250 each) and control (n=200). The chickens body weight ranged to 10%. Weighing was carried out on OHAUS PA2102C scales.

Chickens were infected with museum strains of Escherichia coli serotype 078, Klebsiella pneumoniae subspecies rhinoscleromatis, Salmonella enteritidis. Infection of 2-day-olds chickens was performed orally with suspension of 24-hours agar cultures of microorganisms using a special syringe. The bacterial cells concentration was determined using MacFarland turbidity standards. Chickens of experimental group I were infected with Klebsiella pneumonia (2.5 x10⁹ CFU / 1 ml) at an infecting dose of 0.4 ml / head, chickens of experimental groups II and III - Escherichia coli and Salmonella enteritidis (2.0 x10⁸ CFU / 1 ml), 0.2 ml / head, respectively. Control group chickens were given oral saline solution in a volume of 0.4 ml / head.

Chickens were sacrificed by decapitation with preliminary ether anesthesia at 1–4th, 6–8th, 10th, 15th, 21th and 30th days of life per 15 heads. Chickens’ liver pieces were fixed in a 10% buffered formalin solution for 7-10 days. After washing for 24 hours in running water, the liver pieces were dehydrated in an alcohol baths (from 60 % to 100 %) with subsequent paraffin embedding [10].

Histological samples with a thickness of 4-7 microns were made from paraffin blocks with use of the microtome "Mikrom HM450" (Germany). They were stained with the fluorochrome DTAF by the author’s method [3]. Luminescence microscopic investigation of unstained and stained samples was carried out using a universal color analyzer – a microscope-spectrophotometer MSFU-K (Russia) [11].

3. Results and discussions
When luminescence microscopic investigation of unstained and DTAF - stained histological chickens’ liver samples, areas with luminescence of bright green color with different intensity degrees were observed against the background of a weak blue-green the tissue fluorescence.

The most pronounced luminescence intensity was observed in histological samples of the chickens control group liver, and the least pronounced - in the I experimental group chickens (klebsiellosis). When studding of histological samples, prepared on 1-4, 6-8, 10, 15, 21 and 30 days of life of chickens with experimental intestinal infectious diseases, visual differences in the fluorescence intensity were not revealed. This method does not allow us to study the dynamics of proteins quantitative content in chickens’ hepatocytes, which depends on the age of poultry and the pathomorphogenesis of klebsiellosis, escherichiosis and salmonellosis of chickens.

In this regard, to assess the functional status of chickens’ hepatocytes, we used a single-wave method of developed by us luminescence spectral assay [8].

The data on proteins quantitative content in chickens’ hepatocytes (relative units) on different days of life are presented in the table 1. The dynamics of the indicators are shown in figure 1.

| Life day | Control | Experimental |
|----------|---------|--------------|
|          | I (klebsiellosis) | II (escherichiosis) | III (salmonellosis) |
| 1        | 1.27±0.01 | 1.28±0.01 | 1.27±0.01 | 1.28±0.01 |
| 2        | 1.47±0.02 | 1.46±0.02 | 1.47±0.01 | 1.46±0.02 |
| 3        | 2.18±0.03 | 1.93±0.01*** | 1.94±0.04*** | 1.95±0.04*** |
| 4        | 2.46±0.02 | 1.84±0.02*** | 2.15±0.01*** | 1.91±0.04*** |
| 6        | 2.74±0.04 | 1.72±0.02*** | 2.15±0.02*** | 1.82±0.04*** |
| Life days | Control | I (klebsiellois) | II (escherichiosis) | III (salmonellosis) |
|----------|---------|-----------------|---------------------|---------------------|
| 7        | 2.87±0.03 | 1.92±0.02***   | 2.27±0.05***   | 1.80±0.03***         |
| 8        | 2.88±0.04 | 2.17±0.01***   | 2.53±0.03***   | 1.87±0.04***         |
| 10       | 2.89±0.04 | 2.38±0.02***   | 2.72±0.02***   | 1.99±0.05***         |
| 15       | 3.18±0.12 | 2.53±0.01***   | 2.86±0.03***   | 2.46±0.07***         |
| 21       | 3.48±0.16 | 2.65±0.01***   | 2.99±0.05***   | 2.74±0.05***         |
| 30       | 4.19±0.09 | 2.84±0.01***   | 3.15±0.01***   | 2.83±0.05***         |

Note: statistically significant difference between the experimental and control groups (*- P ≤ 0.05, ** - P ≤ 0.01, *** - P ≤ 0.001).

Figure 1. Protein content (IB) in the chickens’ hepatocytes of the control and experimental groups I – III (RU).

In the control group chickens, the quantitative proteins content in hepatocytes gradually increased from 1.27±0.01 to 4.19±0.09 RU during the experiment (1 - 30 life days). The functional activity of hepatocytes increased by 229.9% during this period.

In chickens under experimental klebsiellois (experimental group I) the proteins content in liver cells decreased from 1.93±0.01 RU (on the 3th life day) to 1.72±0.02 RU (on the 6th day of life) compared with the control group. Consequently, the functional activity of liver cells decreased by 10.9%. Starting from 7th life day, the IB index values gradually increased and by the experiment finish reached to 2.84±0.01 RU. In total, during the period from 1th to 30th life days, the functional hepatocytes activity increased by 121.9%. However, on the 30th day of life, the value of the IB indicator in the experimental group I chickens was 47.5% less than the same indicator in the control group poultry.

In chickens under experimental escherichiosis (experimental group II), the proteins quantity in hepatocytes gradually increased over the experimental period, to the exclusion of the 6th life day (2.15±0.02 RU), when the indicator was recorded at the level of 4th life day (2.15±0.01 RU). At the same time, the IB indicator values in chickens of the experimental group II were less than those in the control group over the experimental period. During the period from 1th to 30th life days, the functional hepatocytes activity increased by 148.0%. But by 30th days of life, the indicator values were less than in the control group by 33.0%.

In chickens under experimental salmonellosis (experimental group III) the amount of proteins in liver cells decreased from 3th (1.95±0.04 RU) to 7th days of life (1.80±0.03 RU) in comparison with the control group chickens. Starting from 8th life day, there was a gradual increase in the IB indicator values. At the
same time, the \( I_b \) indicator values in the experimental group III chickens were less than those in the control group poultry over the experimental period. During the period from 1\(^{th}\) to 30\(^{th}\) days of live, the hepatocytes functional activity increased by 121.1\%. Although, by 30\(^{th}\) live day, the indicator values were 48.1\% lower than in the control group chickens.

4. Conclusion

The obtained results brings us to the conclusion that the luminescence spectral assay using 5-((4.6-Dichlorotriazin-2-yl)amino) fluorescein hydrochloride allow to reveal the features of protein distribution in histological samples of chickens liver. And self - developed a single-wave method of luminescence spectral assay lets us to calculate their quantitative content. What can have an important differential value in determining the functional status of hepatocytes \[12\].

Normally the protein quantity in hepatocytes is characterized by gradual increasing. But under intestinal infectious diseases (klebsiellosis, escherichiosis and salmonellosis), a decrease of the proteins content (RU) is reviled.

Thus, the indicator of the quantitative protein content in hepatocytes, detected by the method of luminescence spectral analysis using fluorochrome DTAF, can be considered as one of the biological markers of the health of the poultry gastrointestinal tract.

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