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«Microspectral method for assessing the functional state of chickens' hepatocytes under intestinal infectious diseases»

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- **The purpose of our research** to study the functional state of hepatocytes of intact chickens and chickens under experimental klebsiellosis, escherichiosis and salmonellosis using the luminescence spectral assay.

The object of the study was Hisex brown chickens (cockerels). The chickens were divided into 4 groups: 3 experimental (n=250 each) and control (n=200).

As a luminescent marker, **5-([4.6-Dichlorotriazin-2-yl]amino) fluorescein hydrochloride (DTAF)** was used.

Materials and methods.

Chickens were infected with museum strains of *Escherichia coli* serotype 078, *Krebsiella 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 $x10^9$ 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 $x10^8$ 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. Histological samples were stained with the fluorochrome DTAF by the author's method. Luminescence microscopic investigation of unstained and stained samples was carried out using a universal color analyzer – a microscope-spectrophotometer MSFU-K (Russia).

Results

Table 1. Protein content (IB) in the chickens' hepatocytes of the control and experimental groups (RU).

Life day	Groups			
	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***
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 (*- $P \leq 0.05$, ** - $P \leq 0.01$, *** - $P \leq 0.001$)

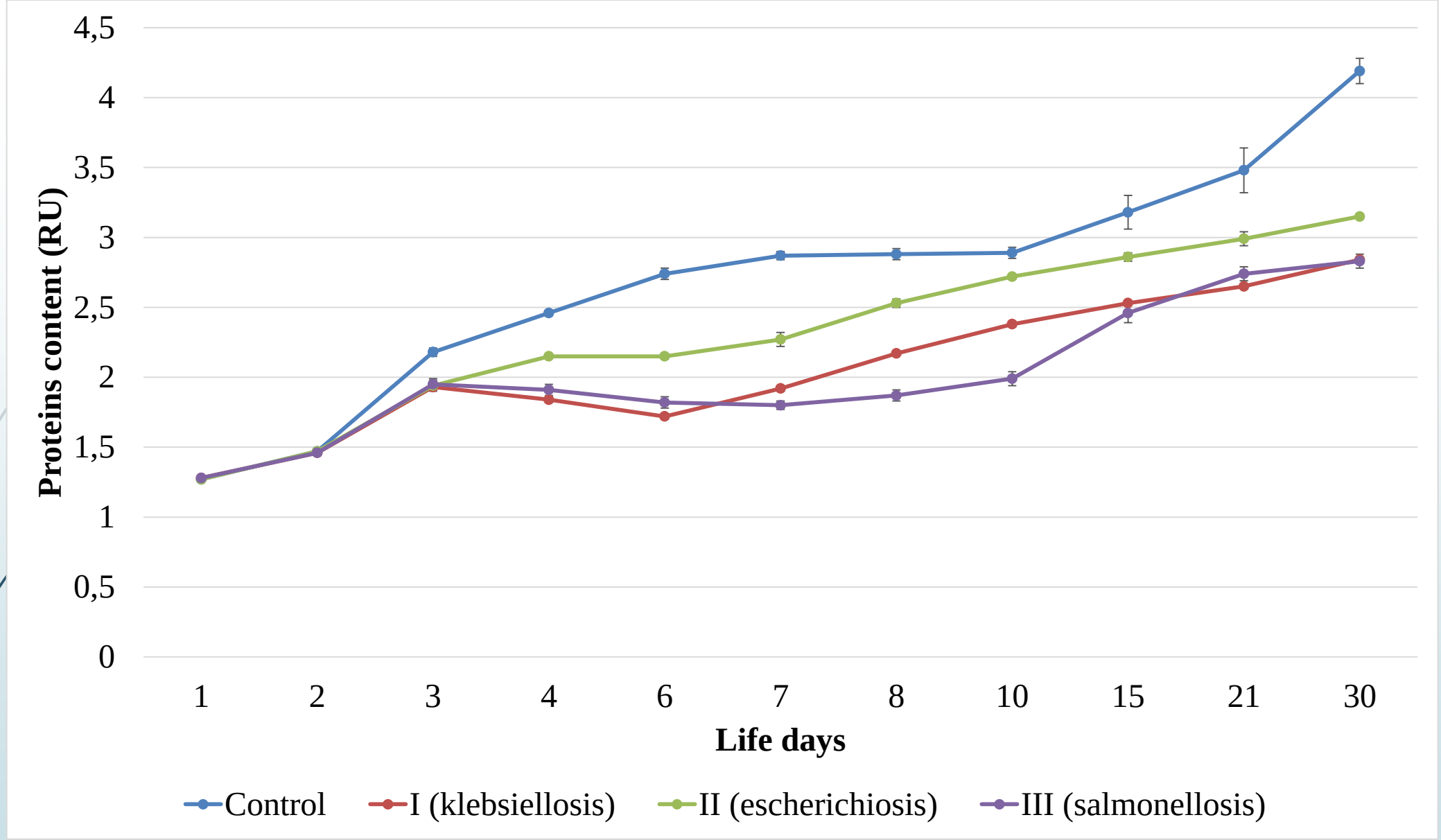

Figure 1 – Protein content (IB) in the chickens' hepatocytes of the control and experimental groups I – III (RU)

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.

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.