THE EVALUATION OF CHOLESTEROL CONCENTRATION IN EGG YOLKS CONTAINING OXYTETRACYCLINE RESIDUE

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Abstract

The purpose of this paper is to calculate the percent of cholesterol and the triacyglycerols which are very important for the pharmaceutical, cosmetic and aquaculture products. We have chosen the cholesterol thanks to its important share from the lipid overall in the egg yolk, considering that this compound might be the marker of chemical denaturation, induced by the medicinal residue over the nourishing components in the egg. We considered that it is important to the correlation between the level of oxytetracycline and the level of cholesterol in the yolk in the case on administration through drinking water and after intramuscular administration. The concentration of cholesterol in the yolk is not a direct consequence of the presence of antibiotics in the egg. The medium value of the cholesterol in eggs coming from hens under treatment, differs based on how the antibiotic is being administered, being larger in the case of the hens who received the antibiotic through drinking water (5.9g cholesterol/100 g yolk), than the ones who have been treated through intramuscular administration (5.5 g cholesterol/100 g yolk); a resembling situation can be observed in the case on cholesterol values, measured after ending the oxytetracycline treatment.

Key words: administration, cholesterol, oxytetracycline, residue, triacyglycerols

INTRODUCTION

The cholesterol is an important component of egg yolk, representing 1,6% of its mass and 5% of its fat [6].

The free cholesterol represents 84% of all the remaining 16% cholesterol, being represented by cholesterol esters [5]. As a forerunner of the bile acids, sexual hormones and adrenal hormones, the cholesterol plays an important role in the well-functioning of cells as well as in the metabolism of lipoproteins. As recognition of its special attempts properties, the of selectively extracting the cholesterol and fatty acids from the yolk without deteriorating the proteins and phospholipids can be mentioned. In order to extend the investigations concerning the effects of oxytetracycline residue on the chemical composition of the egg, an attempt of evaluation of cholesterol concentration in egg yolks containing oxytetracycline residue was considered well-timed.

MATERIALS AND METHODS

For this study, 3 eggs were used for each day of analysis and for each individual group of hens. In order to obtain some information about associating oxytetracycline the treatment with the chemical pollution of the internal environment of the egg, a batch of egg hatching hens, weighting the same and having similar maintenance and health state, was used. The batch was formed of 20 egg hatching hens, which were submitted to the analysis regarding this study, after dividing them into two groups. The oxytetracycline administration was performed differently for each one of the two groups, as following:

Group 1 – intramuscular administration;

Group 2 – oral administration, through drinking water.

The study was based on experimental, intramuscular administration of oxytetracycline, under the form of the commercial product Egocin 10% L.A. (1 ml injectable solution contains 200 mg

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oxytetracycline dehydrate), to the 10 hens in the first group, at a dosage of 2 ml/kg, each day, for five days.

In the second group, the oxytetracycline hydrochloride was given through drinking water during a week, at a dosage of 2 g/liter of water. The eggs were collected twice a day (in the morning and in the evening), during the and also after treatment the final administration, until residue could not be detected anymore. Since the monitoring was realized within 20 days for both groups of hens included in the experiment (7 days of treatment + 13 days of post-treatment, in case of administration through water; 5 days of treatment + 15 days of post-treatment, in case of intramuscular administration). The determination of oxytetracycline residue levels was performed using the high pressure liquid chromatographic with post column derivatization fluorescence and detectiontechnique. Hereby, in the case of the intramuscular treated hens, the last two days of treatment were chosen (4 and 5), corresponding to values of residual antibiotic of 3.63 μ g/g and 4.21 μ g/g, as well as days 3 and 4, after the final administration, corresponding to values of 9.53 μ g/g and 5.43 μ g/g. In the case of the orally treated hens, there were chosen for sampling, the last two days of treatment (6 and 7), corresponding to values of 2.87 μ g/g and 2.96 μ g/g, as well as the first two days after the final administration, corresponding to values of 3.30 μ g/g and 1.67 μ g/g (μ g oxytetracycline/g yolk). Thanks to the fact that the determinations were achieved within 4 days for each group of hens (corresponding to different types of administration of the antibiotic), using 3 eggs for each group and for each day of control, 12 eggs were analyzed for each group, totaling 24 eggs necessary to the study. The method used for extracting the cholesterol implies a working protocol based on the next steps:

A quantity of 0.5 ml of yolk, along 20 ml of methanolic potassium hydroxide and 10 ml of isopropanol, are kept on a water-bath, with refrigerator, for about 30 minutes.

After cooling, the mix is completed with isopropanol, until reaching 50 ml.

The filtering of the homogenized is realized. The clear obtained filtered is used for the enzyme dosage of the cholesterol.

The principle of the dosing method:

The cholesterol is oxidized by the cholesterol oxidase into cholesteron. In the presence of catalase, the resulted hydrogen peroxide from this reaction, oxidizes the methanol into formic aldehyde [2]. The formic aldehyde reacts with the acetyl-acetone, a substance which produces, in the presence of ammonium ions, the color yellow. The concentration of the colored compound is directly proportional with the concentration of the cholesterol and with the intensity of the color, evaluated at a wave length of 405 nm [1]. The reactions involved in the chemical transformations described above are the following:

Cholesterol $O_2 \xrightarrow{\text{cholesterol oxidase}} Cholesterol + H_2O_2 \xrightarrow{\text{Oxygenated water}} Cholesterol$

 $\begin{array}{c} \text{catalase} \\ \text{Methanol} + H_2O_2 & \longrightarrow & \text{Formaldehyde} + \\ 2H_2O \end{array}$

 $\begin{array}{rrrr} Formaldehyde \ + \ NH^{+}_{4} \ + \ 2 \ Acetyl-acetone \\ Lutidine \ + \ \ 3H_{2}O \end{array}$

Yellow compound

The working technique and the calculus of the results were realized according to the usage recommendations of the kit promoted at the Boehringer-Munheim firm, and the concentration was expressed in g cholesterol/100g yolk probe.

RESULTS AND DISCUSSIONS

The evaluations of the lipid content of the yolk, amongst other biochemical compounds, can provide information about what the presence of antibiotic residue doe the biochemical composition of the egg. The obtained results (Table 1) show that high concentrations of antibiotics (oxytetracycline) in the yolk (9.53 maximum oxytetracycline concentration (9.53 μ g/g) cause dropping of the cholesterol concentration (4.90 g/100g). An important aspect is represented by the fact

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that the concentration of the antibiotic (4.21 μ g/g), as well as the dropping of the level of cholesterol (5.20 g/100g), are significantly modified when the medicine is injected.

Table 1. The correlation between the level of cholesterol in the yolk and the concentration of oxytetracycline, in the case of intramuscular administration (medium values).

| | | Concentration | Concentration of |
|-------------------------------------|--------|-----------------------|------------------|
| | Time | of OTC ⁽³⁾ | cholesterol |
| | (days) | μg/g | g/100g |
| During the treatment ⁽¹⁾ | 4 | 3.63 | 5.80 |
| | 5 | 4.21 | 5.20 |
| After the treatment ⁽²⁾ | 3 | 9.53 | 4.90 |
| | 4 | 5.42 | 5.05 |

Source: Own calculations

- 1-numbering from the beginning of the treatment
- 2 numbering from the final administration

3 – Oxytetracycline



Fig. 1. The connection between the level of oxytetracycline and the level of cholesterol in the yolk, in the case of intramuscular administration Source: Own calculations

The reverse proportionality between the concentrations of oxytetracycline and the level of cholesterol from the yolk shows evidently from the graphic representation in the table 1, as well as the figure 1, state the fact that the minimum value of cholesterol (4.90 g/100g), was determined in the probes with the maximum oxytetracycline concentration (9.53).



Fig. 2. The oxytetracycline residue effect concerning the cholesterol level in the yolk (after intramuscular administration) Source: Own calculations

The oral administration of the medicine. unlike the intramuscular one, didn't cause large variations concerning the cholesterol content of the eggs gathered during the days in which the elimination of the antibiotic through the yolk was at its maximum 3,30 μ g/g, the values being shown in the figure 2. The results, presented highlight a relatively constant concentration of this biochemical compound in the egg yolk, derived from oxytetracycline treated hens. the oxytetracycline being administered through drinking water. As well as shown in the Table 2, in the six day the concentration of OTC was 2,87 μ g/g and the concentration of cholesterol was 5, 90 g/100g.

Table 2. The correlation between the level of cholesterol in the yolk and the concentration of oxytetracycline in the case on administration through drinking water (medium values)

| | Time (days) | $\begin{array}{c} Concentration \\ of \\ \mu g/g \end{array} \\ \end{array}$ | Concentration of cholesterol g/100g |
|----------------------------|----------------|--|---|
| During the treatment | 6 | 2.87 | 5.90 |
| | 7 | 2.96 | 5.91 |
| After the treatment (2) | 1 | 3.30 | 5.82 |
| | 2 | 1.67 | 6.01 |

Source: Own calculations

- 1 numbered from the beginning of the treatment
- 2 numbered from the last administration

3-oxytetracycline



Fig. 3. The correlation between the level of oxytetracycline and the level of cholesterol in the yolk in the case on administration through drinking water Source:Own calculations

These results suggest the fact that there is a correlation between the lipid metabolism of the antibiotic treated birds and its level in the egg. The obtained results are in conformity with data from the literature [7] which notice a drop of the concentration of the serum

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cholesterol, following the injectable oxytetracycline treatment.

Table 3. The effect of oxytetracycline residues on the cholesterol level in the yolk (after oral administration).



Source: Own calculations

The same results were obtained by other researchers [4], who, after the injectable oxytetracycline treatment aiming a group of hens, he noticed the reduction of cholesterol and triglycerides in the yolk of the analyzed eggs, without recording any effect on the phospholipidic content.

CONCLUSIONS

The drop of the concentration of cholesterol in the yolk is not a direct consequence of the presence of antibiotics in the egg, but of the effects that these have on the bird's organism, the low level of cholesterol in the eggs being correlated with a decreased cholesterolemy.

The investigation of egg yolks from the birds who had taken the antibiotic through drinking water, mark out the fact that the concentration of the antibiotic, as well as the drop of the cholesterol's concentration, are much less important.

The medium value of the cholesterol in eggs coming from hens under treatment, differs based on how the antibiotic is being administered, being larger in the case of the hens who received the antibiotic through drinking water (5.9g cholesterol/100 g yolk), than the ones who have been treated through intramuscular administration (5.5 g cholesterol/ 100 g yolk); a resembling situation can be observed in the case on cholesterol values, measured after ending the oxytetracycline treatment.

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REFERENCES

[1]Awade, A.C., Moreau, S., Molle, D., Brule, G. and Maubois J.L., 1994, Two-step chromatographic procedure for the purification of hen egg white ovomucin, lysozyme, ovotransferrin and ovalbumin and characterization of purified proteins. Journal of Chromatography A 677(2):279-288.

[2]Bade, H., Stegemann, H., 1984, Rapid method of extraction of antibodies from hen egg yolk. Journal of Immunological Methods 72(2):421-426

[3]Balevi, T., Coskun, B., 2004, Effects of dietary copper on production and egg cholesterol content in laying hens. Br Poult Sci. 2004 Aug:45(4):530-4.

[4]Bărzoi, D., Meica, S., Neguț, M., 1999, Alimentaire ToxinfectionT, Ed. Diacon Coresi, București;

[5]Beia Violeta, Pop Aneta, Savu, C., Beia, S.I., 2005, Concentration of slug, cadmium and copper in chicken eggs in suburban areas and the rural area, Papers of Scientific Symposium U.S.A.M.V., Faculty of Veterinary Medicine – Bucharest.

[6]Beia Violeta, Savu, C., Beia, S.I., Florea, C., Beia Daniela, 2005, Study importance of microbiological determinations for eggs and egg products, Scientific International Symposium ,,Results and perspectives in agriculture ,,Agrarian State University from Republic of Moldova, Chişinău.

[7]Shaddad, S.A., Wasfi, I.A., Maglad, M.A., Adam, S.E., 1985, The effect of oxytetracycline on growth and lipid metabolism in poultry. Comp. Biochem.Physiol., 80C: 375-80.