MONITORING ANTIBIOTIC RESIDUES IN HONEY

MONITORIZAREA REZIDUURILOR DE ANTIBIOTICE DIN MIERE

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Abstract

Next to the β-lactam antibiotics in veterinary medicine, streptomycin is one of the mostly used antibiotics. High concentration of streptomycin could lead to ototoxic and nephrotoxic effects. Low concentration – as found in food – may cause allergies, destroy the intestinal flora and favor immunity to some pathogenic microorganisms. In 1948 chlortetracycline was isolated by Duggan as a metabolite and this was the first antibiotic substance of the group of tetracyclines. In the present paper there are presented the monitoring of the antibiotic residues in honey from Timiş County. The residues of tetracycline and streptomycin in honey were determined by the method ELISA – a quantitative method of detection. The microtitre wells are coated with tetracycline and anti-streptomycin antibodies. Free antibiotic and immobilized antibiotic compete with the added antibiotic antibody (competitive immunoassay reaction). Any unbound antibody is then removed in a washing step. Bound conjugate enzymes convert the colorless chromogen into a blue product. The addition of the stop reagent leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm. The absorption is inversely proportional to the antibiotic concentration in the sample. [9]

Key words: *monitoring, antibiotic, residues, honey*

Rezumat

După antibioticele pe bază de betalactamice, în medicina veterinară cel mai folosit antibiotic este streptomicina. Concentraţiile mari de streptomicină pot avea efecte ototoxice şi nefrotoxice. În concentraţii mici – găsite în alimente – pot cauza alergii, pot distruge flora intestinală şi pot da rezistenţă la anumite microorganisme. În 1948 clortetraciclina a fost izolată de Duggan şi a fost primul antibiotic din clasa tetraciclinelor. În lucrarea de faţă se prezintă ca studiu de caz monitorizarea reziduurilor de antibiotice din mierea de albine în judeţul Timiş, în perioada 2006-2008. S-au determinat reziduuri de tetraciclină şi streptomicină din miere prin metoda Elisa, metodă cantitativă de decelare. Godeurile prezente în kit sunt căptuşite cu tetraciclină, respectiv anticorpi anti-streptomicină. După adăugarea standardelor, probelor şi a reactivilor necesari, antibioticul liber şi cel imobilizat competiţionează cu anticorpul de antibiotic adăugat (reacţie imunoenzimatică de competiţie). Orice anticorp nelegat este eliminat în faza de spălare. Enzimele conjugate legate transformă culoarea cromogenului în albastru. Adăugarea apoi, a soluţiei de stopare schimbă culoarea din albastru în galben. Citirile se fac fotometric la lungimea de undă de 450nm. Absorbanţa este invers proporţională cu concentraţia de antibiotic din probă.

Cuvinte cheie: *monitozare, antibiotic, residuu, miere*

Introduction

After beta-lactamase based antibiotics the most widely used veterinary antibiotic is streptomycin.

High concentrations of streptomycin may have ototoxic and nephrotoxic effects. In low concentrations - found in foods - can cause allergies, can destroy intestinal flora and may give resistance to certain microorganisms.

In 1948 chlortetracycline was isolated by Duggan as a metabolite and this was the first antibiotic substance of the group of tetracyclines.

Honey is mainly used in food for nutrition, diet therapy and has great value. Honey is the sweetest natural product and is made by processing plant nectar of flowers or mane.

The chemical composition of honey is water, glucose, fructose, sucrose, dextrin, vitamins of group B, C, provitamin A, P, K, pantothenic acid, folic minerals Ca, Na, P, Al, Fe, Si , Mg and micronutrients such as Ni, Ag, Vd, Cr.

Proteins are present in honey in small amounts as free amino acids such as lysine, valine, threonine, tryptophan, leucine [1].

By keeping honey, it deposits crystals consisting of levulose, remained fluid consisting mostly of dextrose [4].

All of these bioactive substances have toning action, energizing action in states of convalescence and anemia.

Honey is a unique food in terms of acceptance by the consumer.

It is seen as a natural product that does not undergo any treatment of the human [2].

Quality control is considered to mark the food industry, a prerequisite for a product to be competitive.

The smallest deviation in terms of flavor, color, flavor or consistency of honey from a variety of ordinary quality product will destined the product to be discarded or downgraded.

To establish rules to ensure the highest quality honey possible, we must take into consideration certain physical and chemical properties.

Recent years have shown an increased interest for Romanian honey composition and the place it might be provided according to the proposed EU rules

Honey exports abroad, especially in the European Union, have raised several issues relating to alignment with EU quality standards.

The honey processors were forced to seek veterinary laboratories authorized to perform the required quality consignments for export, being established surveillance and control measures of certain substances: streptomycin, tetracycline, sulphonamides.

Characteristics of honey

The color of honey is given by the plant pigments it contains.

The colour in honey becomes more pale during ageing (pigments accentuates their color), under the influence of high temperature (liquefaction), by keeping it exposed to light to long (transparent packaging), or in packaging that corrodes (iron).

Honey consistency (viscosity) is determined by the relationship: carbohydrate substances - water, which in turn is dependent on the ratio between fructose and glucose.

The floral honey fluidity is the consequence of prolonged fructose compared to glucose prevalence.

Crystallization of honey is determined by increasing glucose ratio, equal or, – less frequent-, overcoming fructose.

High quality honey contains: 18% water and 82% dry substance.

The dry component is 94.6% sugar and 3.6% other substances.

Out of the weight of floral honey, simple sugars (fructose and glucose) represent 70-75% while disaccharides (sucrose and maltose) represent only 5%. [3].

Bee diseases and their treatment with antibiotics

Disease prevention in beekeeping shall meet the following particular:

Selection of resistant breeds

Practical application of favoring a good resistance to diseases and preventing infections such as: regular renewal of queen bees, systematic control of hives to detect any form of disease, control juvenile male bee families, disinfecting materials and equipment, materials or destruction contamination sources, regular renewal beeswax and respectively establishment of sufficient reserve of honey and bee pollen families.

Antibiotics are used to treat bacterial diseases. Diseases caused by bacteria are: European foulbrood, American foulbrood, salmonellosis and septicemia. [8].

Temperature variations between 0 and 50 degrees C did not influence the amount of antibiotics present in the sample.

These antibiotics are increasingly used by beekeepers in the preventive treatment and control of bee diseases.

Therefore, to eliminate the risk of contamination of food with residues of tetracycline and streptomycin is necessary to monitor them.

European foulbrood

Etiology of this disease is complex. In the intestine of dead or sick larvae with the disease, there is a bacterian flora formed *of Bacilus Alvei, Bacilus Orpheus, Eurydice bacterium, Streptococcus and Streptococcus Apis Pluto.* It occurs mainly in the spring before the harvest of Acacia.

Infection occurs by mouth, administered through feeding juveniles by nurse bees.

Put in larval gut microbes multiply in the intestine on account of their existing food and eliminate toxins that spread through the intestinal wall throughout the body, causing serious physiological disturbance and ultimately death of larvae.

The apiary disease can be transmitted through drones, infected inventory, etc. [6].

In combating this disease is recommended: weak family unification, changing queens, disinfection of hives and making medical treatment.

This disease can be treated with one of the following substances:

- Oxytetracycline (tetracycline) from 0.5 to 0.75 g per liter of syrup, are each managing 4-5 every 250 - 500 ml of syrup every 4-5 days.

- Locamycin mix 2.5 g to 1 kg powdered sugar, consists of powdering frames with 80-100g mixture of three times in three days and another two times at intervals of 5-7 days[7].

- Streptomycin 0.5 g per liter of syrup is given ~ 100 ml syrup for one frame at 5-7 days. Due to increasing demands on the use of antibiotics in their honey for export must be done with great discernment [7].

The European Union through legislation tried to lower the maximum admitted limits, as low as possible to protect the consumers.

American foulbrood

Etiologic agent of this disease is *Bacillus larvae* that develop when the living conditions of bees' families are unfavorable.

It appears after picking *Acacia.*

Contamination is achieved by feeding larvae aged 2-3 days by contaminated nurse bees.

The disease is terminated by destroying weak families or only the weak honeycombs with signs of illness from strong families.

The treatment of sick families is made with oxytetracycline or with locamycin administered as for European foulbrood or by administering negamicin and erythromycin. [5].

Apipest consists of oxytetracycline that acts on the vegetative form of *Bacillus larvae.*

To treat bee brood antibiotics are used. It was found that antibiotics reduce resistance to bee fungal diseases.

Salmonellosis

Etiologic agent is *Bacillus paratyphi alvei* that can be found in the digestive tract of healthy bees that becomes pathogen when the natural resistance of the family decreases under the influence of unfavorable factors. In these situations it multiplies rapidly, penetrates in to the haemolymfa and causes death through septicemia.

This disease usually occurs in the spring and not so often in the summer.

Sick bees shows distended abdomen, cannot fly, paralyze and die.

Fighting these diseases is achieved through measures of hygiene by ensuring abundant harvest or by feeding sugar syrup and strengthening the sick bee families periodically.

Treatment consists of administration of oxytetracycline or streptomycin as with European foulbrood.

Healing takes place within 10-20 days after the treatment.

Septicemia

Pathogen is *Bacillus apisepticus* agent who meets frequently inside the hive, but becomes virulent only when the natural resistance of the family decreases.

Disease occurs in adult bees at any time of year, is favored by high humidity of hives, lack of harvest, etc. [7].

The disease can be prevented by better standards of maintenance, strengthening and stimulating the bee families, as well as changing the queen.

Although there is no specific treatment of septicemia, it was found that antibiotics (oxytetracycline) prevent the occurrence of other problems [6].

Study objectives

Aim of the study is the monitoring of residues of antibiotic (tetracycline, streptomycin) of honey.

Results and discussion

Timis County was used to carry out the investigations.

This sample was analyzed for 30 days, with screening test Elisa [10], in order to follow of the antibiotic's (tetracycline) evolution, used to treat bees.

Next Sample preparation was performed according to Work Protocol [10].

Ridawin program calculates concentration values depending on absorbency, also recording semi-logarithmic chart and calculating the real concentration of tetracycline in the sample.

To obtain the actual concentration of tetracycline in the sample, the value obtained must be multiplied with the dilution factor for honey – 50 [10].

Calibration μg/kg (ppb) curve should be linear in the field from 0.150 to 1.350 μg/kg and the corresponding concentrations of tetracycline in μg/kg according to the absorbency of every sample can be read from the calibration curve [10].

Table 1.
Tetracycline concentration depending on time

DAY	Concentration of tetracycline (ppb)
1	199.00
2	198.88
3	198.74
4	198.61
5	198.00
6	197.87
7	197.74
8	197.60
9	197.42
10	196.00
11	195.71
12	195.20
13	195.00
14	194.70
15	194.13
16	193.98
17	193.72
18	193.00
19	193.76
20	192.98
21	192.74
22	192.51
23	192.39
24	192.00
25	191.88
26	191.75
27	191.62
28	190.33
29	189.86
30	189.79

In Figure 1, it is noted that the tetracycline concentration decreases linearly, with linear regression equation:

$$y = - 0,322x + 199,5.$$

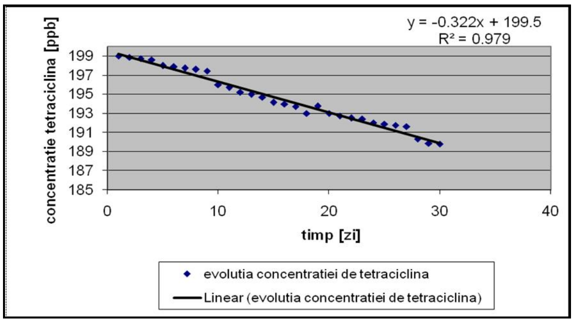

Fig. 1. Evolution of concentration of tetracycline

In Figures 2, 3 and 4 is observed that the three curves are polynomial - R2 between 0.846 and 0.961. To follow the evolution of streptomycin antibiotic depending on temperature, a honey sample was analyzed, which was previously tested to see if no antimicrobials are present.

The sample was then fortified with 20 ppb, 30 ppb and 40 ppb of streptomycin.

After preparation, samples were kept for 30 minutes under different conditions of temperature (0, 10, 20, 30, 40, 50°C).

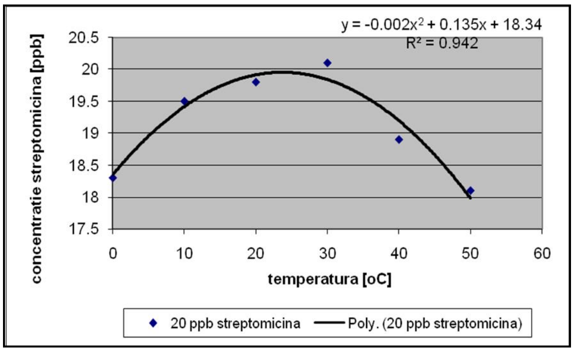

Fig. 2 Evolution of concentration of streptomycin (20 ppb)

Sample preparation, preparation of reagents and standards were made in accordance with existing protocol work in kit. Standard solutions provided in the kit are concentrated and must be diluted 1:10 (1 9) with buffer solution [8].

For the determination Elisa the work protocol must be followed and the absorbency is mesured at 450 nm against a blank of air. Medium values of absorbance of standards and samples are divided to the absorbance value of the first standard (zero standard) and then multiplied by one hundred.

Ridawin program calculates the concentration values depending on absorbency, recording the semi-logarithmic chart and calculating the real concentration µg/kg of streptomycin in the sample.

Calibration µg /kg (ppb), curve should be linear in the field 2-32 µg/kg, and the concentrations of streptomycin in µg/kg, corresponding to the absorbance of each sample can be read from the calibration curve.[9]

To obtain the real concentration of streptomycin in the sample, the value obtained must be multiplied by the dilution factor as follows – for honey is 20 [9].

Table 2
Concentration of streptomycin (ppb)

Temperature (°C)	Concentration of streptomycin (ppb)		
	20 ppb	30 ppb	40 ppb
0(°C)	18.3	28.8	38.1
10(°C)	19.5	29.2	39.4
20(°C)	19.8	30.1	40.0
30(°C)	20.1	29.9	39.8
40(°C)	18.9	28.5	39.2
50(°C)	18.1	28.0	38.5

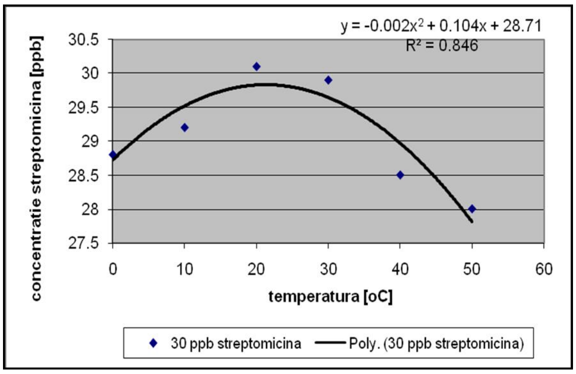

Fig. 3 Evolution of concentration of streptomycin (30 ppb)

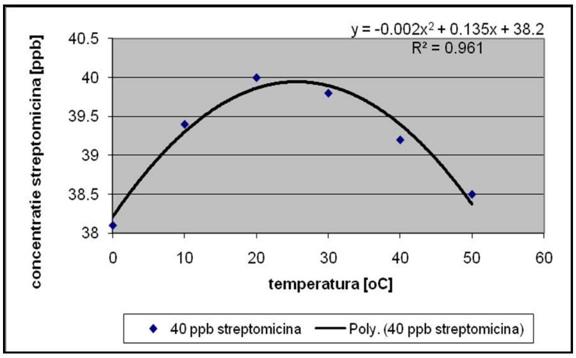

Fig. 4 Evolution of concentration of streptomycin (40ppb)

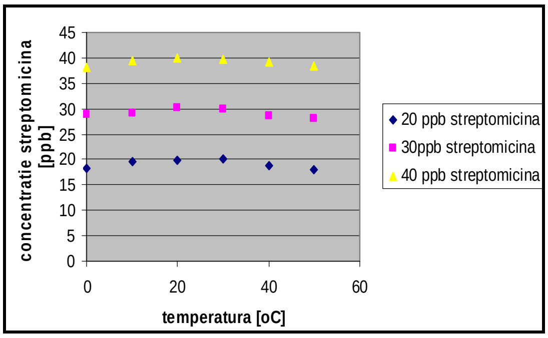

Fig. 5 Evolution of concentration of streptomycin

CONCLUSIONS

1. Putting them in the same graph we can see a similar comportamet of curves for all three concentrations of streptomycin followed, and the variation is several ppb units to the initial concentration.
2. 2. Due to the increasing requirement for antibiotics in honey exported their use should be made with great discernment. In this respect, it was.
3. To eliminate contamination of food with residues of tetracycline and streptomycin their monitoring is required. In this concern an annually sanitary – veterinary survelliance plan was established to control these residues.

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