PREMI®TEST - FAST SCREENING TEST FOR DETECTION OF SULPHADIMIDINE RESIDUES IN POULTRY TISSUES

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SUMMARY

The aim of the study was to detect the residue concentrations of sulphadimidine (SD) in the edible tissues of laying hens after oral administration up to the 15th day of the withdrawal period (WP) by the two microbiological methods – The Four plate test (FPT) and the Premi®Test. Finally, the results were confirmed by high-performance liquid chromatography system (HPLC). The FPT detected positive results of SD residues only the first day of the withdrawal period in all edible tissues, the Premi®Test detected positive results in the muscle and in the liver till the fifth day and in the gizzard till the third day of the withdrawal period. The Premi®Test was more sensitive than FPT and the results obtained by Premi®Test were confirmed by HPLC.

INTRODUCTION

The four plate test is the reference method for the detection of the presence of sulphonamides residues in the food producing animals and in the foods of animal origin (Nagy et al., 1996; Jevinova et al., 2003; Kožárová et al., 2001). The agar medium inoculated with the *Bacillus subtilis* BGA (pH 7.2) with the addition of trimethoprime (0.05 μg.ml^{-1}) is the most sensitive testing microorganism to the sulphonamides residues in the comparison with another test strain used in four plate test. Recently a new broad spectrum screening test for the detection of antibiotic residues and sulphonamides, Premi®Test, has been developed (Kožárová and Labanská, 2005; Lohajová et al., 2004., Popelka et al., 2001).

The aim of our experiment was to detect the residues of SD in the edible tissues of the laying hens by the four plate test and the Premi®Test and the results were confirmed by HPLC.

MATERIAL AND METHODS

Experimental animals. In our experiment, 35 laying hens (ISA Brown, from the farm AGRONÁKUP T, Zemplínsky Branč, Slovak Republic) in the 35th week of laying period were used. Sulfadimidin PG plv. sol. a.u.v. (PHARMAGAL, Nitra, Slovak Republic) in a dosage of 120 mg (dissolved in water) per kg of body weight and per day (recommended dosage by producer) with a probe into the oesophagus was administered. Medicated drinking water was prepared each day. SD was applied three days and after three days of break was applied next three days. Six laying hens free of residues were used as a control. After the end of administration of SD two laying hens were slaughtered every day during fifteen days of withdrawal period. The muscle and internal organs were used for the detection of SD residues and 6 samples were prepared for each method.

Detection by four plate test. The stock solution, agar media, assay plates and the procedures were described by Kožárová and Máté (2000).

Detection by the Premi®Test. Premi®test was obtained from DSM (Netherlands). From the sample of lean muscle, liver and gizzard was obtained

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250 µl of tissue fluid by the garlic press. A total of 100 µl tissue juice was applied to an ampoule and preincubated 20 min at room temperature. After preincubation the residual tissue fluid was washed away and medium in the ampoule was washed two times by deionised water. The ampoules were then incubated 3 h ± 15 min at 64 °C ± 0,5 °C. The colour of medium was evaluated. The concentration of SD residues below the limit of detection (LOD) or their absence was visualized as a colour change from purple to yellow and in the presence of SD residues at or above the LOD the colour wasn't changed.

Detection of sulphadimidine residues by HPLC. Method was described by Sokol (2001).

RESULTS AND DISCUSSION

Current legislation has established the MRL of 0.1 $mg.kg^{-1}$ for sulphonamides in foods of animal origin (EEC No. 2377/90; 1990).

By means of FPT, the presence of SD residues in all tested samples was detected only on the first day after the end of the drug administration. From the second day of withdrawal period the negative results were obtained in all tested samples of meat and organs. The confirmation of results by HPLC showed that FPT detected false negative results from second to fifth day of withdrawal period.

The Premi®Test integrates a strategy of detection of antibacterial substances below MRL for a wide spectrum of biological matrices. The Premi®Test is based on inhibition of growth of test microorganism *Bacillus stearothermophilus* visualized as a colour change of the agar medium in case of absence the residues or any colour change in their presence.

The results of detection of SD residues by the Premi®Test are recorded in Table 1. The presence of SD residues above a limit of detection (LOD 0.05 $mg.kg^{-1}$) of the method was in the samples of breast and leg muscle detected during the first five days of the withdrawal period. From the sixth day of the withdrawal period all samples were negative. In the liver of laying hens was the presence of SD residues above the LOD till fifth day after the end of the drug administration. From the sixth day of withdrawal period all samples were negative. In the samples of gizzard was the presence of SD residues above the LOD recorded only first three days after end of the oral administration of the drug. The negative result of the test was recorded from the fourth day of the withdrawal period.

The results obtained by Premi®Test were confirmed by quantitative HPLC method (Table 2). The results indicated that the Premi®Test could be used for the detection of SD residues in the edible tissues of laying hens. A reliability and sensitivity of the

▼ **Table 1.** Detection of the SD residues by the Premi®Test in the edible tissues (n=6)
▼ **Tablica 1.** Određivanje ostataka sulfonamida u jestivim tkivima (n=6) Premi®Testom

Day WP/ Dan izlučivanja	Liver/jetra		Gizzard/želudac		Muscle/mišić	
	Sample/ uzorak	Control/ kontrola	Sample/ uzorak	Control/ kontrola	Sample/ uzorak	Control/ kontrola
1.	+	–	+	–	+	–
2.	+	–	+	–	+	–
3.	+	–	+	–	+	–
4.	+	–	–	–	+	–
5.	+	–	–	–	+	–
6.	–	–	–	–	–	–
7.	–	–	–	–	–	–
8.	–	–	–	–	–	–
9.-15.	–	–	–	–	–	–

▼ **Table 2.** The residual concentrations of the SD residues (mg.kg^{-1}) detected by HPLC (n=6)
▼ **Tablica 2.** Koncentracija ostataka sulfonamida (mgkg^{-1}) utvrđena HPLC metodom (n=6)

Tissue/ Tkivo	Withdrawal period/ vrijeme izlučivanja Day/dan							
	1.	2.	3.	4.	5.	6.	7.	8.-15.
Breast muscle/ prsni mišić	37,22 ± 3,42	25,44 ± 2,37	1,97 ± 0,17	1,68 ± 0,13	1,48 ± 0,11	0,043 ± 0,008	0	0
Leg muscle/ nožni mišić	37,22 ± 3,42	25,44 ± 2,37	1,97 ± 0,17	1,68 ± 0,13	1,48 ± 0,11	0,043 ± 0,008	0	0
Liver/jetra	8,116 ± 0,75	0,404 ± 0,32	0,155 ± 0,015	0,119 ± 0,011	0,104 ± 0,009	0,099 ± 0,009	0,035 ± 0,003	0
Gizzard/želudac	18,495 ± 1,32	0,241 ± 0,021	0,212 ± 0,020	0,038 ± 0,002	0,034 ± 0,003	0	0	0

Premi®Test for the SD residues was also described by Stead et al. (2004) and Kožárová and Labanská (2005). Above mentioned authors achieved limit of detection of the Premi®Test ranged from 0.01 to 0.1 mg.kg^{-1} and the sensitivity of the method was in accordance with requirements of the European legislation (EEC No. 2377/90; 1990).

PROŠIRENI SAŽETAK

PREMI®TEST – BRZI TEST ZA DOKAZ OSTATAKA SULFONAMIDA U TKIVU PERADI

Four Plate Test referentna je metoda za otkrivanje ostataka sulfonamida u hrani životinjskog podrijetla. Bacillus subtillis BGA (pH 7.2) uz dodatak trimetoprima (0.05 µg ml-1) najosjetljiviji je na ostatke sulfonamida u usporedbi s drugim indikatorskim mikroorganizmima. Nedavno je razvijen još jedan "screening" test za otkrivanje ostataka antibiotika i sulfonamida - Premi®Test. Cilj ovog istraživanja bio je detektirati ostatke sulfonamida u jestivim tkivima nesilica pomoću Four Plate i Premi® testa te rezultate provjeriti HPLC-om.

U istraživanju su korištene nesilice (n=35) u 35. tjednu nesenja. Sulfadimidin PG plv. sol. a. u. v. apliciran je sondom u dozi 120 mg/kg (preporučena doza proizvođača). Sulfonamid je primjenjivan tijekom 3 dana te ponovno trokratno nakon 3 dana stanke. Nakon prestanka davanja preparata nesilice su privedene klanju (n=2) tijekom 15-dnevnog perioda izlučivanja (karencija). Ostaci sulfonamida određivani su u mišićju i unutrašnjim organima.

Za Premi®Test iz mišićja, jetre i želuca izdvojeno je 250 µl tkivnog soka, a 100 µl je prebačeno u testne ampule i preinkubirano 20 minuta na sobnoj temperaturi. Nakon preinkubacije zaostali tkivni sok je ispran. Ampule su potom inkubirane 3 sata ± 15 min na 64 °C ± 0,5 °C, nakon čega je provjerena boja medija. Koncentracije sulfonamida ispod granice detekcije ili negativan nalaz očitovali su se promjenom boje medija iz ljubičaste u žutu, a u prisustvu ostataka sulfonamida u količini iznad granice detekcije boja medija je ostala nepromijenjena.

Postojeća legislativa propisuje 0.1 mg/kg kao najviše dopuštenu količinu ostataka sulfonamida u animalnim namirnicama. Four Plate testom ostaci sulfonamida utvrđeni su samo prvog dana nakon prestanka njegove aplikacije nesilicama. Provjerom rezultata HPLC metodom uočeno je da je Four Plate Test davao lažno negativne rezultate od 2. do 5. dana 15-dnevnog perioda izlučivanja. Uporabom Premi Testa prisutnost ostataka sulfonamida iznad granice detekcije (0.05 mg/kg) zabilježena je u uzorcima mišićja i jetre tijekom prvih 5 dana izlučivanja, a u uzorcima želuca tijekom prva 3 dana. Rezultati Premi testa potvrđeni su kvantitativno na HPLC-u, što upućuje na prikladnost Premi testa za detekciju ostataka sulfonamida u jestivim tkivima nesilica.

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ČIMBENICI KAKVOĆE PRŠUTA

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SAŽETAK

Zdravstvena ispravnost sirovine, proizvodnja i prerada u odgovarajućim registriranim objektima i veterinarsko-sanitarni nadzor od farme do maloprodaje preduvjeti su bez kojih nema legalne proizvodnje pršuta. Tek uz navedene uvjete zdravstvene ispravnosti može se govoriti o kakvoći proizvoda, odnosno o čimbenicima koji utječu na proizvodnju više ili manje kvalitetnog proizvoda. Proizvodi koji se proizvode s namjerom stavljanja na tržište moraju zadovoljiti propisane veterinarsko-sanitarne uvjete proizvodnje. Spomenuti čimbenici koji u lancu proizvodnje pršuta od farme do stola utječu na konačnu kakvoću pršuta, iako brojni, mogu se u osnovi podijeliti na čimbenike izbora sirovine i čimbenike načina prerade, odnosno preradbene tehnologije. Izbor sirovine, odnosno kakvoća buta prvenstveno ovisi o genotipu, dobi i tjelesnoj masi svinja, ali i o brojnim drugim čimbenicima kao što su tehnologija uzgoja i tova svinja, postupku sa svinjama ante i post mortem, morfološkim osobinama svinja, odnosu mišićnog i masnog tkiva, fizikalno – kemijskim osobinama mesa (pH, WHC, sastav enzimskog sustava itd.) i dr. Definiranje odgovarajućih genotipova svinja pogodnih za proizvodnju pršuta, te tehnologije njihova uzgoja i tova uz uvođenje standarda u tehnološki postupak prerade pršuta, osobito tradicionalnih tipova (dalmatinski i istarski pršut) značajno bi doprinijelo rješavanju problema neujednačenosti kakvoće finalnog proizvoda. Time bi se, uz definiranje osobina finalnog proizvoda i zakonsku zaštitu imena (zaštita izvornosti i zemljopisnog podrijetla temeljem važećih zakonskih propisa) doprinijelo očuvanju i unaprjeđenju visoke kakvoće i tržišne vrijednosti naših tradicionalnih tipova pršuta, te njihova konkurentnost na domaćem i svjetskom tržištu.

Ključne riječi: *kakvoća, pršut*

UVOD

U lancu proizvodnje i prerade mesa, od farme do stola (Slika 1.) veliki je broj čimbenika koji utječu na sirovinu, a u konačnici i na kakvoću pršuta. Svi se oni mogu u osnovi svrstati u dvije osnovne grupe: čimbenici kakvoće sirovine i čimbenici preradbene tehnologije. Jedan od najvećih problema u domaćoj proizvodnji pršuta, osobito tradicionalnih tipova (dalmatinski i istarski pršut) je neujednačena kakvoća butova namijenjenih preradi što uz nestandardiziranu preradbenu tehnologiju rezultira velikom varijabilnošću u kakvoći pršuta. Stoga se na tržištu pod istim nazivom mogu naći pršuti najrazličitijih osobina. Osnovni razlog tomu je uvoz sirovine (od živih svinja do butova) različitog podrijetla.

Procjena udjela mesa u trupu i debljina leđene slanine, osnovni je kriterij kod ocjene ekonomske vrijednosti svinja. Stoga je cilj selekcijskih programa u svinjogojstvu dugi niz godina bio povećanje

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