Coccidiostats in table eggs, liver and poultry meat on the market in Bosnia and Herzegovina

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Abstract. Poultry meat production is one of the most dynamic sectors in agriculture, recording the quickest growth in the food industry, while egg production has shown strong growth in the last twenty years. Combined with meat production, it is achieving the highest growth when it comes to meeting protein needs for the global population. In economic terms, coccidiosis is one of the most significant poultry diseases. Effective application of coccidiostats in poultry feed has been playing a key role in development of commercial poultry production for more than 50 years. The aim of this research was to estimate occurrence and residue concentrations of coccidiostats in table eggs, poultry liver and meat, available on the market in Bosnia and Herzegovina (B&H). Residues of lasalocid were found in table eggs, while residues of nicarbazin, maduramicin and diclazuril were detected in broiler meat and liver.

1. Introduction
Large bird agglomerates in poultry facilities are at risk of fast, massive spread of various infectious diseases. From an economic perspective, coccidiosis is one of the most significant invasive diseases in intensive poultry farming, especially in young animals [1].

Coccidiosis is a parasitic disease of various food production animal species, and is one of the most frequent health problems in intensive poultry farming [2,3]. The prevalence of coccidiosis in commercial poultry flocks is directly proportionate to production intensity, which is characterized by high animal density per unit, i.e., population density, in commercial production facilities [3]. Dense populations, along with other stress factors, help the occurrence and spread of the disease in flocks [4].

The prevention of coccidiosis is based on fundamental factors: zoohygienic measures, genetics, vaccination and administration of coccidiostats [5]. Among them, prudential and effective use of coccidiostats in poultry is essential for modern commercial poultry production. Coccidiostats interfere
in various stages of intestinal development of *Eimeria*, and are the only veterinary drugs allowed as additives in animal feed to prevent and treat poultry coccidiosis [1,6].

This paper reports the results of a study conducted on products at retail in B&H to estimate the occurrence and residue concentrations of coccidiostats in table eggs, poultry liver and meat.

### 2. Materials and methods

Ninety samples of table eggs of domestic origin were collected to study the presence of coccidiostat residues. One representative sample was made up of twelve table eggs. Sampling of broiler meat and liver on B&H’s retail market was conducted in three groups. The first and second groups (Group A and Group B) included samples from two different local poultry producers that had been distributing their products to the whole B&H market. The third group (Group C) included samples from an importer, whose products were also available on the entire market in B&H. Random sampling was conducted between April 2017 and April 2018 in large retailers across B&H.

Screening identification of residues of diclazuril (DICL), lasalocid (LAS), maduramicin (MAD), monensin (MON), narasin (NAR), nicarbazin (DNC), robenidine (ROBN), salinomycin (SAL) and toltrazuril sulfone (TOLT) in samples of table eggs, broiler meat and liver was conducted using liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS) with a Waters ACQUITY detector connected to TQD mass spectrometer (Waters, Milford, MA, USA), controlled by MassLynks software version 4.1 in the Laboratory for Residues and Food Quality Testing of the Veterinary Faculty, University of Sarajevo, B&H. Confirmation testing was conducted at the Institute of Meat Hygiene and Technology of Serbia, using a Shimadzu LCMS-8040 Triple Quadrupole Liquid Chromatograph Mass Spectrometer (Shimadzu, Japan), operated in positive ion mode, controlled by LabSolution software.

Statistical data analysis of grouped data was conducted using the IBM SPSS Statistics v. 17 software (IBM Inc., USA). Fisher’s exact test and chi-square test ($\chi^2$) were used to test the statistical significance of difference in frequency of coccidiostat-positive samples that contained residues of DNC, MAD, LAS and/or DICL among three types of tested matrices (eggs, broiler meat and liver), as well as to test statistical significance of difference in frequency of samples positive for residues of MAD, DNC and/or DICL, given the difference in sample origin (local and imported). P-values less than 0.05 were considered statistically significant.

### 3. Results and discussion

Table 1 shows the results of screening tests for presence of residues of the nine coccidiostats by using the LC-MS/MS technique.

| Table 1. Occurrence and frequency (%) of coccidiostat residues in table eggs, broiler meat and liver that screened positive$^a$. |
| --- |
| Table eggs (n=90) | Group A$^b$ | Group B$^c$ |
| (n=30) | (n=30) | (n=30) |
| Lasalocid | 10 (11.1%) | - | - |
| Nicarbazin | 4 (13.3%) | 12 (40.0%) | 2 (6.6%) |
| Diclazuril | - | - | 2 (6.6%) |

$^a$ concentration of coccidiostats > CClB (5 µg/kg)

$^b$ samples from local producer A

$^c$ samples from local producer B

Note: Residues of maduramicin, narasin, monensin, salinomycin, toltrazuril sulfone and robenidine were not detected in any of the tested samples. None of the nine tested coccidiostats were detected in the imported broiler meat and liver (Group C).

Confirmation testing included 36 samples, out of which 10 samples of table eggs were positive for LAS residues, 6 samples of muscle tissue and 16 samples of liver for DNC residues, and 2 liver samples for...
DICL and MAD residues. LAS residues were confirmed in all 10 samples of table eggs (m/z: 613.2→359.3 and 613.2→377.3), with concentrations ranging from 7 µg/kg to 33 µg/kg (mean value of 20.8 µg/kg), while DNC residues (m/z: 301→107 and 301→137) were confirmed in all 22 samples of muscle tissue and liver of broilers. The concentrations of DNC residues in broiler liver samples ranged from 6 µg/kg to 165 µg/kg, with a mean value of 43.4 µg/kg, and in broiler meat from 6 µg/kg to 41 µg/kg (mean value of 25.0 µg/kg). In two liver samples, DICL residues (m/z: 406.60→336.00 and 405.0→334.0) were confirmed in concentrations of 49 µg/kg and 65 µg/kg, as well as MAD residues (m/z: 934.8→629.5, 934.8→647.5) at 1 µg/kg concentrations in both matrices (Figure 1). This finding may be explained by the fact that the used screening method could not identify such low concentrations of MAD, given that its CCB value for broiler meat and broiler liver was estimated at 5 µg/kg. According to the relevant B&H legislation [7,8], the estimated concentrations of the detected coccidiostats were far below maximum residual limits (MRL).

![Figure 1. MRM chromatogram of maduramicin (m/z MAD: 934.7→629.5 and 934.7→647.4) in a sample of broiler liver (1 µg/kg of MAD).](image)

A statistically significant difference (p=0.001) in the presence of LAS residues was found among three tested types of matrices (table eggs, broiler meat and liver). Similarly, a statistically highly significant difference (p<0.001) was observed in the presence of DNC residues in the three types of tested matrices. Also, statistically significantly higher (p<0.001) occurrences of DNC residues in muscle tissue and broiler liver were found in the domestic products as compared to the imported ones.

Our results were also compared with results of coccidiostat residue monitoring in table eggs and broiler livers, provided by the Veterinary Office of Bosnia and Herzegovina (VOB&H) for the period 2010-2016 [9]. In 2011, two samples (1.6%; n=122) of table eggs were positive for MAD residues, while in 2012, five samples (3.1%; n=160) were positive for coccidiostats, with four being positive for MAD and one for SAL. In addition, one positive sample (0.7%) was found among 143 samples tested for SAL residues in 2014. Our study found 11.1% of 90 table egg samples were positive for LAS residues, but in very low concentrations ranging from 7 µg/kg to 33 µg/kg. In 2010, 2013, 2015 and 2016, coccidiostat residues were not identified in table eggs.

According to the VOB&H results [9], coccidiostat residues in samples of broiler liver were not identified in 2010 and 2012, while 4 out of the 11 broiler liver samples tested (36.4%) in 2011 were positive (3 samples positive for MAD and 1 for MON). Out of 20 samples tested in 2013, 6 positive samples were reported (30%), of which 3 were for DNC, 2 for MAD and 1 for DICL. In 2014, out of 19 tested samples of broiler liver, 7 (36.8%) were positive, of which 5 were for DNC and 2 for DICL. These results argue in favour of our findings of DNC, MAD and DICL in broiler liver.

Additionally, an increasing trend in the number of broiler livers positive for DNC residues was detected in 2015 and 2016 [9]. Of 20 samples of broiler liver tested during 2015, 15 samples (75%) were positive, of which 14 (70%) contained DNC residues. The residues were observed in low concentrations, ranging from 17 µg/kg to 229 µg/kg, which includes the DNC residue concentration range observed in our current study (from 6 µg/kg to 165 µg/kg).
High rates of positive broiler livers and animal feeds were also recorded in 2016 [9]. Out of 27 tested samples of broiler liver, 13 (48.1%) were positive, of which 11 (40.7%) contained DNC residues in high concentrations ranging from 8 to 1,200 μg/kg. However, the frequency of broiler feed (finisher) samples positive for LAS and SAL residues (40%) was recorded during the same year, which probably points to the occurrence of cross-contamination from non-target feed for broilers. In that, the highest concentrations of coccidiostat residues in finisher feed were 5,850 mg/kg for LAS, and 1,030 mg/kg and 900 mg/kg for SAL.

The presence of coccidiostat residues in broiler meat and table egg samples, as identified in our research, was most probably the consequence of cross-contamination of animal feed that occurred during the feed production and/or in further processing of the feed on the farm itself, as confirmed by others [10,11,12,13,14,15]. Feed cross-contamination is possible at farm level [16,17]. Inappropriate storage and/or marking of animal feed intended for different animal categories or species, inadequate cleaning of feed reservoirs and equipment, illegal addition of coccidiostats in feed, and disrespecting the coccidiostat withdrawal periods are all known risk factors for cross-contamination of non-target feed and the consequential detection of coccidiostats residues in broiler meat and table eggs [18].

4. Conclusion

This study is the first research into coccidiostat residues in poultry products on the B&H retail market. Study results show that concentrations of identified coccidiostats were below MRL values imposed by the current B&H legislation. This finding probably implies satisfactory handling of poultry feed, as well as adequate preventive and therapeutic applications of coccidiostats. The control and surveillance of coccidiostats in animal feed and poultry products has an essential role to maintain Bosnia and Herzegovina’s status as a poultry exporting country on the EU market.

References
[1] Radičević T, Janković S, Stefanović S, Nikolić D, Đinović-Stojanović J and Spirić D 2017 IOP Conf. Ser.: Earth Environ. Sci. 85 012080
[2] Noack S, Chapman H D and Selzer P M 2019 Parasitol. Res. 118 2009–26
[3] Tewari A K and Maharana B R 2011 J. Paras. Dis. 35 (1) 10–7
[4] Lindahl J F and Grace D 2015 Infect. Ecol. Epidemiol. 5 30048
[5] Petričević S M, Ilić T and Dimitrijević S 2006 Vet. Glas. 60 (5–6) 271–82
[6] Chapman HD 1999 Avian Pathol. 28 (6) 521–35
[7] Off. Gaz. B&H no. 74/2019
[8] Off. Gaz. B&H no. 61/2011, 67/2012 and 45/2016
[9] Veterinary Office of Bosnia and Herzegovina 2018 Results of Monitoring of Coccidiostats Residues in Bosnia and Herzegovina, 2010 – 2016. Vedrana Jelušić, Senior Associate for Veterinary Public Health, Veterinary Office of Bosnia and Herzegovina. Personal e-mail communication
[10] Kennedy D G, Blanchflower W J, Hughes P J and McCaughey W J 1996 Food Addit. Contam. 13 (7) 787–94
[11] Kennedy D G, Smyth W G, Hewitt S A and McEvoy J D 1998 Analyst 123 (12) 2529–33
[12] Kennedy D G, Hughes P J and Blanchflower W J 1998 Food Addit. Contam. 15 (5) 535–41
[13] Daeseleire E, Mortier L, Delahaut P and Huygebaert G 2006 Accred. Qual. Assur. 11 44–8
[14] Danaher M, Campbell K, O’Keeffe M, Capurro E, Kennedy G and Elliott CT 2008 Food Addit. Contam. 25 32–40
[15] Olejnik M, Szprengier-Juszkiewicz T and Jedziński P 2014 Food Chem. 149 178–82
[16] Cannavan A, Ball G & Kennedy D G 2000 Food Addit. Contam. 17 (10) 829–36
[17] O’Keeffe M, Capurro E, Danaher M, Campbell K and Elliott C T 2007 Food Addit. Contam. 24 (9) 923–34
[18] Borras S, Companyo R, Granados M, Guiteras J, Perez-Vendrell A M, Brufau J, Medina M and Bosch J 2011 Trends Anal. Chem. 30 (7) 341 1042–64