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Incidence of ionophore and non-ionophore anticoccidials residues in poultry meat and eggs and their risk characterization

Rossana Roila,[1] Raffaella Branciari,[1] David Ranucci,[1] Dino Miraglia,[1] Elisa Cristofani,[2] Cristiano Carloni,[2] Ivan Pecorelli,[2] Laura Fioroni[2]

[1]Department of Veterinary Medicine, University of Perugia; [2]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy

Abstract

A multi-residue method was applied to investigate the incidence and the concentration of ionophores and non-ionophore anticoccidials residues in poultry meat and hen eggs for the three-year period 2017-2019 in Italy. The risk related to the ingestion of such molecules was also characterized for the entire population. The average incidences of positive samples ranged from 1.35 to 9.45% while the maximum average concentration was of 4.28 µg/kg for non-ionophore molecules. No uncompliant sample was recorded. The overall risk characterization related to the intake of anticoccidials trought chicken meat and eggs reveal a minor concern for consumers of all age. However, the monitoring of coccidiostates residues through official control activity in poultry meat and egg is crucial and it should be continuously conducted to ensure safety of such products and safeguard consumers' health.

Introduction

Poultry products represent the most affordable source of animal protein for human consumption on a global scale. In the last decade European production of poultry meat has increased constantly from the 11 million tons in 2007 to the 15 million tons in 2018; while concerning eggs, the produced volume ranged from 6.1 million tons in 2007 to 6.6 in 2018 (EFSA 2020a). Among the EU Member States, Poland is the main poultry meat producer, followed by UK and France; Italy placed sixth with a production volume of 1.3 million tons recorded in 2018 (EFSA 2020a). European egg production is attributable mainly to France followed by Italy with 800 thousand tons in 2018. A serious threat to commercial poultry and eggs production is represented by infectious diseases such as Coccidiosis which has estimated to cause global economic losses of up to 3 billion dollars per year (Williams, 1999; Clarke *et al*., 2014). This disease can causes extensive damage to the intestinal tract of the birds, and although many infections are subclinical, animals are still affected through reduced weight gain, scarce feed conversion, poor egg production and shedding of infectious oocysts into the surrounding environment. In its acute form, coccidiosis causes high mortality rates (Champman, 2014). Since 1940s the main method of controlling coccidiosis, especially in intensive farming, has been the employment of anticoccidials in combination with preventative measures such as hygiene and biosecurity practices. The molecules primarily used consisted of synthetic non-ionophore anticoccidials such as halofuginone (HFG), robenidine (ROB), diclazuril (DIC), decoquinate (DEC) and nicarbazin (NIC) and, from 1970s, of naturally occurring polyether ionophores, such as monensin (MON), narasin (NAR), lasalocid (LAS), salinomycin (SAL), semduramicin (SEM) and maduramicin (MAD) (Chapman, 2009). Humans can be exposed to residues of such molecules as a consequence of feeding cross-contaminated feed to food-producing animals (Olejnik *et al*., 2014). Albeit coccidiostats are authorized as feed additives for target animal species by European legislation (Regulation (EC) No. 1831/2003), during the production of medicated feed, unintentional (but often unavoidable) transfer from target to non-target feed (feed for which the use of coccidiostats is not authorized) may take place (Vincent *et al*., 2011). The occurrence of anticoccidials carry-over may turn out in the presence of residues of these substances in food (McEvoy, 2002). Following a number of reports of food contamination issues (Clarke *et al*., 2014), in fact, it has been recorded an increasing interest in coccidiostats residues in food of animal origin. Major concerns are addressed to chronic toxicity caused by long-term exposure to low coccidiostat levels (Bacila *et al*., 2017). In this regard the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established Acceptable Daily Intake values (ADIs) for anticoccidials aiming to pursue public health protection. The aim of the study is to evaluate and investigate the incidence and the level of ionophores and non-ionophore anticoccidials residues in poultry meat and hen eggs collected and analyzed within the context of the Italian official residues control plans in the three-years period 2017-2019. Furthermore, specific exposure assessment and risk characterization for these potentially harmful molecules through poultry meat and egg products consumption, were performed for all age population.

Correspondence: Raffaella Branciari, Department of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06126 Perugia (PG), Italy
Tel.: +39.075.5857936 - Fax: +39.075.5857932.
E-mail: raffaella.branciari@unipg.it

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Materials and Methods

Sample collection and preparation

The samples were collected by veterinary inspectors in the Umbria and Marche regions (central Italy) within the framework of official control, according to the national residues control plan (NRCP). Sampling was performed according to the requirements of EU Regulation No 152/2009 amended by Regulation (EU) No 691/2013. The number of samples was calculated every year, taking into consideration animal production and the number of non-compliant results detected within the preceding year. The samples were sent to the Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche 'Togo Rosati' to perform analyses. Egg and muscle samples were homogenized and stored at −20 °C until the day of the analysis.

Five g of whole eggs or muscle were spiked with 250 µL of mixed solution of 11 internal standards. The extraction, purification and preparation of samples were performed as described by Roila *et al*., (2019). The samples were analysed using a multi-residue LC–MS/MS technique for the determination of 11 coccidiostats in meat and eggs samples. Chromatographic separation was performed on a Thermo Electron instrument (San Jose, CA, USA) consisting of a surveyor HPLC and a TSQ Quantum Ultra triple quadrupole mass spectrometer operating in both positive and negative Electrospray Ionisation (ESI) modes. The method used was included in the scope of ISO 17025 accreditation and validated according to Commission Decision 2002/657/EC in food matrices. Furthermore, the performance of the method was evaluated in interlaboratory excercises obtaining a satisfactory Z-scores value (|z| _ 2.0). The limit of determination (LOD) and limit of quantification (LOQ) were fixed at 1 µg/kg for all coccidiostats and selectivity/specificity, linearity, matrix effect, trueness (recovery), precision (repeatability and intra-laboratory reproducibility), decision limit, detection capability, ruggedness (minor changes) and measurement uncertainty were evaluated according to Roila *et al*., (2019). The substitution method has been applied for the management of left-censored data as referred in the scientific report of EFSA concerning dietary exposure assessment of chemical substances (EFSA, 2010a**)**.Therefore, the non-detect results (< LOD) were replaced by 1/2 LOD according to the Medium Bound (MB) approach.

Dietary exposure assessment and risk characterization

The dietary exposure to anticoccidials was assessed as already reported in literature (Roila *et al*., 2018), by combining food contamination results with specific food consumption values.

Food consumption detailed data was obtained from the Comprehensive Food Consumption Database of EFSA (EFSA, 2020b). The exposure assessment is based on Italian survey's mean and 97.5th percentile of "Chicken fresh meat" (level 5 database exposure hierarchy) and "Eggs and egg products" (level 1 database exposure hierarchy) consumption data (g day^{-1}) of total population ("all subjects" and "all days") of each population groups: infants (0 – <1 years), toddlers (1 – <3 years), children (3 – <10 years), adolescents (10 – <18 years), adults (18 – <65 years), elderly (65 – <75 years), and very elderly (>75 years).

The anticoccidials Estimated Daily Intake (EDI: mg kg^{-1} bw day^{-1}) of the population groups were calculated as reported by Branciari *et al*., (2020).

Three-year average EDI values were calculated from the corresponding mean concentration values (MB) of ionophoric and non-ionophoric molecules in foodstuff in the specified period of time and for average and high consumers (97.5th percentile). Furthermore, aiming to perform a quantitative evaluation of the harmful potential of anticoccidials molecules on targeted consumer population, the risk was characterized by comparing the results of the dietary exposure assessment for all age groups with the reference health-based guidance values and expressed as percentage contribution to the ADI (Altissimi *et al*., 2017; Roila *et al*., 2018).

Results and Discussion

As shown in Table 1 a total of 1000 and 836 samples of poultry meat were tested for ionophoric and non-ionophoric anticoccidials residues, in that order, during the three-year period 2017-2019; concerning eggs in the same time frame, 1554 and 1295 units where tested for the two types of molecules, respectively. Among all tested samples no incompliance was recorded for any of molecules considered.

The average incidences of positive samples is higher for the presence of non–ionophoric molecules compared to ionophoric considering both food categories (Table 1). Indeed, the maximum value of positive samples is recorded for non-inonoforic anticoccidials in poultry meat samples (9.45 %) while the minimum is referred to ionophoric compounds in eggs (1.35 %). Among non-ionophoric NIC was the molecule with the highest incidence in the three years study in meat samples with values of 44, 33, and 25% in 2017, 2018 and 2019, respectively. DIC was detected in meat samples in all the three years with incidence of 12, 5 and 3% showing a decrease along the period considered. Furthermore, a high incidence of DEC was registered in 2018 (16%) while in the other two years considered was equal to zero. In the same year; positive samples were found for all the non-ionophoric anticoccidials, although HFG and ROB were characterized by very low incidence. Regarding this type of coccidiostats, a high number of poultry meat samples were found positive to NIC by other authors (Danaher *et al*., 2008), reporting an incidence of 26% nearly similar to the one reported in this study for 2019. Concerning egg products, DEC and NIC were the molecules with the highest rate of positive samples. DEC registered a higher incidence then NIC in 2017 (17% *vs* 8%) while in the following two years the molecule's incidence decreased to low values (3% in 2018 and 1% in 2019). On the other hand for NIC the incidence reached

Table 1. Incidence and average concentration of ionophoric and non-ionophoric anticoccidials residues in chicken meat and eggs.

	Ionophoric				Non-ionophoric			
	Tested samples	Positive samples	Incidence (%)	Average (MB)	Tested samples	Positive samples	Incidence (%)	Average (MB)
Chicken meat								
2017	204	1	0.49	0.52	170	19	11.18	1.24
2018	436	7	1.60	0.55	366	42	11.46	10.37
2019	360	6	1.67	0.53	300	18	6.00	1.24
Total	1000	14	1.40	0.53	836	79	9.45	4.28
Eggs								
2017	72	1	1.39	0.51	60	3	5.00	0.64
2018	678	13	1.92	0.55	565	16	2.83	0.61
2019	804	7	0.87	0.58	670	16	2.39	0.79
Total	1554	21	1.35	0.55	1295	35	2.70	0.68

12 and 10 % in 2018 and 2019, respectively. Concerning ionophoric molecules, NAR was registered in chicken meat samples in all the years considered (3, 1 and 2%) while it was undetected in egg samples. LAS was found in meat in both 2018 and 2019 with the incidence values of 1% and 7%, respectively, while its presence in 2017 was not observed. Furthermore, LAS is characterized by a fluctuating incidence in egg samples along the time frame considered, with values of 0% in 2017, 11% in 2018 and 4% in 2019. The contamination of eggs with ionophores residues is well documented in literature since the late 1990s (Kennedy *et al*., 1998), in particular LAS was frequently identified as the most prevalent coccidiostat residue in eggs (Mortier *et al*., 2005).

Concerning the concentration of coccidiostats residues registered for the analyzed matrices, no samples above the MRL set by the legislation (Regulation (EC) No 1831/2003) were recorded, as mentioned before. This result is in line with what recently reported by EFSA in the latest report on the results from the monitoring of veterinary medicinal product residues in live animals and animal products. In the document, in fact, the Authority states that since 2009, a remarkable decrease has been observed in non-compliant samples for anticoccidials in poultry, most probably due to the effective implementation of the Commission Directive 2009/8/EC setting up maximum levels of unavoidable carry-over of coccidiostats in non-target feed (EFSA 2020a). In spite of this consideration, the results for the frequency of non-compliant samples in the European context for poultry meat (0.17%) and eggs (0.65%) are higher than those observed in this study for the Italian production (EFSA 2020a). Table 1 shows the yearly and three-years average concentration of compounds for the two food groups expressed as MB values, obtained by replacing non-detect samples with 1/2 LOD, as reported in literature for chemical food safety studies (EFSA, 2010a; Moy and Vannoort, 2013; Kabak, 2016, WHO, 2017). On a yearly basis, the results for non-ionophoric molecules are higher than those observed for the ionophoric ones, both for chicken meat than eggs. In particular the highest MB value registered is 10.37 μg/kg related to non-ionophoric molecules in 2018 for poultry meat samples mainly due to the individuation, in that year, of a very high level of NIC in one sample (2397 μg/kg). Consequently, the three-years average concentration is higher for non-ionophoric compounds in chicken meat (4.28 μg/kg) than the other values reported in Table1.

Table 2 summarise the mean and 97.5th percentile food consumption data obtained from the Comprehensive Food Consumption Database (EFSA, 2020b) for the seven above mentioned population groups. The percentage of chicken meat and egg products consumers of the respective population groups and the mean body weight are also reported. Chicken meat mean consumption ranges from 1.07 to 19.51 g day $^{-1}$ for infants and children respectively, while egg products are consumed at a minimum value of 0.33 g day $^{-1}$ by infants and at a maximum value of 20.84 g day $^{-1}$ by adults. Concerning high consumers (97.5th percentile) the lowest value refers to infants for both chicken meat and eggs consumption (17.07 and 5.33 g day $^{-1}$ respectively), while the most upper value is reached by elderly for chicken meat (88.86 g day $^{-1}$) and by adolescents for egg products (86.95 g day $^{-1}$). Regardless the population age grouping, the mean consumption of egg products is slightly higher than the one of chicken meat (15.72 and 14.36 g day $^{-1}$, respectively), while for high consumers the situation is inverted with values of food intake of 61.43 and 66.46 g day^{-1}, in the order. Concerning the exposure assessment, the three-years mean values of EDIs of ionophoric and non-ionophoric coccidiostats through the consumption of chicken meat and egg products is shown in Figure1A and B. The EDI calculation has been reported for all population groups and for average and high consumers. As shown in Figure 1A, the EDI attributable to chicken meat average consumption is slightly higher for non-ionophoric anticoccidials, varying from 1.02 (infants) to 4.63 (toddlers) ng kg bw^{-1} day $^{-1}$, in comparison to the EDI of ionoforic molecules that ranged from 0.11 (infants) to 0.67 (elderly) ng kg bw^{-1} day^{-1}. Similarly, the EDIs related to chicken meat high consumption is higher for non-ionophore anticoccidials varying from 20.50 (toddlers) to 5.13 (very elderly) ng kg bw^{-1} day $^{-1}$ in comparison to the EDI of ionoforic molecules that ranged from 0.57 (very elderly) to 2.27 (toddlers) ng kg bw^{-1} day $^{-1}$.

As reported in Figure 1B, on a general basis, the consumption of egg products contributes to the EDI of coccidiostats at a lower degree than chicken meat. Differently to what previoulsy reported for meat (Figure 1A), the two types of coccidiostatic molecules contribute similarly to the total EDI due to egg products (Figure 1B). The intake of non-ionophore compounds ranged from 0.04 (infants) to 0.47 (children) ng kg bw^{-1} day^{-1} for average consumption from 0.18 (elderly and very elderly) to 1.93 (children) ng kg bw^{-1} day $^{-1}$ for 97.5th percentile consumtion rate. The EDI attributable to ionophoric anticoccidials varied from 0.04 (infants) to 0.41 (children) ng kg bw^{-1} day $^{-1}$ and from 0.51 (elderly) to 1.69 (children) ng kg bw^{-1} day^{-1}, for average and high consumers respectively.

In order to esteem the severity of the potential adverse health effects in the given population related to the ingestion of coccidiostat residues, the risk characterization was performed through the comparison of

Table 2. Mean body weight and chicken meat and egg products consumption data utilized for Estimated Daily Intake (EDI) assessment in different age groups.

Population group	Mean body weight (kg)	% consumers	Chicken meat Mean consumption (g day $^{-1}$)	97.5th percentile consumption (g day^{-1})	% consumers	Egg products Mean consumption (g day $^{-1}$)	97.5th percentile consumption (g day^{-1})
Infants	5.0	6.3	1.07	17.07	6.3	0.33	5.33
Toddlers	12.0	38.9	11.60	51.37	58.3	8.31	30.26
Children	26.1	46.1	19.51	85.35	76.7	19.79	80.82
Adolescents	52.6	43.7	18.03	61.85	79.4	20.33	86.95
Adults	70.0	34.7	16.54	85.56	72.8	20.84	82.93
Elderly	70.1	34.1	17.31	88.86	71.7	20.58	78.43
Very elderly	70.1	38.6	16.46	75.17	70.2	19.88	65.26

the results of the exposure assessment with the health-based guidance value. For this purpose the three-years mean EDI values calculated in the present study, were expressed as contribution to the ADI of all the molecules considered and for all age groups (Table 3). The heat map shows that the maximum level of contribution to the guidance value (ADI) is reached in high consuming toddlers for HFG (11.53%) followed by high consuming children for the same molecule (10.76%). HFG is characterized by the highest contribution to the reference value albeit this molecule is rarely detected in food samples considered. This can be likely due to the extremely low ADI attributed to this molecule combined with the adoption of the middle bound approach for the management of undetects.

For the other molecules considered the risk characterization higlighted an insignificant (<0.01%), minor (0.01-0.10%) and moderate (0.11-1.00%) contribution to the ADI (Table 3), therefore the severity of the potential adverse health effects related to the intake of anticoccidials trought chicken meat and egg products consumption for the population considered, is low. These results differ from what reported in litterature for NIC, referring a contribution to the respective ADI from up to 24% (EFSA, 2010b). Furthermore, Dorne *et al.* (2013) reported a risk characterization of coccidostats for human health related to the consumption of

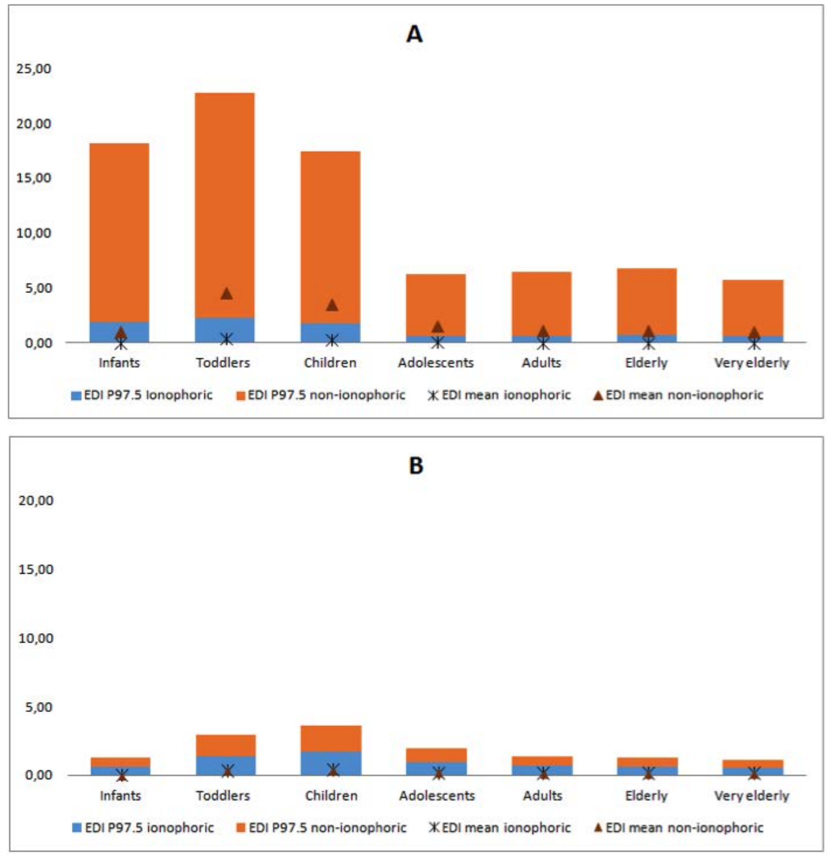

Figure 1. Estimated Daily Intake (EDI) values for ionophore and non-ionophore anticoccidials in the Italian population (A & B figures). Three-years mean EDI values by age for average and high consumers (97.7th percentile) and related to chicken meat (A) and egg products (B) consumption. Values are expressed as ng kg bw-1 day $^{-1}$.

Table 3. "Heat map" (scale: green-yellow-red) of the three-years mean values of contribution (%) to the ADI* in the Italian population by age (2017–2019).

	Infants		Toddlers		Children		Adolescents		Adults		Elderly		Very elderly	
	Average	97.5th	Average	97.5th	Average	97.5th	Average	97.5th	Average	97.5th	Average	97.5th	Average	97.5th
Ionophoric														
Monensin	0.00	0.07	0.02	0.11	0.02	0.11	0.01	0.05	0.01	0.04	0.01	0.04	0.01	0.03
Narasin	0.00	0.05	0.01	0.07	0.01	0.07	0.01	0.03	0.01	0.03	0.01	0.03	0.01	0.02
Lasalocid	0.00	0.05	0.02	0.09	0.01	0.08	0.01	0.04	0.01	0.03	0.01	0.03	0.01	0.03
Salinomycin	0.00	0.05	0.01	0.07	0.01	0.07	0.01	0.03	0.01	0.03	0.01	0.03	0.01	0.02
Semduramicin	0.01	0.18	0.05	0.27	0.05	0.25	0.03	0.11	0.02	0.10	0.02	0.10	0.02	0.08
Maduramicin	0.01	0.23	0.06	0.35	0.06	0.33	0.04	0.14	0.03	0.12	0.03	0.12	0.03	0.10
Non-ionophoric														
Halofuginone	0.29	7.62	2.11	11.53	1.91	10.76	1.23	4.77	0.90	4.07	0.91	4.03	0.87	3.39
Robenidine	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diclazuril	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01
Decoquinate	0.00	0.02	0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01
Nicarbazin	0.00	0.04	0.01	0.05	0.01	0.04	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01

ADI values (mg kg bw^{-1} day^{-1}): MON= 0.003, NAR= 0.005, LAS=0.005, SAL= 0.005, SEM= 0.00125, MAD= 0.001, HFG= 0.00003, ROB= 0.0375, DIC= 0.030, DEC= 0.075, NIC= 0.77 (Roila *et al.*, 2018).

<0.01%
0.01 % - 0.10 %
0.11 % - 1.00 %
1.01 % - 10.00 %
>10%

animal products from non-target species fed cross-contaminated diets, of higher concern in comparison with the one present in this study. The author, indeed, referred percentage contribution to the ADIs up to 27% for LAS, 3.7% for MAD, 0.6% for MON, 0.17 % for NAR, 0.8% for SAL, 10% for SEM, 0.75% for DEC, 0.08% for DIC, 0.18% for NIC, and 4.3% for ROB.

Conclusions

The reported results show that the presence of anticoccidials residues in eggs and chicken meat actually occurs, most likely as a consequence of anticoccidials unavoidable carry-over to non-target feedstuff. As shown; the moderate contribution to the ADIs, confirms, indeed, that the intake of anticoccidials residues through the consumption of poultry products, does not represent an health concern niether for mean nor for high Italian consumers, inrespective of the age. This outcomes were confirmed by the evidences of analyzed samples demostrating that official control activities are thoroughly conducted on the Italian context, keeping the issue of coccidiostatic residues in foodstaff under control. Meanwhile, we recommend that these official control activites as well as monitoring of coccidiostates residues in chicken meat and egg are crucial and should be continuously conducted to safeguard consumer health.

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