Pilot survey of hen eggs consumed in the metropolitan area of Rio de Janeiro, Brazil, for polyether ionophores, macrolides and lincosamides residues

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A liquid chromatography–tandem mass spectrometry (LC–MS/MS) method, which has recently been developed and validated, was used for the identification and quantification of polyether ionophore, macrolide and lincosamide residues in commercial eggs sold in the metropolitan area of Rio de Janeiro, Brazil. The method was applied to 100 samples and the results showed a high incidence of polyether ionophore residues (25%). Salinomycin was detected in 21% of samples, but only two non-compliant results (5.3 and 53 µg kg⁻¹) were found if maximum limits (tolerances) established by European Union were adopted in Brazil and if a method decision limit (CC₁₀) of 3.4 µg kg⁻¹ was considered. In 8% of analyzed samples, more than one studied coccidiostat was found. The lincosamide, lincomycin, and the macrolide, tylosin, were detected at trace levels in 4 and 1% of the samples, respectively. Lasalocid, clarithromycin and erythromycin were not found.

Keywords: eggs; veterinary drug residues, antibiotics, tylosin

Introduction

Eggs are an important source of animal protein and also contribute to everyday needs for minerals, vitamins and fatty acids. The consumption of eggs has increased in Brazil despite prejudices in relation to their intake. In 2007, per capita annual consumption was 132 eggs – unimpressive when compared to Mexico (375 eggs), Japan (347 eggs) and USA (258 eggs) (Quevedo 2009) – even though Brazil was seventh in the world ranking of egg producers, only behind China, USA, India, Japan, Mexico and Russian (FAO 2009). The egg export sector has increased significantly since 2004; thus, to participate in the strictest markets, such as European Union, Brazil’s egg production chain needs to monitor quality, wholesomeness, traceability and animal welfare standards and control residues and contaminants in hen eggs.

Macrolides, lincosamides and polyether ionophores antibiotics are administered via feed or drinking water to prevent or treat bacterial and coccidial infection diseases in poultry and other food-producing animals. However, the failure to follow good veterinary practices or cross-contamination of feed can lead to potentially harmful levels of residues in commercial eggs. Unlike polyether ionophores, which are used only in animals, the other mentioned classes of veterinary drugs are also used in humans, increasing concerns of cross-resistance with respect to foodborne pathogens and reinforcing the continuance of research on the impact of antibiotic use in agricultural on resistance (American Society for Microbiology 2009).

The ionophore coccidiostats are registered in Brazil as follows: lasalocid (LAS) for chickens, turkeys, beef cattle, ovine and rabbits; monensin (MON) for chickens, turkeys and beef cattle; salinomycin (SAL) for poultry and quail and maduramicin (MAD), narasin (NAR) and semduramicin (SEM) for chickens only (Brasil 2008). Use of the macrolide, tylosin (TYL), the lincosamide, lincomycin (LIN) and the coccidiostats, LAS and MON, as zootechnical feed additives, among other drugs, is prohibited in Europe, but allowed in Brazil to improve the rate of growth and efficiency of feed utilization in swine (LIN, SAL, TYL, ERY), chickens (LIN, TYL), laying hens (TYL), ovines (MON), beef cattle (LAS, MON, SAL) and dairy calves (LAS, MON) (Brasil 2008). For the lincosamides, there are veterinary medicinal products registered in Brazil to treat and control infections in laying hens based on the association of lincomycin and spectinomycin, with the recommendation that the eggs must be discarded 10 days after the last administration. Other products based on the same association are not indicated for animals producing eggs for human consumption. Macrolide-based products, mainly

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tylosin-based, are generally not for use in laying hens (Compêndio de Produtos Veterinários 2009).

A search of the Rapid Alert System for Food and Feed (RASFF) online database showed that residues of tylosin (7.3 mg kg\(^{-1}\)) were found in whole fresh liquid eggs from Spain in 2008. In 2007, lasalocid (5 μg kg\(^{-1}\)) was detected in quail eggs from France and, in 2006, salinomycin (4 μg kg\(^{-1}\)) was detected in raw eggs from Poland (RASFF 2010). Polyether ionophores are not for use in animals from which eggs are produced for human consumption, but the occurrence of residues in eggs is well documented. Monitoring results in several countries have revealed that it was mainly due to cross-contamination of feed for poultry reared for meat production (Kennedy et al. 1996, 1998a,b; Lynas et al. 1998; Rösen 2001; Mortier et al. 2005a). Assuming that feed cross-contamination was unavoidable and to protect public health and assure good functionality of the domestic market in Member States, the Commission of the European Communities (2009) recently established maximum limits (ML) or tolerances for coccidiostats in foodstuffs as follows: for cod liver oil, EU MRLs can be defined for eggs. Tolerance levels for LAS and MON in eggs are not required by US regulations, but MRLs due limits (MRL) defined by Regulations (EC) No. 470/2009 (European Parliament and Council of the European Union 2009) and No. 37/2010 (European Commission 2010), which lists a MRL of 150 μg kg\(^{-1}\) in eggs for LAS alone, among the polyether ionophores. European Union (EU) MRLs of 150, 200 and 50 μg kg\(^{-1}\) for ERY, TYL and LIN, respectively, are defined for eggs. Tolerance levels for LAS and MON in eggs are not required by US regulations, but MRLs of 200 μg kg\(^{-1}\) for TYL and 25 μg kg\(^{-1}\) for ERY have been established for this matrix (US Code of Federal Regulations 2009). In Brazil, the MRLs are generally those recommended by Codex Alimentarius. Since no Codex MRL or ML has previously been set for polyether ionophores in eggs, EU limits can be adopted. Regarding macrolides, a MRL of 300 μg kg\(^{-1}\) of TYL in eggs was proposed by Codex (Codex Alimentarius Commission 2009a). Table 1 summarizes the tolerances and the MRLs for the studied drugs in eggs adopted by the various authorities.

Although significant endeavors have been made in Brazil in recent years as regards food safety issues, currently eggs are screened by the National Residues and Contaminants Control Plan of the Secretariat of Animal and Plant Health and Inspection of the Ministry of Agriculture, Livestock and Food Supply only for nitrofuran metabolites, chloramphenicol and sulfonamides (Brasil 2010). Hence, coccidiostats and a wider range of antibiotics need to be evaluated.

To the best of our knowledge, no data on the presence of polyether ionophores, macrolides or lincosamides residues in Brazilian eggs is available in the literature. Therefore, the aim of this study was to determine the occurrence and contamination level of 10 drugs from the three mentioned classes in eggs sold within the metropolitan area of Rio de Janeiro. The analyses were carried out with an in-house-validated liquid chromatographic–tandem mass spectrometric (LC–MS/MS) method (Spisso et al. 2010) applied to a hundred samples.

Materials and methods

Sample collection and preparation

Since no previous information was available, analytical and economic considerations were also taken into account when adopting the statistically based Codex sampling strategy (Codex Alimentarius Commission 2009b). The number of samples for non-biased sampling admitted a defined confidence of 99% that the prevalence of non-compliant results in the general population would not exceed a percentage of 5%. As the minimum number of samples following these statements is 90, a total of 100 samples were collected. Samples (sample size of 12 eggs) of 33 brands and different lots of shell eggs were purchased from retail markets in the Rio de Janeiro metropolitan region between November and December 2009. Whole eggs were homogenized, transferred into first-use polypropylene bottles and stored at −80°C until analysis.

Chemicals and materials

Methanol (MeOH) and acetonitrile (ACN) were HPLC grade and obtained from J.T. Baker (Phillipsburg, NJ, USA) and Merck (Darmstadt, Germany). HPLC–MS/MS grade formic acid and acetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methyl methanesulfonate (MMS) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

Table 1. MRLs and MLs (tolerances) values for the studied antibiotics in eggs.

| Analyte | EU | US | Codex Alimentarius |
|---------|----|----|-------------------|
| LAS     | 150| n.r.| –                 |
| MAD     | 2* | –  | –                 |
| MON     | 2* | n.r.| –                 |
| NAR     | 2* | –  | –                 |
| SAL     | 3* | –  | –                 |
| SEM     | 2* | –  | –                 |
| CLA     | –  | –  | –                 |
| ERY     | 150| 25 | –                 |
| TYL     | 200| 200| 300               |
| LIN     | 50 | –  | –                 |

Note: *MLs (tolerances); n.r. = not required.
Germany), respectively. Sodium acetate (NaOAc) and formic acid (FOA) were Merck Suprapur® reagents. Ultra pure water was provided by a Milli-Q system (Millipore, Bedford, MA, USA). Narasin (NAR), salinomycin (SAL) and nigericin sodium (NIG) were supplied by Sigma-Aldrich (St. Louis, MO, USA). The sodium salts of lasalocid (LAS), monensin (MON) and the ammonium salt of maduramicin (MAD) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The sodium salt of semduramicin (SEM) from Phibro Animal Health was a gift from Dr Petra Gowik (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Berlin, Germany).

Tylosin tartrate (TYL) and lincomycin hydrochloride (LIN) were certified reference standards from US Pharmacopeial Convention (Rockville, MD, USA). Clarithromycin (CLA) and erythromycin (ERY) were certified reference standards from the Brazilian Pharmacopeial Convention (Santa Maria, RS, Brazil) and WHO Collaborating Centre for Chemical Reference Substances (Stockholm, Sweden), respectively.

Stock standard solutions of 1 mg ml\(^{-1}\), except for LAS (that was prepared at 10 µg ml\(^{-1}\)) as the standard is provided as an acetonitrile solution at 0.1 mg ml\(^{-1}\), were made up by dissolving each standard in methanol and stored in Eppendorf micro-tubes at −80 °C.

Intermediate and working standard solutions were prepared freshly at several concentrations by appropriate dilution of stock standard solutions in methanol.

**Sample extraction**

To a sample of 2 g of egg, 50 µl of NIG at 0.6 µg ml\(^{-1}\) was added as a surrogate standard to assess possible losses during the analytical procedure. After homogenizing and standing for 10 min, the sample was extracted with 8 ml of acetonitrile (2 × 4 ml portions) using a vortex for 15 s at each solvent addition and a mechanical shaker for 30 min at 240 rpm after the total volume of the solvent has been added. Centrifugation was performed for 5 min at 12,000 g and 4°C. Then, 250 µl of the supernatant was evaporated under nitrogen at 46–48°C, reconstituted with 1 ml of 5 mmol l\(^{-1}\) NaOAc: MeOH (70:30, v/v) and filtered directly to the HPLC vial using a 0.22-µm polyvinylidene fluoride (PVDF) membrane filter.

Calibration curves were constructed by spiking blank samples with six different concentration levels of standards (including zero) and extracting as described for the samples. In addition to standard solutions to verify system suitability, quality control samples, including blank samples and spiked samples at 1 MRL, 1 ML and 1 CC\(_{0}\) (for CLA), were added to the batch to monitor requirements of analytical quality assurance.

**Liquid chromatography conditions**

HPLC analysis was carried out on a Shimadzu Prominence HPLC instrument (Kyoto, Japan) equipped with a quaternary pump (LC-20AD), a membrane degasser (DGU-20A5), an auto-sampler (SIL-20AC), a column oven (CTO-20AC) and a system controller (CBM-20A). Sample aliquots (stored at 4°C in the auto-sampler) of 5 µl were injected on a 50 × 2.1 mm ACE C\(_{18}\) analytical column (Advanced Chromatography Technologies, Aberdeen, Scotland), with a guard column containing the same sorbent. Gradient elution was performed with water, acetonitrile and methanol all containing 0.1% formic acid (mobile phases A, B and C, respectively) at 35°C and at a flow rate of 0.3 ml min\(^{-1}\). The run started at 7% B, followed by a 4-min linear gradient to 80% B, immediately changed to 95% B (4.10 min), followed by a linear gradient to 100% B at 6 min. This eluent was maintained up to 8 min, when a 0.5-min linear gradient to 100% C was performed. The column was washed for 3 min in 100% C, the initial condition was reestablished in 0.5 min, while 6 min was required to re-equilibrate. The total run time was then 18 min.

**Tandem mass spectrometry conditions**

A triple quadrupole mass spectrometer, API5000 (Applied Biosystems/MDS Sciex, Foster City, CA, USA), equipped with electrospray ionization (TurboIonSpray® source) was employed in positive multiple reaction monitoring (MRM) acquisition mode, acquiring the three most intense fragment ions. The optimization of MRM parameters was performed by direct infusion of standards. Table 2 shows these parameters. An ionspray potential of 4500 V, an entrance potential of 10 V and a source temperature of 600°C were set. Nitrogen was used as nebulizer and dryer gas (55 psi), as collision gas (10 arbitrary unit) and as Curtain™ gas (10 psi).

**Identification and quantitation**

The most intense MRM transition was selected for quantification and the two additional MRM transitions monitored for confirmation. Identification of one MRM transition, in addition to the quantitation transition, was considered sufficient for conclusions regarding the detection of the analyte, as described in 2002/657/EC (European Commission 2002). IntelliQuan algorithm in Analyst® software (Applied Biosystems/MDS Sciex) was chosen for peak integration. A signal-to-noise ratio of at least 3 was required for detection of MRM peaks. Samples were considered contaminated when the calculated concentrations were higher than the method limits of detection.
All quantifications were done using matrix-matched calibration curves (samples spiked before extraction) fitted by weighted regression analysis ($1/x^2$ weight) using Analyst®. Thus, recovery corrections were not necessary.

**Validation studies**

The procedure was validated at five concentration levels around the MRL, ML or the concentration selected as the lowest validation level (LVL): 25–225 mg kg$^{-1}$ for LAS and ERY (corresponding to 0.5–4.5 Codex MRL for ERY and 1/6–1.5 EU MRL for LAS and ERY), 1–5 mg kg$^{-1}$ for MAD, MON, NAR and SEM (corresponding to 0.5–2.5 EU ML), 1.5–7.5 mg kg$^{-1}$ for SAL (corresponding to 0.5–2.5 EU ML), 100–450 mg kg$^{-1}$ for TYL (corresponding to 1/3–1.5 Codex MRL and 0.5–2.25 EU MRL), 25–125 mg kg$^{-1}$ for LIN (corresponding to 0.5–2.5 EU MRL) and 5–15 mg kg$^{-1}$ for CLA (corresponding to 1.0–3.0 LVL). NIG was used as a surrogate compound and to calculate relative retention times. A full description of the validation procedure and results will be reported elsewhere (Spisso et al. 2010). Recoveries over 100% were observed for MAD, MON, NAR, SAL and SEM when spiked samples were calculated against matrix-matched calibration curves constructed with standards added to the dry residues obtained after the extraction and evaporation of the samples. Notwithstanding, recovery corrections were implicitly included in result calculations since matrix-fortified calibration curves (constructed with samples spiked at the beginning of the sample preparation procedure, before sample extraction) were used. Overall RSD was below 12% for all analytes, except for LIN (20 and 22% at 25 and 75 mg kg$^{-1}$, respectively), in repeatability conditions (three different days, same operators). Table 3 summarizes method critical concentrations and overall recoveries. Limits of detection (LOD) and limits of quantification (LOQ) were estimated

| Substance | Molecular mass (Da) | Precursor ion (m/z) | Product ion (m/z) | Dwell time (ms) | DP | CE | CXP |
|-----------|-------------------|-------------------|------------------|----------------|----|----|-----|
| LAS A     | 590.4             | 613.3             | 377.3(100)       | 20             | 316 | 49 | 30  |
|           |                   |                   | 595.4(39)        |                | 39 | 14 |     |
|           |                   |                   | 577.3(39)        |                | 45 | 20 |     |
| MAD a     | 916.5             | 939.6             | 877.5(100)       | 20             | 301 | 45 | 32  |
|           |                   |                   | 895.5(17)        |                | 65 | 32 |     |
|           |                   |                   | 859.5(13)        |                | 81 | 30 |     |
| MON A     | 670.4             | 693.4             | 675.3(100)       | 25             | 341 | 51 | 24  |
|           |                   |                   | 479.3(52)        |                | 69 | 18 |     |
|           |                   |                   | 461.2(47)        |                | 67 | 32 |     |
|           |                   |                   | 531.2(52)        |                | 63 | 20 |     |
| NAR A     | 764.5             | 787.4             | 431.2(100)       | 25             | 341 | 73 | 34  |
|           |                   |                   | 531.2(52)        |                | 63 | 20 |     |
|           |                   |                   | 403.3(25)        |                | 83 | 16 |     |
| NIG       | 724.5             | 747.5             | 703.4(100)       | 25             | 341 | 75 | 26  |
|           |                   |                   | 729.4(67)        |                | 55 | 24 |     |
|           |                   |                   | 501.3(38)        |                | 77 | 18 |     |
| SAL A     | 750.5             | 773.5             | 431.1(100)       | 20             | 346 | 67 | 32  |
|           |                   |                   | 531.1(49)        |                | 61 | 20 |     |
|           |                   |                   | 265.2(49)        |                | 71 | 22 |     |
| SEM       | 872.5             | 895.5             | 833.4(100)       | 20             | 246 | 39 | 20  |
|           |                   |                   | 705.4(39)        |                | 81 | 18 |     |
|           |                   |                   | 851.5(36)        |                | 51 | 26 |     |
| CLA       | 747.5             | 748.6             | 158.1(100)       | 15             | 106 | 37 | 10  |
|           |                   |                   | 590.3(46)        |                | 37 | 20 |     |
|           |                   |                   | 116.2(23)        |                | 53 | 22 |     |
| LIN       | 406.2             | 407.2             | 126.4(100)       | 30             | 166 | 35 | 8   |
|           |                   |                   | 359.3(16)        |                | 25 | 28 |     |
|           |                   |                   | 389.1(4)         |                | 23 | 12 |     |
| ERY       | 733.5             | 734.5             | 158.2(100)       | 15             | 181 | 41 | 12  |
|           |                   |                   | 576.3(55)        |                | 25 | 20 |     |
|           |                   |                   | 116.2(32)        |                | 61 | 16 |     |
| TYL       | 915.5             | 916.6             | 174.2(100)       | 25             | 246 | 51 | 14  |
|           |                   |                   | 772.5(45)        |                | 39 | 26 |     |
|           |                   |                   | 116.2(9)         |                | 73 | 16 |     |

Note: DP, declustering potential (V); CE, collision energy (eV); CEP, collision exit potential (V). NIG was used as a surrogate compound.

Table 2. LC–MS/MS parameters for the target analytes. Relative abundances are given in parentheses.
using a signal-to-noise ratio of \( \geq 3 \) and \( \geq 10 \), respectively.

**Results and discussion**

Baseline resolution was obtained for all peaks. Short dwell times had to be adjusted to give sufficient acquisition data points for correct peak integration, since very sharp peaks were obtained using the described chromatographic conditions. Protonated molecular ions \([\text{M}+\text{H}]^+\) were observed for LIN, ERY, CLA and TYL, while all the ionophores were detected as the sodiated ions \([\text{M}+\text{Na}]^+\), due to their high affinity for mono- and bi-valent metal cations, especially \(\text{Na}^+\). This is consistent with published data (Matabudul et al. 2001, Matabudul and Lumley 2002, Dubois et al. 2004, Mortier et al. 2005b, Dubreil-Chêneau et al. 2009). The reduced injection volume, the dilution of the sample extracts and the improved chromatography reduced but did not eliminated matrix effects, which were negated by using matrix-matched calibration curves (matrix calibration points spiked before extraction).

Table 4 shows the occurrence and levels of antibiotic residues found in the collected samples. Of the 100 samples analyzed, at least one drug was detected in 30 samples (30%). More than one analyte was found in eight samples. NAR and SAL were detected simultaneously in four samples, MON and SAL in one sample, SAL and SEM in two samples and MON, NAR and SAL in one sample. SAL was the residue most frequently found (21% of samples), showing contamination levels ranging between 0.05 \(\mu\text{g kg}^{-1}\) (near LOD) and 53 \(\mu\text{g kg}^{-1}\). SAL mean concentration was 0.90 \(\mu\text{g kg}^{-1}\) (excluding the extreme value of 53 \(\mu\text{g kg}^{-1}\)), around 1/3 of the ML established in EU legislation. LAS was the only polyether ionophore not found in any sample. No samples contained residues of the macrolides ERY and CLA, as was foreseen for CLA since it is not registered for animals reared for food production. TYL was detected at trace levels in one sample only, with an estimated concentration of 0.61 \(\mu\text{g kg}^{-1}\) (near to the LOQ of 0.53 \(\mu\text{g kg}^{-1}\)), well below the Codex MRL of 300 \(\mu\text{g kg}^{-1}\) and the EU MRL of 200 \(\mu\text{g kg}^{-1}\). LIN was detected in four samples, but the mean value did not exceed 1/20 the MRL.

For 87% of contaminated samples (26 of 30 samples), the levels of antibiotics were below the MRLs recommended by either Codex Alimentarius or the European Commission or below European MLs in the case of polyether ionophores. Only 2% of the total number of collected samples (7% of contaminated samples or two of 30 samples) was non-compliant, i.e. the obtained results were higher than CC\(\alpha\). The concentration of SAL exceeded the ML by a factor of over 17 in one sample. This very high concentration (53 \(\mu\text{g kg}^{-1}\)) gives rise to suspicion of abuse of the coccidiostat in laying hens or a severe problem of feed cross-contamination. When the results were evaluated regarding the 33 different commercial brands of eggs, surprisingly, two of the four most contaminated samples with salinomycin were from the same brand (all three samples collected from this brand were contaminated). Also, two other brands showed an incidence of contamination of 100%, as five positive results were found in five collected samples from each brand.

Residues incidence seems to be higher in red (34%) than in white (27%) eggs.

These results confirm the widespread contamination of eggs with residues of polyether ionophores described by several authors. Mortier et al. (2005b)
Table 4. Occurrence and levels of the studied antibiotics in 100 egg samples from markets in Rio de Janeiro.

| Analyte | n.d. (<LOD) | LOD to <LOQ | 1LOQ to 2LOQ | >2LOQ ≤ CCα | >CCα | Mean ± SD | Minimum | Maximum | Contaminated samples (%) | Violative samples (%) |
|---------|-------------|-------------|--------------|-------------|-------|-----------|---------|---------|------------------------|---------------------|
| LAS     | 100         | 0           | 0            | 0           | 0     | 1.33 ± 0  | 1.33    | 1.33    | 0                      | 0                   |
| MAD     | 99          | 0           | 1            | 0           | 0     | 0.08 ± 0.04 | 0.05 | 0.12 | 3                      | 0                   |
| MON     | 97          | 3           | 0            | 0           | 0     | 0.19 ± 0.26 | 0.06 | 0.66 | 5                      | 0                   |
| NAR     | 95          | 4           | 0            | 1           | 0     | 0.90 ± 1.39a | 0.05 | 53.0 | 21                     | 2                   |
| SAL     | 79          | 5           | 7            | 7           | 2     | 0.28 ± 0.15 | 0.10 | 0.47 | 4                      | 0                   |
| SEM     | 96          | 2           | 2            | 0           | 0     | 0.61 ± 0  | 0.61    | 0.61    | 1                      | 0                   |
| CLA     | 100         | 0           | 0            | 0           | 0     | 2.16 ± 0.38 | 1.74 | 2.66 | 4                      | 0                   |
| ERY     | 100         | 0           | 0            | 0           | 0     | –         | –       | –       | –                      | –                   |
| TYL     | 99          | 0           | 1            | 0           | 0     | –         | –       | –       | –                      | –                   |
| LIN     | 96          | 4           | 0            | 0           | 0     | –         | –       | –       | –                      | –                   |

Note: LOD, method limit of detection; LOQ, method limit of quantification; S.D., standard deviation.

aMean concentration and standard deviation, excluding the extreme value of 53.0 μg kg⁻¹.

Figure 1. MRM chromatograms of an egg sample with an estimated concentration of salinomycin at 0.18 μg kg⁻¹ and traces of semduramicin (concentration between LOD and LOQ).
found a similar incidence of coccidiostats contamination (35.6%) in eggs from eight European countries, and SAL as one of the most frequently detected drug (18.8%), in addition to LAS. Also, SAL was responsible for the highest concentration found – 63 μg kg⁻¹. In Northern Ireland, several surveys showed that LAS was often found in analyzed eggs, with incidence rates decreasing from 66.5% in 1994 to 20.5% in 1998 (Kennedy et al. 1996, 1998b). In Sweden, narasin was found in 12 of 24 eggs analyzed in 1999 (Rösen 2001). As shown from feeding trials (Kennedy et al. 1998b), LAS has the highest potential for accumulation compared to MON and SAL; nevertheless, the drug was not found in any samples, indicating that it is probably not much used in Brazil. This study also suggests that SAL is probably the most widely used polyether ionophore in the Brazilian poultry industry, as is true in Denmark (Hansen 2009). National consumption data of ionophores were not found.

MRM chromatograms of an egg sample with an estimated concentration of salinomycin at 0.18 μg kg⁻¹ and traces of semduramicin (concentration between LOD and LOQ) is shown in Figure 1.

Conclusions
The results indicate a high incidence of contamination of Brazilian eggs with polyether ionophores (25%), although most of residue levels were near to the LOQ. Macrolide and lincosamide contamination were limited to a few samples (5%). Even though the incidence of samples with residues of coccidiostats above the permitted limits was small, the extreme value of 53.0 μg kg⁻¹, almost 18-fold higher than the permitted limit, and the frequency of contamination in commercial brands suggest that efforts are necessary to intensify monitoring controls regarding the use of coccidiostats. This high concentration might pose a consumer risk due to salinomycin inotropic effect, i.e., its ability to increase myocardial contractility (European Food Safety Authority 2004). Also, low levels of antibiotics at a high incidence may contribute to resistance and environmental concerns. Investigations should be carried out on non-compliant samples by the competent authorities to identify the origin of the contamination. Finally, more samples should be taken to improve the statistical significance of the data, enabling a dietary exposure assessment, taking into account consumption patterns of foods of animal origin in Brazil.

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