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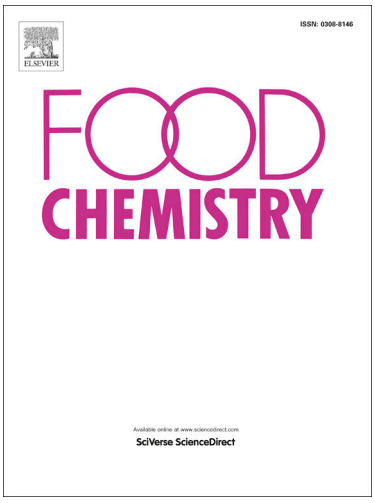

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Highlights

- Implementation of a HPLC-DAD method for the determination of enrofloxacin in egg.
- Analysis of the presence of enrofloxacin in white egg, egg yolk, and lyophilized samples
- Study of the long-term administration of ENR (100 days) to laying hens on the characteristic of the eggs.
- Analysis of the consumer exposure assessment after the intake of lyophilized samples.

Determination of residual enrofloxacin in eggs due to long term administration to laying hens. Analysis of the consumer exposure assessment to egg derivatives

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Abstract

The use of the antibiotic enrofloxacin (ENR) in poultry is controversial. A high-performance liquid chromatography coupled to fast-scanning fluorescence detection (HPLC-FSFD) method for the determination of ENR in egg white, egg yolk, and lyophilized samples was developed. In a first analysis, the long-term administration of ENR (100 days) to laying hens was carried out to determine its presence in egg white, yolk, or both. The predominance of ENR was observed in egg white and variations in the weight of egg white and eggshell was evidenced, showing a potential problem in the industry. Eventually, the presence of ENR was confirmed in commercial lyophilized egg white samples in concentration values around 350 μg kg^{-1}. The consumer exposure assessment was estimated for children, adolescents, and adults. The result displayed that, in an intake of lyophilized egg white with food-producing animals, the %ADI exceeds 100%, showing toxicological levels.

Keywords: Enrofloxacin (ENR); lyophilized egg; egg white; egg yolk; consumer exposure assessment.

1. Introduction

A variety of foodstuffs containing eggs are available for the consumers. Due to their versatility, these products are becoming one of the most popular food products of animal origin (Instituto de Estudios del Huevo, 2009). Moreover, the use of egg in different forms (pasteurized, dehydrated or lyophilized) is growing daily in the industry (Abeyrathne, Lee, & Ahn, 2013; Chen, Sheng, Gouda, & Ma, 2019; Laca, Paredes, Rendueles, & Díaz, 2014; Suman, Riani, & Dalcanale, 2007).

In this sense, in the industry, eggs are replaced with derivatives to improve the sanitary conditions related to the bacteriological control of products. Moreover, the use of egg derivates (as lyophilized and pasteurized eggs) upturns the storage and reduce the total volume to be transported (Ministerio de Agroindustria, 2016; Piątkowska et al., 2017). In Argentine, since 2015, fifteen industries process fresh egg to produce their derivatives (Ministerio de Agroindustria, 2016). Therefore, consumers are exposed to egg residues in a wide variety of products.

In the poultry industry, the use of veterinary drugs is a common practice. Nevertheless, in the laying hen industry, their use is reduced and, in some cases, forbidden (Gaugain et al., 2019). In Argentine, only 28 compounds are approved for their use in laying hens, with their corresponding maximum residue limits (MRLs) in eggs (Servicio Nacional de Sanidad y Calidad Agroalimentaria, 2011).

Fluoroquinolones (FQs) are a group of antimicrobials exhaustively used in human and veterinary medicine. Their broad-spectrum and capability of inhibiting DNA gyrase and topoisomerase IV of most bacteria make FQs suitable for the treatment of many infections in farmed animals and fish (Zeng, Dong, Yang, Chen, & Huang, 2005). Several publications can be found in the literature related to the presence of FQ residues in different matrices due

to their extensive use in farms (Alcaraz, Culzoni, & Goicoechea, 2016; Teglia, Peltzer, Seib, Lajmanovich, Culzoni, & Goicoechea, 2017; Teglia, Perez, Michlig, Repetti, Goicoechea, & Culzoni, 2019; Vera-Candioti, Teglia, & Camara, 2016). Although the approved compounds for the laying hen industry are scarce around the world, the appearance of FQs residues in eggs is a reality.

Studies carried out *in vitro* have shown that FQs possess a long half-life after administration owing to their lipophilic characteristics, so their half-life, the liberation period, and their metabolization are slow (Cabrera Pérez, González Díaz, Fernández Teruel, Plá-Delfina, & Bermejo Sanz, 2002; Lemus, Blanco, Arroyo, Martínez, & Grande, 2009). Moreover, due to their physicochemical characteristics, the target tissues included fat and visceral organs, such as the liver and kidney (Cabrera Pérez, González Díaz, Fernández Teruel, Plá-Delfina, & Bermejo Sanz, 2002). In this sense, their presence can affect the well-being of the consumers, for example, due to the possibility of causing various diseases as diarrhea, typhoid fever, and infections. Moreover, the intake excess can generate development of drug resistance and increase allergic reactions (Dincel, Yildirim, Caglayan, & Bozkurt, 2005; Kurrey et al., 2019; Piątkowska et al., 2017; Piatkowska, Jedziniak, & Zmudzki, 2016).

The use of FQs is prohibited in the laying hen industry, not only in Argentine but also in the European Union (EU) (Gbylik-Sikorska, Posyniak, Gajda, & Błądek, 2013) and the USA (Goetting, Lee, & Tell, 2011). However, the presence of these residues in eggs has been widely reported in commercial samples (Garrido Frenich, Aguilera-Luiz, Martínez Vidal, & Romero-González, 2010; Lu et al., 2019; Piatkowska, Jedziniak, & Zmudzki, 2012; Zhou et al., 2018). This can be explained by the presence of cross-contamination, and/or uncontrolled and illegal usage of FQs (Gbylik-Sikorska, Posyniak, Gajda & Błądek, 2013; G. Yang, Dong,

Zeng, Huang, & Chen, 2006; Yang, Liu, Wang, Zhou, Zhang, & Wang, 2020). Moreover, in the poultry systems, it is feasible to detect FQs for long periods of time either due to the treatment of the animals at sub-therapeutic levels or the off-label and unnecessary use.

In this context, to assure the innocuousness of foodstuffs, the Food and Agriculture Organization of the United Nations (FAO) defines the maximum concentration of drugs and pesticides that a person can incorporate with the diet without causing negative effects. In the case of ENR, the acceptable daily intake (ADI) value is 2.30 μg kg^{-1} b.w. (Food and Agriculture Organization of the United Nations, 1997).

For these reasons, the determination of FQs residues in eggs, as well as the analysis of their distribution between egg white and egg yolk become of utmost importance. After the administration, the residues of FQs will appear in white or yolk, or both, and the different deposition will depend on the physicochemical properties of the drug and the physiology of the chicken, among others (Alaboudi, Basha, & Musallam, 2013). Currently, few works describe and analyze the distribution and concentration of ENR in eggs after its administration in short periods of time (Gorla et al., 1997; Lolo, Pedreira, Fente, Vazquez, Franco, & Cepeda, 2005). In this work, the analysis was carried out during a regular period of administration. Nevertheless, due to the previous work mentioned, the possibility of a long-term exposition is possible. The herein proposed work focused on the study of the continuous administration of ENR during the egg-laying and the analysis of the effects on the poultry production system, including analysis of egg derivates. As mentioned in the manuscript, although the use of enrofloxacin has been banned in poultry, its presence in eggs is still a reality.

Because the distribution of the veterinary drug residues not always follows a defined pattern, the monitoring of these compounds in egg derivates is mandatory to establish the

safety of the final product. Until now, in Argentine, like in other countries, the monitoring of veterinary drug residues in eggs is a common practice, but there is no obligation to monitor these residues in egg powders. In this sense, the presence of ENR in free-dried and lyophilized egg albumen was confirmed by Piątkowska et al. (2017) in 17 samples from Poland. Furthermore, in the case of egg powder, the Argentinian regulation does not define an MRL for ENR and, in consequence, the analysis of this FQ is not mandatory. Therefore, the absence of ENR in this kind of product is not guaranteed.

In this context, it is mandatory to have efficient analytical methods to determine FQs in these kinds of sample. In this regard, and considering the native fluorescence of FQs, the use of high-performance liquid chromatography (HPLC) coupled to fluorescence detection was the preferred option. Then, to validate the method, the use of UHPLC coupled to triple quadrupole tandem mass spectrometry (MS/MS) detection was followed.

In the present work, firstly, the presence and concentration of residual ENR in eggs of laying hens after oral administration of 5 mg kg^{-1} day^{-1} during long term exposure (100 days), and the comparison with a control group, were studied. Secondly, the monitoring of commercial dried samples of egg white and yolk, and the analysis of the consumer exposure were carried out.

2. Materials and methods

1. 2.1. Chemicals and reagents

Enrofloxacin (ENR) was provided by Fluka (Buchs, Switzerland). HPLC-grade acetonitrile (ACN) and methanol (MeOH) were obtained from Merck (Darmstadt, Germany). Milli-Q water was obtained from a Millipore system (Bedford, MA, USA). Sodium hydrogen

phosphate, phosphoric acid, ethylenediaminetetraacetic acid (EDTA), acetone, and potassium chloride (KCl) were purchased from Cicarelli (San Lorenzo, Argentina).

The stock standard solution was prepared by exactly weighing and dissolving a portion of ENR standard in MeOH to reach a concentration of 1.00 mg mL^{-1}. This solution was stored for a week at 4 ºC in a light-resistant container, which allowed to reach room temperature before use. The stability of the standard solution was tested daily. When necessary, a working standard solution was prepared by diluting the stock solution in water.

For the depletion study, enrofloxacin KW oral 10% (m/v) from KW S.R.L (Rosario, Argentina) was used.

2.2. Instrumentation

All experiments were performed on an Agilent 1100 (Agilent Technologies, Waldbronn, Germany) series liquid chromatography instrument equipped with a quaternary pump, membrane degasser, thermostated column compartment, autosampler, fast-scanning fluorescence detector (FSFD), and the ChemStation software package (all from Agilent Technologies) to control the instrument, the data acquisition, and the data analysis. The separation was performed on a Zorbax Eclipse XDB-C18 column (4.6 × 75 mm, 3.5 µm particle size) (Agilent Technologies, Waldbronn, Germany) in gradient mode at 1.00 mL min^{-1} flow rate and 40 °C. The mobile phase consisted of a mixture of 10 mmol L^{-1} phosphate buffer pH 3.50, ACN and MeOH (80:10:10). During the run time, the mobile phase composition was 10% of each organic solvent between minutes 0 to 1, 30% of each organic solvent between minutes 6 and 7, and was reverted to 10% of each organic solvent at 8 min. The emission fluorescence chromatograms were acquired exciting at 280 nm and emitting at 450 nm.

2.2.1. UHPLC coupled to triple quadrupole mass spectrometry detection

The lyophilized samples were also analyzed by UHPLC using an ACQUITY UPLC™ System (Waters, Milford, USA). The software package MassLynk Mass Spectrometry (Waters, Milford, USA) was used to control the instrument, the data acquisition, and the data analysis. The separations were achieved using an ACQUITY UPLC® BEH C18 RP (2.1 × 100 mm, 1.7 μm particle size) column (Waters, Milford, USA). The binary mobile phase consisted of 0.1% (v/v) formic acid in water (A) and ACN (B), at a flow rate of 0.20 mL min^{-1}. The analysis was carried out in an isocratic mode with 80.0% of A and 20.0% of B for 3.00 min. The injection volume was 10 μL and the column temperature was set at 35 °C.

Mass spectrometry detection was achieved using a Quattro Premier™ XE Micromass MS Technologies triple quadrupole mass spectrometer with a ZSpray™ ESI source operated in positive ion mode (Waters, Milford, USA). For ionization, the temperature source was set at 150 °C with a 50 L h^{-1} cone gas rate and a 400 L h^{-1} desolvation gas flow. The cone voltage was set at 24 V, the capillary voltage at 4.75 kV, the extractor voltage at 4 V, and the radio frequency voltage at 0 V. Concerning the MS spectrometry detection, the triple quadrupole mass spectrometer was used in multiple reaction monitoring (MRM). The fragmentation patterns in MRM mode were used following a previous work (Teglia, Guiñez, Goicoechea, Culzoni, & Cerutti, 2020). The retention time of the analyte was 1.43 min, its precursor ion $[M+H]^+$ of m/z 360, with product ions of *m*/*z* 245 and *m*/*z* 316 which were considered for verification and quantification purposes; respectively.

2.3. Hens

The study was performed in accordance with ethics requirements and authorized by the official ethical committee of our university (FBCB-CE2017-260705).

The experiments were carried out on five Rhode Island (Red) laying hens housed in individual cages. The administration of ENR started at week 21 when the animals laid the first egg. At the beginning, the weight of the animal under study was controlled, being the normal average of the hens of 1980 g per animal.

The animals were fed with a standard balanced diet, free from antibiotics, and water. The hens were separated into two groups: (1) control (two hens) and (2) treatment (three hens).

The experiments were carried out during four months, from October 2018 to January 2019, which correspond to spring and summer seasons in Argentine. In these months, the minimum temperatures fluctuated between 13.2 °C and 19.5 °C, while the maximum temperature between 25.0 °C and 32.1 °C, and the rainfall varied from 99 mm to 114 mm (per month), with 8 rainy days each month. The animals were exposed at natural light for at least 12 h per day.

Every day the eggs were collected at 8 a.m. and 6 p.m., and the weight, quantity, and size of eggs were registered (see Figure SM1).

2.3. Treatment of hens with ENR

The hens were orally treated with 5 mg kg^{-1} day^{-1} of 10% ENR, during 100 days, starting three months after they were born, when their egg-laying stage begins. Daily, an ENR known concentration was added to the drinking water with the aims of analyzing the effect of a chronic exposure of the animals to ENR through water, i.e., taking the ENR as a contaminant.

During 100 days, the eggs were daily collected starting 24 h after the first administration of ENR and, for the determination of ENR, egg yolk and white were separately handled. The samples were weighted and then 1.00 g was transferred to a centrifuge tube.

2.4. Samples

2.4.1. Fresh eggs

For the extraction of ENR, the sample was mixed with 1.00 mL of buffer phosphate 10 mmol L^{-1} pH=3.50, 1.00 mL KCl, and 1.00 mL EDTA 0.20 mol L^{-1}, vortex mixed for 1 min, sonicated for 5 min and centrifuged at 3500 rpm for 10 min. The supernatant was added with 1.00 mL MeOH and 1.00 mL ACN, vortex mixed for 1 min, sonicated for 5 min, and kept at 4 °C for 24 h. After that, the tube was centrifuged at 3500 rpm for 10 min and the supernatant was mixed with 1.00 mL of acetone, vortex mixed for 1 min, sonicated for 5 min, and centrifuged at 3500 rpm for 10 min. The supernatant was evaporated under a nitrogen stream and re-suspended with 500 μL of a mixture of water, ACN, and MeOH (80:10:10).

2.4.2. Lyophilized eggs

For the monitoring of the concentration of ENR in commercial albumen (egg white) and yolk samples, six albumen and four yolks (1.00 g of each sample) from stores as dietary, grocers and wholesalers of Santa Fe city were firstly reconstituted in 1.00 mL of water and then processed as described the *Section 2.4.1.* The concentrations of ENR found in the commercial samples were used to perform the consumer exposure assessment (see *Section 2.6*). Due to the lack of control of the presence of ENR in these samples, the name of the stores is maintained anonymous.

2.5. Method validation

For the HPLC-FLD analysis, a calibration set of seven standard samples was prepared in triplicate by transferring appropriate aliquots of ENR stock solution to 5.00 mL volumetric flasks and completing to the mark with a mixture of water, ACN, and MeOH (80:10:10). For the calibration curve, solutions of ENR at 0.05, 0.1, 0.25, 0.48, 1.00, 1.50 and 2.00 μg mL^{-1} were prepared. Standard and sample solutions were filtered through 0.22 μm syringe nylon membranes before injection into the chromatographic system.

Besides, a calibration set with concentrations of 0.058, 0.096, 0.26, 0.38 and 0.62 μg mL^{-1} of ENR was prepared in triplicate by transferring appropriate aliquots of ENR stock solution to 5.0 mL volumetric flasks and completing to the mark with a mixture of water, ACN and MeOH (80:10:10), and analyzed by UHPLC-MS/MS. The matrix effect was evaluated by comparing slopes from both the standard calibration curve (Enrofloxacin in water:acetonitrile:methanol; 80:10:10) and spiked samples after they had been treated as above mentioned and then calculating signal suppression or enhancement (SSE%) using an equation that compares the slopes obtained: SSE% = [Slope(spiked extract)/ Slope(spiked solvent)]× 100. The percentage of the quotient of the slopes in the spiked and pure solvent samples was used as an indicator of the extent of ion suppression or signal enhancement.

Limits of detection (LOD) and quantification (LOQ) were calculated according to Currie (Danzer & Currie, 1998).

Precision (repeatability and reproducibility) and recovery were assessed in two different weeks by replicate (n=3) analysis of egg white and yolk spiked at three different concentration levels: (1) level 1 at 0.200 μg mL^{-1}, (2) level 2 at 0.384 μg mL^{-1} and (3) level 3 at 1.200 μg mL^{-1}. The solutions were prepared by spiking a blank sample with appropriate aliquots of ENR standard solution and proceed as described in *Section 2.4*.

2.6. Consumer exposure assessment

To obtain information about consumer exposure, a theorical analysis was evaluated for children, adolescents, and adults. Following the description of Piątkowska et al. (2017), the children were separated into three groups according to their ages: 6 months-1 year, 7 kg; 1-3 years, 12 kg; 3-10 years, 23.1 kg (Committee, 2012). Meanwhile, the adolescents were separated into three groups according to their ages: 10-13 years, 41.2 kg; 14-16 years, 57.2 kg; 17-18 years, 66.6 kg (del Pino, Bay, Lejarraga, Kovalskys, Berner, & Rausch Herscovici, 2005), and the group of adults (19-65 years) were discriminated according to their weight as normal weight, 67.9 kg (Walpole, Prieto-Merino, Edwards, Cleland, Stevens, & Roberts, 2012) and overweight, 100 kg (Ministerio de Salud de la República Argentina, 2020). The consumption of egg was set at 100 g per day of egg product for adults, adolescents, and children from the last group (aged 3-10 years). For smaller children, the intake was calculated based on nutritionist on data provided by the Ministry of Health of the Argentine Republic (Ministerio de Salud de la República Argentina, 2020), who recommend eating 4 and 6 eggs weekly for smaller and older children, respectively. Assuming an average weight of 60 g per egg, this corresponds to a daily intake of 34.3 g and 51.4 g for smaller and older children, respectively. At the same time, the exposure of bodybuilders was evaluated analyzing a daily consumption of 105 g per day and 70, 80, 90, and 100 kg of body weight.

To assess the acceptable daily intake (ADI) and, later, to calculate the % of ADI, the highest concentrations of ENR detected in the commercial lyophilized egg white or yolk were included in the following equation:

$$\%\ ADI = \frac{daily\ intake\ of\ the\ substance\ (\mu g\ kg^{-1}\ b.w.)}{ADI\ (\mu g\ kg^{-1}\ b.w.)} \times 100 \qquad (1)$$

where b.w. corresponds to body weight.

3. Results and discussion

3.1. Method validation

One of the main drawbacks of a method using LC-atmospheric pressure ionization-MS for complex samples is related to ionization effects because of co-eluting matrix components. Matrix effects can cause suppression or enhancement of the target analytes and might hamper accurate mass spectrometric quantitation, leading to false results. As mentioned in Section (2.5), the matrix effect was evaluated. As result, the sample matrix has a negligible none statistically significant effect on the ENR determination. Therefore, quantification was performed by means of external calibration.

The analytical performances of the methods are displayed in Table SM1. In this sense, the HPLC-FSFD method was used to evaluate the presence of ENR in all samples, with the posterior confirmation of the results of the lyophilized samples by UHPLC-MS/MS. Thus, the calibration curve obtained by HPLC-FSFD was used to evaluate recovery.

The goodness of the fit was evaluated testing the variance of the lack of fit against the pure error variance. The adequacy of each model was estimated by the application of the *F*-test recommended by IUPAC (Danzer & Currie, 1998). For both methods, the linearity and the LOD and LOQ were computed according to Currie (Currie, 1999). Since the HPLC-FSFD method is more sensitive to the detection of ENR, it was selected for the determination of this analyte in both validation and real samples (see Table SM1).

The recoveries (%R) were evaluated at three concentration levels for both egg white and egg yolk. The %R was between 86 and 91% for egg white, and 90% for egg yolk (see Table SM2), endorsing the applicability of the HPLC-FSFD method to quantitate ENR in eggs

(EMEA, 2012). Moreover, the results for the assessment of the intra- and inter-day precisions(%RSD) were between 1.5 – 2.8% and 1.8 –3.1%, respectively, in the case of the egg white, and between 1.6 – 4.0% and 1.2 – 4.3%, respectively, in the case of the egg yolk (see Table SM3). The *F*-test used to verify the results evidenced that there is no statistical difference between the mean values.

3.2. Long term administration: analysis of the presence of ENR in egg white and egg yolk

Long term administration (100 days) was carried out to evaluate the presence of residual ENR in eggs laid by hens in continuous contact with the analyte due, for example, to proximity with another productive system. In this sense, the objective was to map the distribution of ENR in egg white and egg yolk, and evaluate changes in the normal production of eggs, if the production system receives unintentional ENR during the life of the laying hens.

Firstly, zootechnical parameters such as quantity, weight, and size of the eggs obtained after the first day of treatment and during the posterior 99 days were measured. Figures SM1 and SM2 showed these results. In general terms, the values of weight and size of the treated eggs were smaller than the control. It is important to notice that a laying hen put, normally, one egg each 24 – 26 h (Instituto de Estudios del Huevo, 2009), and, in this case, the total collected eggs in the treatment group (3 laying hens) were 64 (see Figure SM1). During the treatment period, there were periods (no more than 5 consecutive days) where egg-laying was not recorded and, after day 91, the hens stopped laying eggs until the end of the treatment.

Secondly, the weight analysis of egg white, egg yolk, and eggshell was carried out. These results, which can be seen in Figure 1, evidence a difference in the distribution between the eggs under treatment and those from the control group. The major difference corresponds to

egg white and eggshell, showing a weight diminution in egg white and an increase in eggshell. Concerning the average results (Table 1), it is important to notice that the normal average of egg white, egg yolk, and eggshell is 60%, 30%, and 10%, respectively (Instituto de Estudios del Huevo, 2009). Our results showed that the values obtained for egg yolk in the two groups were not statistically different, but those for egg white and eggshell were different (p-value <0.001). In this sense, these results demonstrated that the prolonged intake of ENR could affect the normal production of egg white and eggshell, influencing the nutritional properties of eggs.

At this point, one of the factors that might generate the end of laying eggs may be the fact of the increased weight of the shells, with the need for calcium. Since the skeleton of a typical modern breed of egg-laying hen contains only about 20 grams of calcium, each egg represents 10% of the hen's total body calcium. While the skeleton of the hen acts as a calcium reserve to supply the demand for egg production, this reserve is rapidly depleted when external factors generate a major need for this element. In this sense, the weight increase in the eggshell describes that the exposition of the hens to a continuous concentration of ENR affect the ability to bind calcium, and as a hypothesis the compound would affect the metabolic pathway by which calcium binds to the transporter protein calbinida, generating an increase in transport and subsequent deposition.

After that, a quantitative analysis of the concentration of ENR in egg white and egg yolk was carried out as described in *Section 2.4.1*. The results of the present study, which can be seen in Fig. 2 and Table SM3, shows that ENR has different depletion profiles in egg white and yolk. This depletion profile was similar to previously reported ENR depletions in egg (Gorla et al., 1997; Lolo, Pedreira, Fente, Vazquez, Franco, & Cepeda, 2005). It is important to notice that, in contrast with the results herein reported, in those works, the previously

mentioned analysis was carried out only to study ENR concentrations for short periods of time, avoiding the evaluation of other egg features. The first peak of ENR concentration was observed in egg white and egg yolk 24 h after the first day of administration. The highest concentrations of ENR were detected in egg white. In this sense, our results are in accordance with those published by Gorla et al. (Gorla et al., 1997) and Lolo et al. (Lolo, Pedreira, Fente, Vazquez, Franco, & Cepeda, 2005).

During the administration of ENR, its residual concentrations were in the range of 0.12 to 9.72 μg g^{-1} and 0.051 to 4.52 μg g^{-1} in egg white and egg yolk, respectively. The maximum concentrations of ENR, and therefore the maximum concentrations at which the consumer can be exposed in case of eating each tissue correspond to 140.6 μg g^{-1} (day 16) in egg white, and 62.3 μg g^{-1} (day 20) in egg yolk. Finally, the maximum concentration of ENR found in an egg (white and yolk) corresponds to 181.0 μg g^{-1} (day 17).

In a study of the distribution of VDs residues, it is essential to understand the formation and composition of eggs. The formation process of the water-soluble proteins of the white and their secretion by the magnum of the oviduct takes 1 or 2 days (Goetting, Lee, & Tell, 2011). For this reason, the time required to achieve a higher ENR level in the white, in short periods of exposition, is generally 2 or 3 days. As an example, the highest concentration found by Gorla et al. (Gorla et al., 1997) and Lolo et al. (Lolo et al., 2005) corresponds to day 7 (1.56 μg g^{-1}) and to day 3 (6.48 μg g^{-1}), respectively. In this work, since the laying hens were exposed to a continuous concentration of ENR during longer periods of time, the maximum concentration was found at day 16. Moreover, the yolk requires the synthesis of proteins in the liver, so the residues of drugs generally required 8 to 19 days to reach maximum levels (Goetting et al., 2011). In this sense, the maximum values observed in yolk by the previously cited authors in short exposition times were obtained between days 6 and

4 (0.46 and 2.98 μg g^{-1}) (Gorla et al., 1997; Lolo et al., 2005). In our case, the maximum concentration found in yolk was obtained at day 20. As a conclusion, several authors described that, after an oral or intramuscular administration, the residues of FQs appear in eggs around 24 h after the first contact, both in yolk and white eggs, and remain in the tissue for several days after treatment cessation (Goetting, Lee, & Tell, 2011). In this context, the analysis of egg derivatives results of great importance due to the possibility of egg contamination and, subsequently, further consumer intake of FQs.

3.3. Consumer exposure assessment

Table SM4 summarizes the result obtained by HPLC-FSFD for the analysis of the commercial samples. In this sense, only one sample (W2) was positive for ENR with a concentration of 350 μg kg^{-1}. In addition, Figure 3 depicts the chromatogram for sample W2 and a standard solution of ENR. To confirm the presence of ENR in the sample, a new triplicate of sample W2 and W2 spiked with a standard solution of ENR at 0.258 μg g^{-1} was prepared and analyzed by UHPLC-MS/MS (Table 2). Figure SM3 shows the MS/MS chromatogram obtained in all cases, and the most abundant fragment ions, which were used to quantify and confirm the identity of ENR. As a result, the presence of ENR in sample W2 was confirmed.

Subsequently, the consumer exposure assessment was calculated using the concentration found in sample W2 (350 μg kg^{-1}). In this sense, the population was divided as described in *Section 2.6.* Table 3 shows the results obtained. The habitual consumption was set at different values keeping in mind the edges of each group and, in the case of children, adolescents and adults, a dilution factor of 9 was applied following the recommendation of the Argentinian nutritionists, since the intake of powder egg white is not a typical practice, and, in general,

egg powder is consumed in processed products. In the case of adults with overweight and athletes, the intake was fixed at 105 g of egg white per day.

As an example (see Table 3), a daily consumption of 105 g of egg albumen per day containing 350 μg kg^{-1} of ENR in pure form corresponds to an intake of ENR of 36.8 μg (0.53 μg kg^{-1} b.w. in a person of 70 kg), i.e. 22.8% of ADI dose. In the case of children and adolescents, it is important to notice that children between 6 months and 1 year are the most exposed group, with a %ADI of 8.3.

The ADI analysis permits the study of the effect that the intake of veterinary drugs can generate in the population. From a microbiological perspective, the ingestion of residues of FQs in animal-derived food constitutes a potential risk to human health because the change in the human intestinal microbial community may influence the host functions (Ahn et al., 2012). In this sense, changes in the microbial community can generate resistance, shaping of the immune system, and changes in human metabolism. Actually, the emergence and propagation of antibiotic resistance genes increase the concern about the intake of higher levels of antibiotics (Li, Gao, Dai, Wang, & Duan, 2020). Furthermore, Kim et al. (2012) concluded about the increase in the mRNA transcripts and the antimicrobial drug resistance genes on a sample exposed to increased concentrations of ENR. Therefore, the analysis of the complete intake of ENR by food-producing animals should be considered.

In 2019, according to SENASA (Servicio Nacional de Sanidad y Calidad Agroalimentaria, 2019), the average consumption per capita of food animals derivate, was: (a) 329 g of meat, (b) 90 g of fat and skin, (c) 100 g of the liver, (d) 50 g of the kidney, and (e) 501 g of milk. Keeping in mind the intake of 100 g of egg, the percent due to the egg consumption corresponds to the 8.5% of animal derivate per day in a normal diet. Thereby, if the consumption of animal derivatives such as meat, liver, milk, etc. is considered taking

into account the MRL defined for each tissue plus the value obtained for the sample W2, the %ADI for ENR exceeds 100% (107.8%) (see Table SM5). Although the percentage of an egg is lower than 10%, since the value of MRL for ENR in an egg is zero, the appearance of residues can generate problems. This result shows the potential toxicity at which humans are exposed after the consumption of a variety of animal-derived food with individual concentrations of ENR below the MRL defined by the authorities.

4. Conclusions

Considering that the intake of eggs is an extended practice around the world, the analysis of the presence of veterinary drugs and their residues, both in eggs and derivates, is of utmost importance. In the present work, after the analysis of egg white and egg yolk, the results obtained showed that the prolonged intake of ENR could affect the normal production of egg white and eggshell, influencing the nutritional properties of eggs. Moreover, the depletion study showed that ENR followed different depletion profiles in egg white and yolk. The first concentration of ENR was quantified 24 h after the treatment, being the maximum concentration obtained in egg white. In summary, although challenging to compare the two groups at the beginning of the study not only for the size of the groups, but also for the normal biological conditions affecting the laying hens, the developed long-term study allowed to infer the informed results with statistical criterium.

The analysis of lyophilized egg white and yolk resulted of great importance due to the possibility of egg contamination and, subsequently, further consumer intake of FQs. In this sense, the presence of ENR in a lyophilized white egg allowed the analysis of the %ADI to which humans might be exposed when ingesting egg-derived products.

Although ENR is not approved for the use in layer hens and no official MRL has been established for eggs; in this work, the presence of residual in food-derivates has been evidenced.

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Figure captions

Figure 1. Comparison of the weight of treated egg white, egg yolk, and eggshell *vs* control. (A) Weight of treated egg white in light blue circle and control in grey triangle; (B) weight of treated egg yolk in red circles and control in grey triangle, and (C) weight of treated eggshell in violet circles and control in grey triangle. The dotted line describes the start point of the treatment.

Figure 2. The concentration of ENR in treated eggs. Light blue bars represent the concentration of ENR in egg white and orange bars the concentration in egg yolk.

Figure 3: Chromatograms obtained after the analysis of ENR in egg. (A) Chromatogram of egg white (sample W2), (B) chromatogram of a standard solution of ENR at a concentration of 0.10 μg mL^{-1}. In all cases, the chromatograms were registered at an emission wavelength of 450 nm.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Determination of residual enrofloxacin in eggs due to long term administration to laying hens. Analysis of the consumer exposure assessment to egg derivatives

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Table SM21. Analytical performance of the method for ENR monitoring and quantification in eggs

Variable	Method	
	HPLC-FSFD[a]	UHPLC-MS/MS[a]
Linear range (μg g^{-1})	0.051 – 2.100	0.058 – 0.620
Intercept	579.5 (1.4)	88940.1 (223)
Slope	7.48 (1.4)	362.9 (78)
F_{exp}	1.322[b]	2.220[c]
r^2	0.99999	0.99992
Lack of fit (p-value)[d]	0.301	0.106
LOD (ng g^{-1})	17.7	20.5
LOQ (ng g^{-1})	51.1	58.0

[a]Values between parentheses indicate standard deviation
[b]F_{tab}= 4.31
[c]F_{tab}= 6.54
[d]Because the p-value for the lack of fit is greater than or equal to 0.10, the model seems to be adequate for the observed data.

Table SM2. Precision and accuracy results of the HPLC-FLD method.

Sample	Concentration (μg mL^{-1})	Intra-assay precision RSD (%)[a]	Inter-assay precision RSD (%)[a]	*p*-value[b]	Recovery (%)
Egg white	0.200	1.5	2.1	0.680	91
	0.384	1.6	1.8	0.370	86
	1.200	2.8	3.1	0.923	88
Egg yolk	0.200	2.9	3.2	0.892	90
	0.384	1.6	1.2	0.938	90
	1.200	4.0	4.3	0.934	90

[a]Acceptable criterion: RSD (%) ± 15% (EMEA, 2012).
[b]Because the *p*-value is greater than or equal to 0.05, there is no statistical difference between the mean values.

Figure SM1. Amount of eggs during the 100-day treatment. The boxes in pink describe the days in which the hens laid eggs, while the numbers with arrows describe the eggs collected that day.

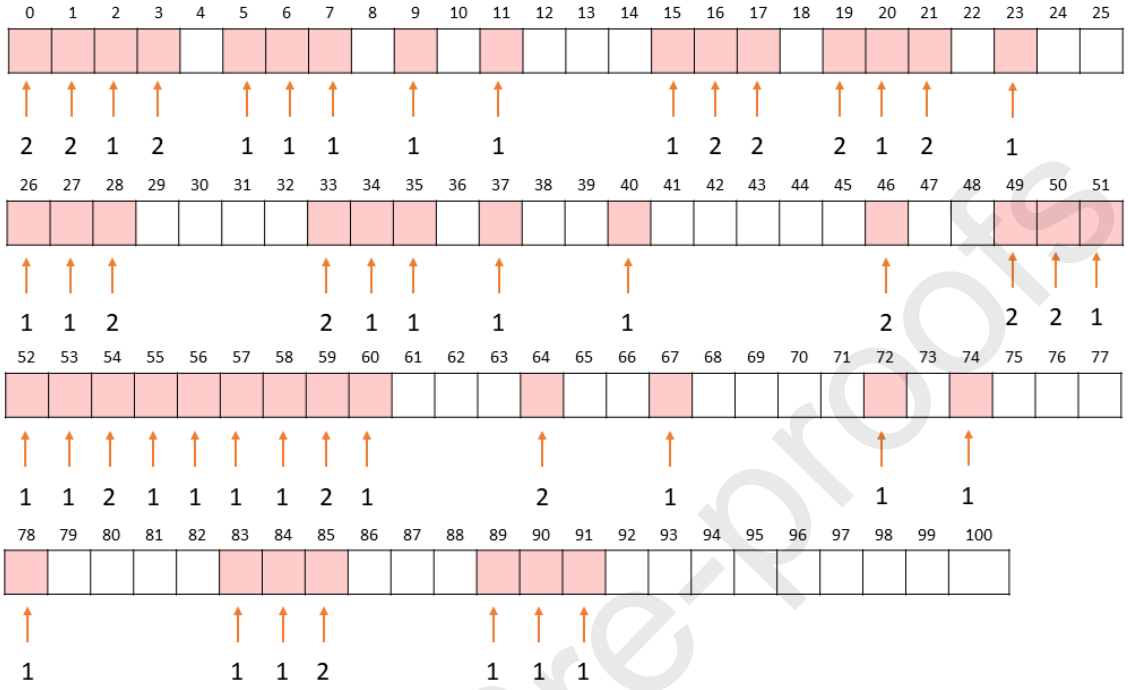

Figure SM2. Weight and size of treated egg *vs* control. (A) Weight of eggs during the treatment in pink circle and control in grey triangle; (B) size of the eggs during treatment in green circles and control in grey triangle. The dotted line described the start of the treatment.

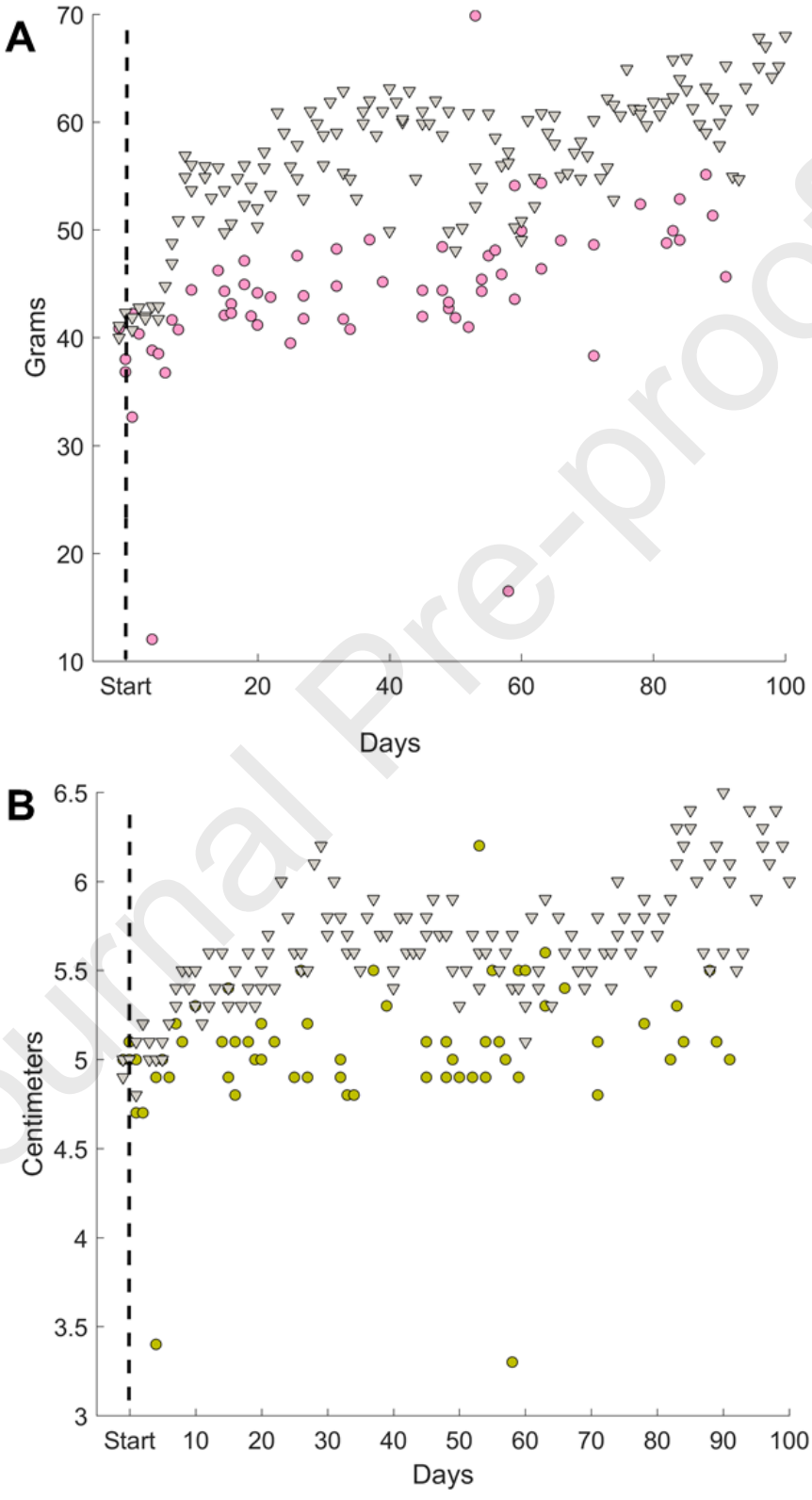

Table SM3. Concentration of ENR in egg white and egg yolk in the analyzed samples.

Day	ENR (μg g^{-1})							
	Egg white (μg g^{-1})	Egg yolk (μg g^{-1})	Ratio white/yolk	Total concentration (μg g^{-1})	Total mass of egg white (μg g^{-1})	Total mass of egg yolk (μg g^{-1})	Ratio white/yolk	Total concentration (μg g^{-1})
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.16	0.05	3.14	0.21	2.89	0.50	5.78	3.30
3	0.45	0.20	2.25	0.65	6.83	2.40	2.85	9.23
7	0.54	0.22	2.45	0.76	7.90	2.35	3.36	10.25
9	0.62	0.34	1.82	0.96	5.58	4.97	1.12	10.6
11	0.44	0.19	2.32	0.63	7.68	2.34	3.28	10.0
15	1.73	1.04	1.66	2.77	41.3	10.8	3.84	52.1
16	9.72	1.84	5.28	11.6	140.6	27.9	5.04	168.5
17	8.38	4.26	1.97	12.6	121.3	59.7	2.03	181.0
20	4.47	4.52	0.99	8.99	82.8	62.3	1.33	145.1
21	3.63	2.62	1.39	6.25	64.1	33.8	1.90	97.8
23	1.65	1.54	1.07	3.19	33.8	16.4	2.07	50.2
26	1.44	0.59	2.44	2.03	16.6	7.74	2.15	24.4
27	1.12	1.10	1.02	2.22	21.0	18.1	1.16	39.1
28	0.85	0.18	4.72	1.03	11.9	2.14	5.57	14.1
33	1.34	0.18	7.44	1.52	30.0	2.14	14.0	32.1
34	0.58	0.33	1.76	0.91	8.35	4.18	2.00	12.5
35	1.08	0.19	5.68	1.27	15.4	3.08	5.01	18.5
46	0.80	0.3	2.67	1.10	14.6	4.84	3.02	19.5
49	0.29	0.13	2.23	0.42	6.91	2.10	3.29	9.01
50	1.17	0.41	2.85	1.58	17.9	6.67	2.68	24.6
51	1.10	0.5	2.20	1.60	14.9	8.30	1.82	23.1
53	2.11	0.87	2.43	2.98	48.9	14.3	3.41	63.2
54	1.33	0.33	4.03	1.66	29.4	5.40	5.44	34.8
55	0.94	0.28	3.36	1.22	16.5	4.57	3.60	21.0
56	1.17	0.46	2.54	1.63	16.3	7.58	2.15	23.9
57	1.19	0.50	2.38	1.69	17.5	8.11	2.15	25.6
60	1.11	0.55	2.02	1.66	19.2	10.1	1.90	29.2
64	0.95	0.62	1.53	1.57	9.30	10.6	0.88	19.9
67	1.15	0.86	1.34	2.01	18.7	14.6	1.28	33.2
72	0.34	0.23	1.48	0.57	4.20	2.46	1.71	6.66
83	0.36	0.18	2.00	0.54	4.80	3.50	1.37	8.30
85	0.65	0.42	1.55	1.07	15.1	6.80	2.22	21.9
89	0.12	0.32	0.38	0.44	1.88	6.88	0.27	8.76

90	0.47	0.27	1.74	0.74	7.47	5.16	1.45	12.6

Table SM4. Concentration of ENR found in the samples by HPLC-FSFD

	Sample	Mass sample (g)	Concentration (μg kg^{-1})	Average	%RSD
Egg white	W1	0.9931	–	-	-
		0.9419	–	-	-
		0.9401	–	-	-
	W2	0.9392	351.2	350(20)	5.9
		0.9652	365.2		
		1.0072	325.0		
	W3	1.0434	-	-	-
		1.0920	-	-	-
		0.9313	-	-	-
	W4	1.1986	-	-	-
		0.9421	-	-	-
		1.2720	-	-	-
	W5	1.1454	-	-	-
		0.9252	-	-	-
		0.9613	-	-	-
	W6	1.0860	-	-	-
		0.9921	-	-	-
		1.1110	-	-	-
Egg yolk	Y1	1.1986	-	-	-
		0.9421	-	-	-
		1.2720	-	-	-
	Y2	0.9852	-	-	-
		0.9958	-	-	-
		0.9742	-	-	-
	Y3	1.0020	-	-	-
		1.0475	-	-	-
		1.1852	-	-	-
	Y4	1.0636	-	-	-
		0.9210	-	-	-
		1.1250	-	-	-

The number in parentheses corresponds to the standard deviation

Figure SM3. Chromatograms of ENR obtained by UHPLC-(+)ESI-MS/MS. (A) ENR standard solution (0.096 µg mL^{-1}), (B) sample W2 spiked with ENR standard solution (0.258 µg mL^{-1}); (C) sample W2, (C1) ion transition, 360→316 (quantification ion) and (C2) ion transition 360→245 (confirmation ion). The figures A, B and C correspond to total ion chromatrogram and the figures C1 and C2 to the extracted ion chromatograms.

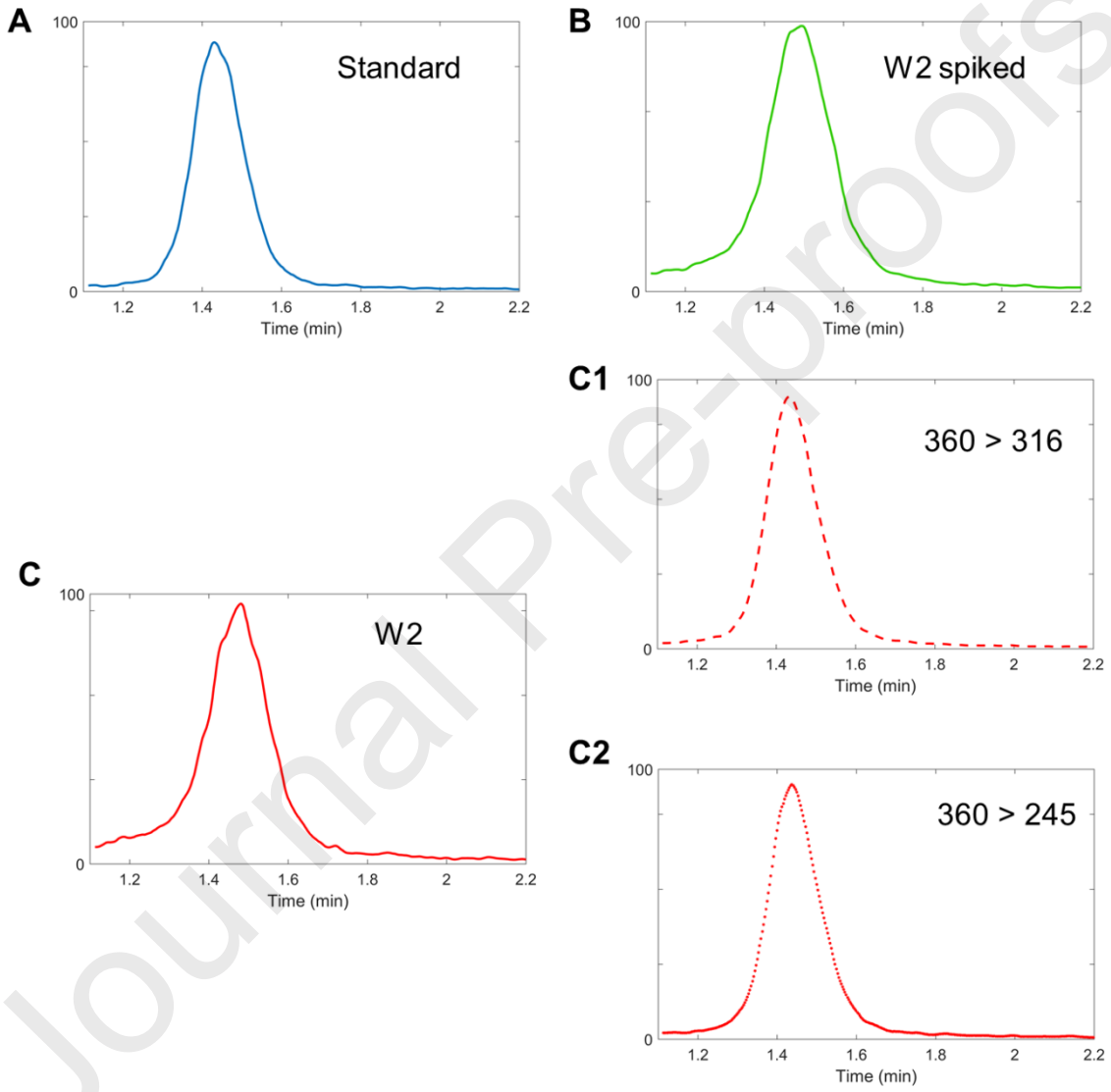

Table SM5. Consumer exposure assessment after detection of 350 μg kg^{-1} of ENR in lyophilized egg albumen; the worst-case scenario; ADI for ENR = 2.30 (μg kg^{-1} b.w.) (Food and Agriculture Organization of the United Nations, 1997).

Edible tissue/animal product	Concentration at the MRL value (μg kg^{-1})[a]	Consumption (kg)	Theoretical highest daily intake (μg kg^{-1} b.w.)[b]	%ADI
Muscles	100	0.329*	0.47	20.4
Fat and skin in natural proportions	100	0.090	0.13	5.6
Liver	300	0.1	0.43	18.6
Kidney	300	0.05	0.21	9.3
Milk	100	0.501*	0.72	31.1
Eggs	0/**350 detected**	0.100/**0.105**	0/**0.53**	0/**22.8**
				Σ 85.0/**107.8**

*Consuption in Argentina per capita in 2019 (Servicio Nacional de Sanidad y Calidad Agroalimentaria, 2019).
[a]Concentration at the MRL value in Argentina (Servicio Nacional de Sanidad y Calidad Agroalimentaria, 2011).
[b]b.w. – body weight, 70 kg.

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Determination of residual enrofloxacin in eggs due to long term administration to laying hens. Analysis of the consumer exposure assessment to egg derivatives

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Table SM21. Analytical performance of the method for ENR monitoring and quantification in eggs

Variable	Method	
	HPLC-FSFD[a]	UHPLC-MS/MS[a]
Linear range (μg g^{-1})	0.051 – 2.100	0.058 – 0.620
Intercept	-7.48 (1.4) ~~579.5 (1.4)~~	362.9 (78) ~~88940.1 (223)~~
Slope	579.5 (1.4) ~~-7.48 (1.4)~~	88940.1 (223) ~~362.9 (78)~~
F_{exp}	1.322[b]	2.220[c]
r^2	0.99999	0.99992
Lack of fit (p-value)[d]	0.301	0.106
LOD (ng g^{-1})	17.7	20.5
LOQ (ng g^{-1})	51.1	58.0

[a]Values between parentheses indicate standard deviation
[b]F_{tab}= 4.31
[c]F_{tab}= 6.54
[d]Because the p-value for the lack of fit is greater than or equal to 0.10, the model seems to be adequate for the observed data.

Table SM2. Precision and accuracy results of the HPLC-FLD method.

Sample	Concentration (μg mL^{-1})	Intra-assay precision RSD (%)[a]	Inter-assay precision RSD (%)[a]	*p*-value[b]	Recovery (%)
Egg white	0.200	1.5	2.1	0.680	91
	0.384	1.6	1.8	0.370	86
	1.200	2.8	3.1	0.923	88
Egg yolk	0.200	2.9	3.2	0.892	90
	0.384	1.6	1.2	0.938	90
	1.200	4.0	4.3	0.934	90

[a]Acceptable criterion: RSD (%) ± 15% (EMEA, 2012).
[b]Because the *p*-value is greater than or equal to 0.05, there is no statistical difference between the mean values.

Figure SM1. Amount of eggs during the 100-day treatment. The boxes in pink describe the days in which the hens laid eggs, while the numbers with arrows describe the eggs collected that day.

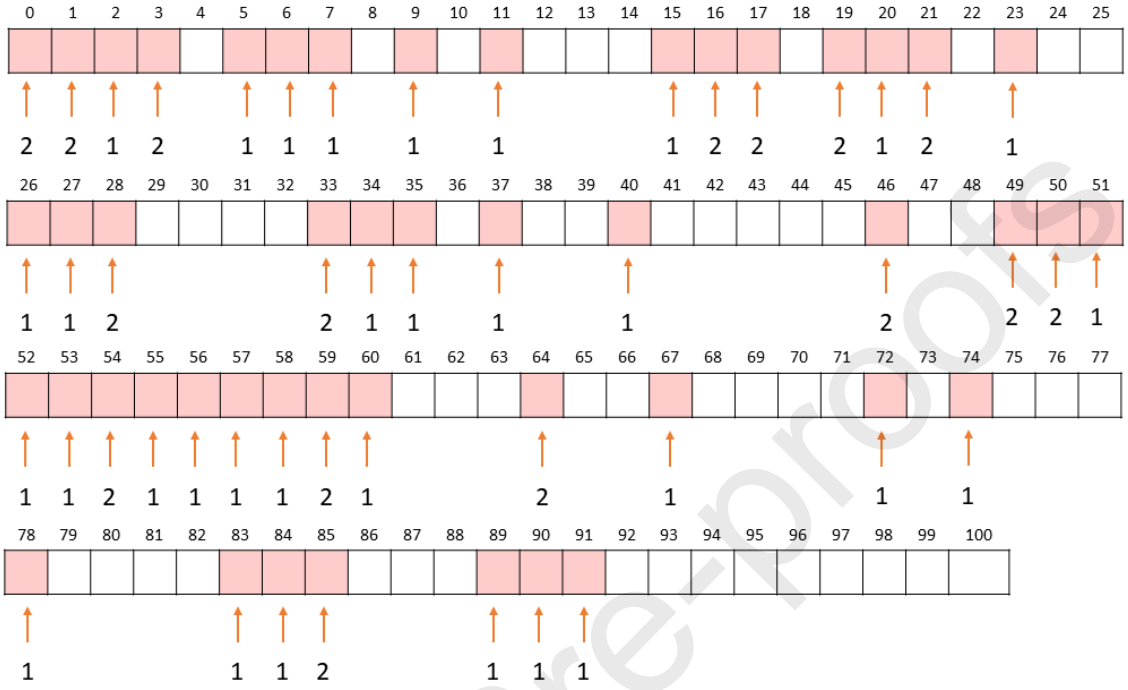

Figure SM2. Weight and size of treated egg *vs* control. (A) Weight of eggs during the treatment in pink circle and control in grey triangle; (B) size of the eggs during treatment in green circles and control in grey triangle. The dotted line described the start of the treatment.

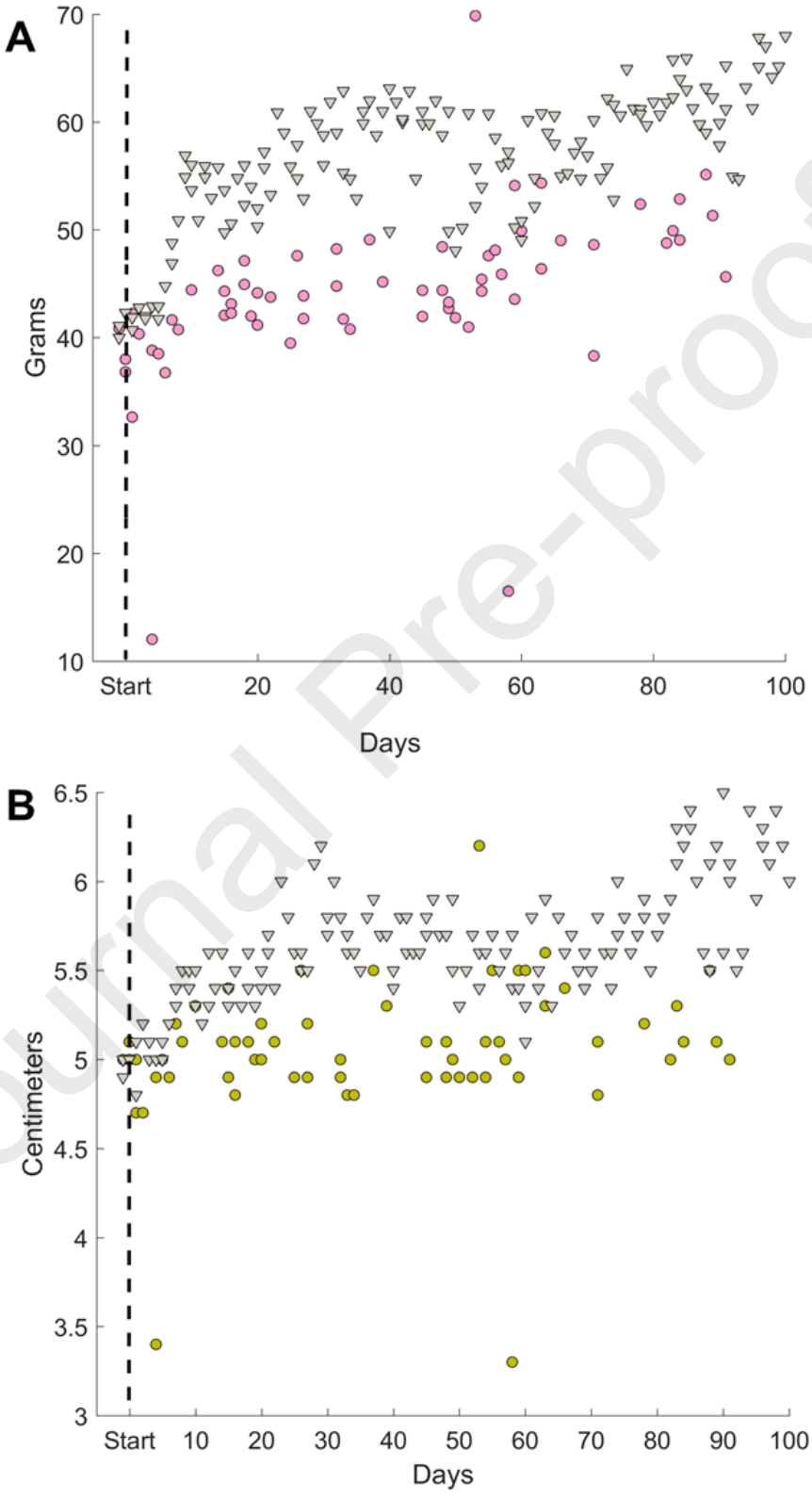

Table SM3. Concentration of ENR in egg white and egg yolk in the analyzed samples.

Day	ENR (µg g^{-1})							
	Egg white (µg g^{-1})	Egg yolk (µg g^{-1})	Ratio white/yolk	Total concentration (µg g^{-1})	Total mass of egg white (µg g^{-1})	Total mass of egg yolk (µg g^{-1})	Ratio white/yolk	Total concentration (µg g^{-1})
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.16	0.05	3.14	0.21	2.89	0.50	5.78	3.30
3	0.45	0.20	2.25	0.65	6.83	2.40	2.85	9.23
7	0.54	0.22	2.45	0.76	7.90	2.35	3.36	10.25
9	0.62	0.34	1.82	0.96	5.58	4.97	1.12	10.6
11	0.44	0.19	2.32	0.63	7.68	2.34	3.28	10.0
15	1.73	1.04	1.66	2.77	41.3	10.8	3.84	52.1
16	9.72	1.84	5.28	11.6	140.6	27.9	5.04	168.5
17	8.38	4.26	1.97	12.6	121.3	59.7	2.03	181.0
20	4.47	4.52	0.99	8.99	82.8	62.3	1.33	145.1
21	3.63	2.62	1.39	6.25	64.1	33.8	1.90	97.8
23	1.65	1.54	1.07	3.19	33.8	16.4	2.07	50.2
26	1.44	0.59	2.44	2.03	16.6	7.74	2.15	24.4
27	1.12	1.10	1.02	2.22	21.0	18.1	1.16	39.1
28	0.85	0.18	4.72	1.03	11.9	2.14	5.57	14.1
33	1.34	0.18	7.44	1.52	30.0	2.14	14.0	32.1
34	0.58	0.33	1.76	0.91	8.35	4.18	2.00	12.5
35	1.08	0.19	5.68	1.27	15.4	3.08	5.01	18.5
46	0.80	0.3	2.67	1.10	14.6	4.84	3.02	19.5
49	0.29	0.13	2.23	0.42	6.91	2.10	3.29	9.01
50	1.17	0.41	2.85	1.58	17.9	6.67	2.68	24.6
51	1.10	0.5	2.20	1.60	14.9	8.30	1.82	23.1
53	2.11	0.87	2.43	2.98	48.9	14.3	3.41	63.2
54	1.33	0.33	4.03	1.66	29.4	5.40	5.44	34.8
55	0.94	0.28	3.36	1.22	16.5	4.57	3.60	21.0
56	1.17	0.46	2.54	1.63	16.3	7.58	2.15	23.9
57	1.19	0.50	2.38	1.69	17.5	8.11	2.15	25.6
60	1.11	0.55	2.02	1.66	19.2	10.1	1.90	29.2
64	0.95	0.62	1.53	1.57	9.30	10.6	0.88	19.9
67	1.15	0.86	1.34	2.01	18.7	14.6	1.28	33.2

72	0.34	0.23	1.48	0.57	4.20	2.46	1.71	6.66
83	0.36	0.18	2.00	0.54	4.80	3.50	1.37	8.30
85	0.65	0.42	1.55	1.07	15.1	6.80	2.22	21.9
89	0.12	0.32	0.38	0.44	1.88	6.88	0.27	8.76
90	0.47	0.27	1.74	0.74	7.47	5.16	1.45	12.6

Table SM4. Concentration of ENR found in the samples by HPLC-FSFD

	Sample	Mass sample (g)	Concentration (μg kg^{-1})	Average	%RSD
Egg white	W1	0.9931	–	-	-
		0.9419	–	-	-
		0.9401	–	-	-
	W2	0.9392	351.2	350(20)	5.9
		0.9652	365.2		
		1.0072	325.0		
	W3	1.0434	-	-	-
		1.0920	-	-	-
		0.9313	-	-	-
	W4	1.1986	-	-	-
		0.9421	-	-	-
		1.2720	-	-	-
	W5	1.1454	-	-	-
		0.9252	-	-	-
		0.9613	-	-	-
	W6	1.0860	-	-	-
		0.9921	-	-	-
		1.1110	-	-	-
Egg yolk	Y1	1.1986	-	-	-
		0.9421	-	-	-
		1.2720	-	-	-
	Y2	0.9852	-	-	-
		0.9958	-	-	-
		0.9742	-	-	-
	Y3	1.0020	-	-	-
		1.0475	-	-	-
		1.1852	-	-	-
	Y4	1.0636	-	-	-
		0.9210	-	-	-
		1.1250	-	-	-

The number in parentheses corresponds to the standard deviation

Figure SM3. Chromatograms of ENR obtained by UHPLC-(+)ESI-MS/MS. (A) ENR standard solution (0.096 μg mL^{-1}), (B) sample W2 spiked with ENR standard solution (0.258 μg mL^{-1}); (C) sample W2, (C1) ion transition, 360→316 (quantification ion) and (C2) ion transition 360→245 (confirmation ion). The figures A, B and C correspond to total ion chromatrogram and the figures C1 and C2 to the extracted ion chromatograms.

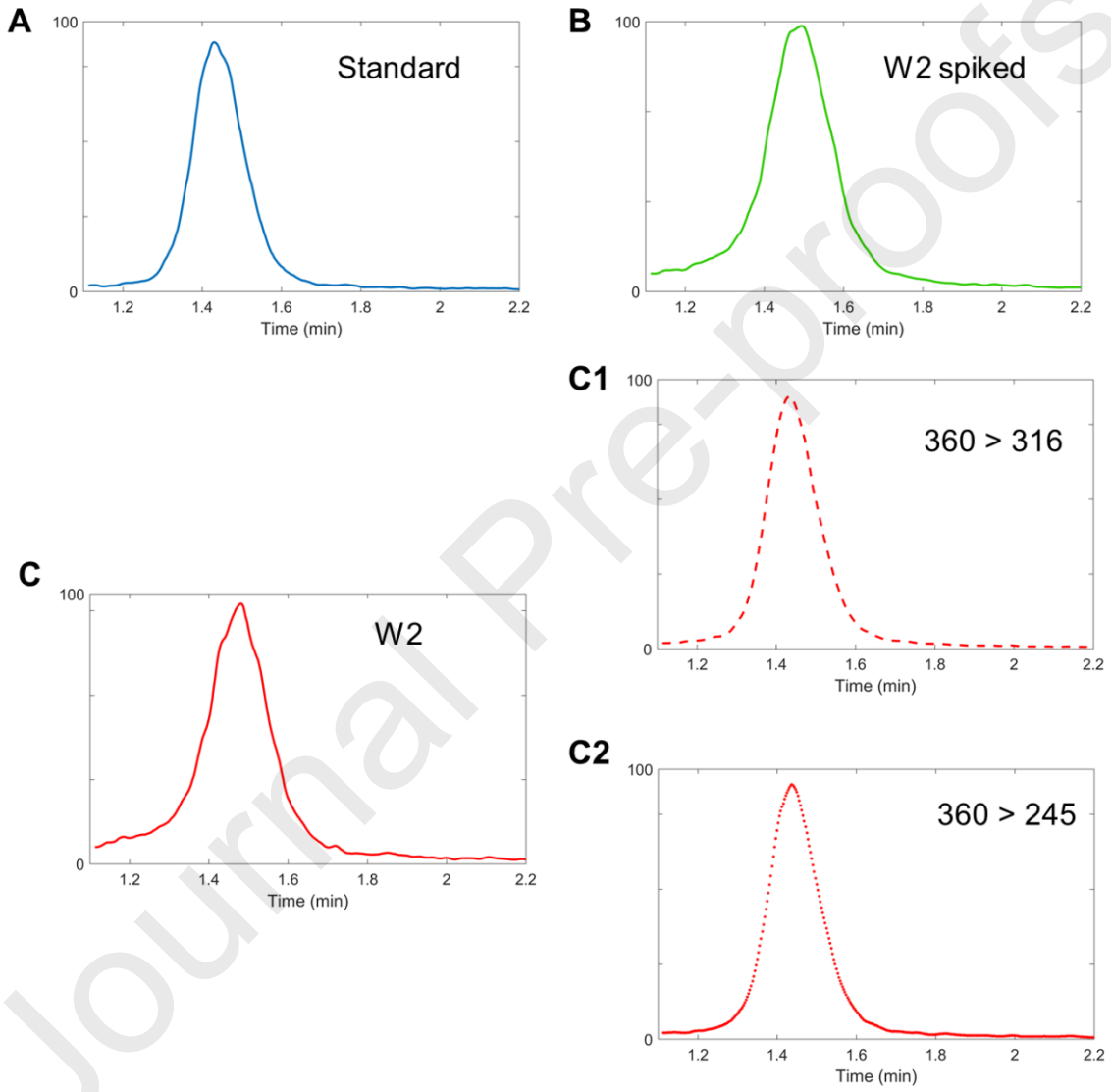

Table SM5. Consumer exposure assessment after detection of 350 μg kg^{-1} of ENR in lyophilized egg albumen; the worst-case scenario; ADI for ENR = 2.30 (μg kg^{-1} b.w.) (Food and Agriculture Organization of the United Nations, 1997).

Edible tissue/animal product	Concentration at the MRL value (μg kg^{-1})[a]	Consumption (kg)	Theoretical highest daily intake (μg kg^{-1} b.w.)[b]	%ADI
Muscles	100	0.329*	0.47	20.4
Fat and skin in natural proportions	100	0.090	0.13	5.6
Liver	300	0.1	0.43	18.6
Kidney	300	0.05	0.21	9.3
Milk	100	0.501*	0.72	31.1
Eggs	0/**350 detected**	0.100/**0.105**	0/**0.53**	0/**22.8**
				Σ 85.0/**107.8**

*Consuption in Argentina per capita in 2019 (Servicio Nacional de Sanidad y Calidad Agroalimentaria, 2019).
[a]Concentration at the MRL value in Argentina (Servicio Nacional de Sanidad y Calidad Agroalimentaria, 2011).
[b]b.w. – body weight, 70 kg.

References

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Table 1. Weight average results for egg white, egg yolk, and eggshell.

	Average (%)[a]		*p*-value
	Egg under treatment	Control egg[b]	
Egg white	36 (8)	62 (5)	<0.001
Egg yolk	31 (5)	29 (3)	0.173[c]
Eggshell	32 (6)	9 (1)	<0.001

[a]Values in parenthesis correspond to standard deviation.
[b]The normal average for egg white, egg yolk, and eggshell are 60%, 30%, and 10% (Instituto de Estudios del Huevo, 2009).
[c]Because the *p*-value is greater than or equal to 0.05, there is no statistical difference between the mean values.

Table 2. Confirmation of the presence of enrofloxacin in the sample by UHPLC-MS/MS.

Sample	Weighed mass (g)	Concentration (μg kg^{-1})	Average	%RSD
W2	1.1330 1.0130 1.0310	347.8 361.3 308.9	340 (30)	8.0
W2 spiked with ENR 258 μg Kg^{-1}	1.0580 0.9970 1.2070	592.4 588.3 535.8	570 (30)	5.5

The numbers in parentheses correspond to the standard deviation.

Table 3. Consumer exposure assessment – daily intake and %ADI estimated using the concentration of ENR found in sample W2 (350 μg Kg^{-1}) and 100 g of egg product consumption (athletes 105 g).

Group of consumers											
Typical body weight (Kg)							Overweight (Kg)	Athletes body weight (Kg)			
Children			Adolescents			Adults	Adults				
7	12	23.1	41.2	57.2	66.6	67.9	100	70	80	90	100
Theoretical daily intake (μg Kg^{-1} b.w.)[1]											
0.19	0.17	0.17	0.09	0.07	0.06	0.06	0.37	0.53	0.46	0.41	0.37
%ADI											
8.3	7.2	7.3	4.1	3.0	2.5	2.5	16.0	22.8	20.0	17.8	16.0

[a]Dilution factor 9
[b]Pure egg white
[1]b.w.: body weight
The albumen dilution factor 9 was included for typical consumers. ADI (μg kg^{-1} b.w.) obtained from Food and Agriculture Organization of the United Nations (1997).