Aquaculture 561 (2022) 738640

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Fluoroquinolones and tetracyclines as growth factors in aquaculture: Increase of biometrical parameters versus emergence of resistant bacteria and residues in meat

Valeria I. Amable [a], María J. Valdéz Amarilla [a], Paula L. Salas [b], Jorge A. Mendoza [a,b,c], Sofía Lizardo Falcón [a,b,c], Silvia I. Boehringer [a], Sebastián Sánchez [b,c], Marcos G. Guidoli [a,b,c,1,*]

[a] Cátedra de Microbiología, Departamento de Tecnología de los Alimentos y Salud Pública, Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, 2139 Sargento Cabral Street, Corrientes, Argentina
[b] Instituto de Ictiología del Nordeste (INICNE), Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, 2139 Sargento Cabral Street, Corrientes, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Keywords:
Piaractus mesopotamicus
Oxytetracycline
Enrofloxacin
Antibiotic pressure selection

ABSTRACT

Antibiotics can be used as growth promoters for fishes in aquaculture. However, its use in animal production induced an increase in the emergence of resistant microorganisms. *Piaractus mesopotamicus* is the second most produced fish species in Argentina. The aim of this work was to evaluate the effect of the administration of subtherapeutic doses of oxytetracycline and enrofloxacin over biometrical parameters, the increase of resistant microorganisms in the intestinal microbiota and the presence of antibiotic residues in meat of *P. mesopotamicus*. 3 months old animals were fed twice a day for 120 days. Commercial balanced feed was added with either oxytetracycline (O), enrofloxacin (E) or water (C). Biometrical parameters, intestinal content (IC) and muscle samples were obtained every 30 days. IC samples were inoculated in plates with and without antibiotics while muscle samples were submitted to a microbiological method to find antibiotic residues. Treatment O maintained survival at 100%, increased mean weight and standard length and was not detected in muscle samples. However, it showed the highest increments in the number of resistant microorganisms. These results indicate that the use of subtherapeutic doses of oxytetracycline for prolonged periods induces increments in biometrical parameters leaving no residues in meat, but also promotes the development of resistant microorganisms when compared with the control group.

1. Introduction

The protein demand for human consumption is expected to increase exponentially, with worldwide fish production expected to expand from 179 million tons in 2018 to 204 million tons in 2030. However, in the last 30 years capture fisheries production decreased or remained stable year after year due to the diminishing of fish stock and the willingness of maintaining the welfare of aquatic biodiversity. Thus, aquaculture has become the main source for aquatic protein (FAO, 2020).

This trend induced the need of increasing productivity. However, intensification of culture systems leads to inconveniences such as stressed animals, decreasing of biometrical and productive parameters all caused by a diminished immune system and the appearance of epizooties (Li et al., 2022).

The addition of growth promoters to balanced feeds allowed an increment in the productive parameters of aquaculture facilities. Some antibiotics are included into this classification for their ability, not only to diminish disease appearance, but also to increase survival, mean weight and appearance and general state of animals. Regardless scarce information about their use in aquaculture, it has been stated that among the top 15 aquaculture-producing countries, 12 use oxytetracycline (Tetracyclines category), 9 oxolinic acid (Fluoroquinolone's category) and 9 chloramphenicol (Amphenicols category), being the most used worldwide (Sapkota et al., 2008). Also, recently has been reported

* Corresponding author at: Cátedra de Microbiología, Departamento de Tecnología de los Alimentos y Salud Pública, Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, 2139 Sargento Cabral Street, Corrientes, Argentina.
E-mail address: marcosguidoli@hotmail.com (M.G. Guidoli).
[1] Personal information: 117 Quiroga Juan Facundo Street, Resistencia, Chaco, Argentina.

https://doi.org/10.1016/j.aquaculture.2022.738640
Received 7 February 2022; Received in revised form 6 July 2022; Accepted 19 July 2022
Available online 22 July 2022
0044-8486/© 2022 Elsevier B.V. All rights reserved.

that enrofloxacin (Fluoroquinolones category) is used in 5 out of the 6 top aquaculture-producing countries (Limbu et al., 2021). Despite all advantages these substances offer, the huge quantities of antibiotics administered with prophylactic or therapeutic purposes in aquaculture has turned on the alarms worldwide. The indiscriminate use of such substances has been associated to the elevated levels of bacterial antibiotic resistance in and around aquaculture production environments, the occurrence of drug residues in meat for human consumption and the potential damage to the environment due to massive liberation to water courses (Sapkota et al., 2008; Limbu et al., 2021).

Piaractus mesopotamicus, also known as pacú, is a native fish species from the Paraná river basin. It is also one of the two most produced species in Argentinian aquaculture, with 1063 tons in 2019 (Panné Huidobro, 2020). There are no official reports on the use of antibiotics in Argentinian aquaculture. However, Griboff et al. (2020) informed that all samples of pacú, shad, trout and salmon obtained from supermarkets in Córdoba, Argentina had residues of enrofloxacin, clarithromycin, roxithromycin, doxycycline and oxytetracycline. Besides, non official data indicate that oxytetracycline and enrofloxacin are the most used antibiotics in Argentinian aquaculture.

Thus, the main objectives of this work were to evaluate if the administration of subtherapeutic doses of oxytetracycline and enrofloxacin to *Piaractus mesopotamicus* juveniles induced significant changes in biometrical parameters, the occurrence of resistant microorganisms in the gastrointestinal tract (GIT) and detectable residues appearance in muscle.

2. Material and methods

2.1. Balanced feed

Commercial balanced feed was added with sterile distilled water, (control group or treatment C) or enough quantity of drug to achieve a concentration of: a) 75 mg kg^{-1} of fish of oxytetracycline (treatment O) and b) 10 mg kg^{-1} of fish of enrofloxacin (treatment E). Once prepared, the feed was dried in oven at 30 °C until constant weighting and kept closed hermetically at room temperature until further use.

2.2. http://2.2.In vivo experiments

A total of 108 juveniles of *Piaractus mesopotamicus* (n = 108), weighing 29,08 ± 6,29 g were distributed in 18 tanks of 70 L (approximately 2570 g m^{3-1} compared with the maximum of 70,000 in intensive culture systems) with constant water exchange and forced aeration to ensure water quality. Animals were fed twice a day with 5% of biomass. After a 15 days acclimation period, fishes were allocated randomly to the 18 experimental units (tanks) which were randomly divided into 3 groups (6 replicates by treatment) and the administration of medicated feed started. Water quality was evaluated at the beginning of the experiment -day 0- and at days 25, 55, 85 and 115 -five days prior to the biometric sampling-. Temperature, dissolved oxygen, pH and conductivity were evaluated.

The present experiment is in allegiance with the ARRIVE guidelines and was carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines. Animals were not sexually mature at the time of the experiment, not allowing differentiation between genders. The number of animals in each experimental unit was chosen in order to have >50% of the initial n after sampling at the end of the experiment. The criteria used for including animals at the beginning of the experiment were being between 2 and 5 months old and weighing between 20 and 60 g. No animals, experimental units or data points were excluded in this experiment.

2.3. Sampling

At days 0, 30, 60, 90 and 120 animals were counted, weighed and measured in order to determine survival, mean weight, standard length and coefficient of condition. Relative condition factor (kn) was obtained as the quotient of the weight of an animal (W) by the mean weight of the population (W′) estimated for an individual with the same standard length (SL) and adjusted by power regression according to the following expression: $kn = W\,W' - 1$, being $W' = a.x^b$ where a and b are parameter of regression that correlate weight an standard length. Besides, three animals of each treatment (three different replicates) were anesthetized with benzocaine 2%, demedullated and dissected aseptically to remove the GIT and obtain the intestinal content by washing and further centrifugation.

2.4. Microbial counts

Suspensions of intestinal content were obtained mixing 1 g of sample with 10 mL of distilled sterile water. Aliquots of 100 μL of the suspension, pure and at different dilutions, were inoculated in plates containing different culture media. After 24 h at different temperatures −10, 25 and 37 °C- counts were performed in those plates containing between 30 and 300 colony forming units (CFU). Results were expressed as the logarithm of the number of CFU per g of intestinal content (Ln CFU/g) and as resistance proportions, calculated as the quotient of the number of resistant over the total. Culture media were: a) Mac Conkey Agar (Britania ®) for the isolation of total *Enterobacteriaceae* and non-fermenting Gram-negative bacilli (E&NFB), b) Mac Conkey Agar (Britania ®) added with 30 μg mL^{-1} of oxytetracycline for the isolation of E&NFB resistant to oxytetracycline, c) Mac Conkey Agar (Britania ®) added with 5 μg mL^{-1} of enrofloxacin for the isolation of E&NFB resistant to enrofloxacin, d) Nutrient Agar (Britania ®) for the isolation of total aerobic bacteria (AB), e) Nutrient Agar (Britania ®) added with 30 μg mL^{-1} of oxytetracycline for the isolation of AB resistant to oxytetracycline and f) Nutrient Agar (Britania ®) added with 5 μg mL^{-1} of enrofloxacin for the isolation of AB resistant to enrofloxacin (Navarrete et al., 2008).

2.5. Statistics

General Linear Mixed Models were fitted to standard length, weight, survival, relative condition factor, total AB and E&NFB count, resistant AB and E&NFB count and proportion between resistant and total AB and E&NFB; assuming a normal conditional distribution of the response.

For standard length, weight, survival and relative condition factor, the linear predictor included the fixed effects of time −0, 30, 60, 90 and 120 days-, treatment -C, and treatments O and *E*- and their 2-way interaction, in addition to the random effects of fish/cage to properly recognize experimental units for each of the factors.

For total AB and resistant E&NFB count, the linear predictor included the fixed effects of time −0, 30, 60, 90 and 120 days-, treatment -C and O or C and E-, antibiotic in plate -with or without-, type of microorganism -aerobic and enterobacteria- and their 4-way interaction, in addition to the random effects of fish/cage to properly recognize experimental units for each of the factors.

Finally, for proportion between resistant and total AB and E&NFB, the linear predictor included the fixed effects of time −0, 30, 60, 90 and 120 days-, treatment -C and O or C and E-, type of microorganism -aerobic and enterobacteria- and their 3-way interaction, in addition to the random effects of fish/cage to properly recognize experimental units for each of the factors.

In all cases, model assumptions were evaluated using externally studentized residuals and were considered to be reasonably met. Variance components were estimated using restricted maximum likelihood. Kenward Roger's method was used to estimate degrees of freedom and make the corresponding adjustments in estimation of standard errors. Models were fitted using the Infostat software (Version 2020). Estimated least square means and corresponding estimated standard errors are presented. Relevant pairwise comparisons were conducted using Tukey-

Kramer or Bonferroni adjustments, as appropriate in each case, to avoid inflation of Type I error rate due to multiple comparisons.

Variables showing a significative effect of the time, were analyzed by orthogonal contrast procedure to evaluate the probable occurrence of linear or quadratic tendences at a temporal scale.

2.6. Microbial detection of enrofloxacin and oxytetracycline residues in muscle

Muscle samples of 1 g were defrosted, macerated with 2 mL distilled sterilized water and neutralized to pH 7. A total of 100 μL of the muscle suspension and 100 μL of a *Geobacillus stearothermophilus* spores' suspension were inoculated in 5 mL of Glucose Bromcresol Purple Agar -in g L^{-1}: pluripeptone 5, meat extract 3, glucose 20, agar, 15 bromcresol purple 0,024-. A tube with only a muscle sample from the control group, a tube with only the spore suspension and a tube without any addition were used as controls. All tubes were incubated at 60 °C for 48 h and checked out every 4 h. Changes in media coloration from purple to yellow indicated absence of detectable residues of antibiotics in the sample. Previously our group evaluated the method, stablishing a minimum detection level of 80 and 60 μg kg^{-1} of enrofloxacin and oxytetracycline, respectively. Both values, lower than the maximum residue limit that the European Union requires for such drugs in meat.

3. Results

Water quality parameters remained stable and between accepted values for the aquaculture of *P. mesopotamicus* (Luchini, 2008).

Statistical analysis showed no significant differences ($p > 0,05$) in survival and relative condition factor values between treatments and control group. Values ranged from 84,17 to 100% for survival and between 0.95 and 1.07 for relative condition factor all along the experiment. However, four animals of treatment E died at days 18 -replicate E3-, 102 -replicate E1-, 107 -replicate E3- and 116 -replicate E3- while three animals of control groups died at days 15 -replicate C1-, 88 -replicate C3- and 105 -replicate C5-. Mean weight and standard length values showed interaction between day and treatment. There were no significant differences in mean weight between treatments in the first 30 days of the experiment. Significant differences ($p < 0,05$) appeared from day 60 and lasted until the end of the experiment, with treatment O significantly higher than the control group and treatment E without significant differences between treatment O and control group. A similar behavior was observed for standard length, with no significant differences among treatments until the last biometry. By the end of the experiment treatment O was significantly higher than the control group, while treatment E remained without significant differences between treatment O and control group. Both variables presented a significant linear tendency for all treatments (Fig. 1).

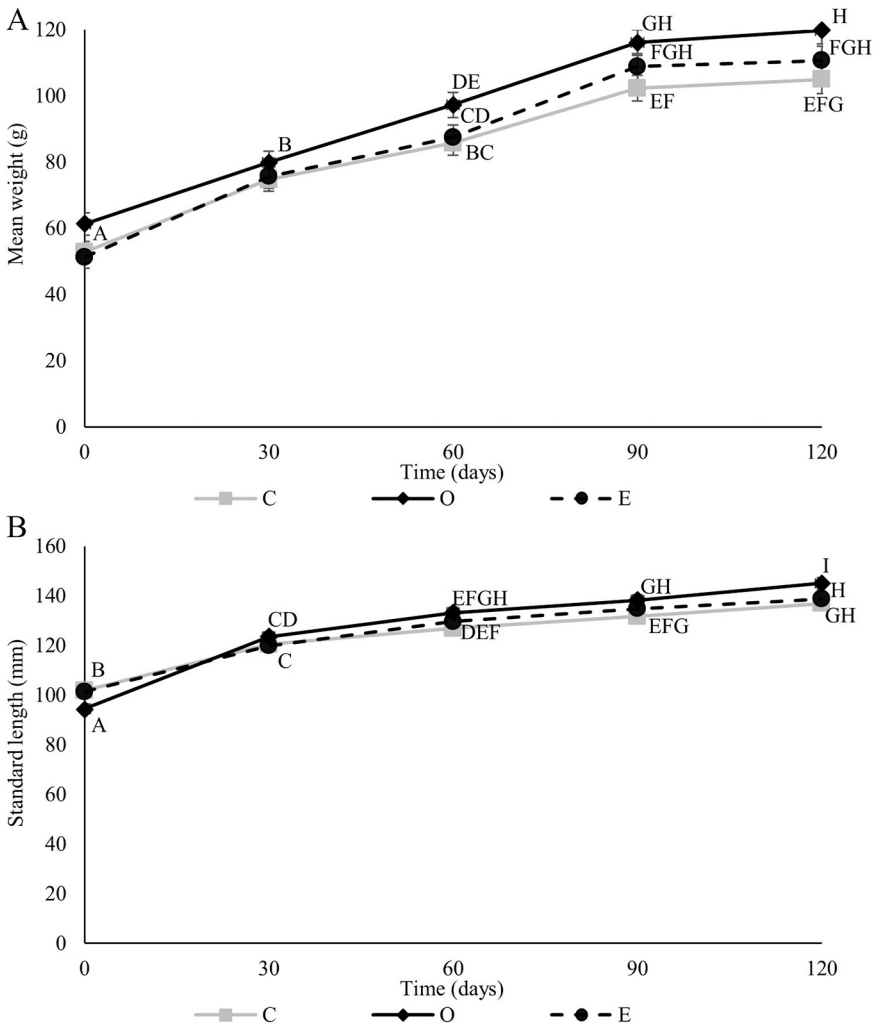

Fig. 1. Mean weight (A) and standard length (B) of *Piaractus mesopotamicus* juveniles fed with balanced feed added with 75 mg kg^{-1} of fish of oxytetracycline (O), 10 mg kg^{-1} of fish of enrofloxacin (E) and without the addition of antibiotics (C) during 120 days. Vertical bars indicate standard error. Different letters indicate significant differences ($p < 0,05$) of values in the same day of experiment.

Plates with samples incubated at 10 °C did not present microbial growth. On the other hand, bacterial growth at 25 °C and 37 °C did not show significant differences among them. Thus, results herein presented are referred to growth at 37 °C.

A triple interaction -Bacteria (total or resistant):treatment:day- was observed when comparing: *i)* total and resistant to oxytetracycline E&NFB in treatment O and control group, *ii)* total and resistant E&NFB in treatment E and control group, *iii)* total and resistant to enrofloxacin AB in treatment E and control group and *iv)* total and resistant to oxytetracycline AB in treatment O and the control group.

Total E&NFB in treatment O and control group present a positive linear tendency through time, with no significative differences between them by the end of the experiment. On the other hand, E&NFB resistant to oxytetracycline have a quadratic tendency in the control group and a positive linear tendency in treatment O. By the end of the experiment E&NFB resistant to oxytetracycline resulted significantly higher ($p <$ 0,05) in treatment O than in control group (Fig. 2).

The total number of E&NFB in treatment E and control group have a linear tendency along time, ending the experiment with no significant differences among them. Conversely, E&NFB resistant to enrofloxacin have a quadratic tendency in the control group and a positive linear tendency in treatment E, with significantly higher values in animals administered with the antibiotic than in the control group at the end of the experiment (Fig. 3).

Total AB show a positive linear tendency in treatment O and the control group, with no significant differences among them by the end of the experiment. Resistant to oxytetracycline AB in the control group show a quadratic tendency along time, while treatment O presents a positive linear tendency. By the end of the experiment, the number of AB resistant to oxytetracycline were significantly higher in treatment O than in the control group (Fig. 4).

Total AB in treatment E and control group do not show linear or quadratic tendency, presenting no significant differences among them all along the experiment. In contrast, resistant to enrofloxacin AB presented a positive linear tendency along time in treatment in E and a quadratic tendency in the control group. By the end of the experiment resistant to enrofloxacin AB in treatment E tended to be significantly higher than those in the control group (Fig. 5).

The effect of both antimicrobials over the resistance proportions of E&NFB and total AB in the GIT was also compared. At the beginning of the experiment there were no significant differences between the resistance proportion in treatments and control groups. However, after 30 days, the resistance proportion of both types of microorganisms in animals administered with oxytetracycline (RO in O) differed significantly from that in the control group (RO in C). These differences persisted all along the experiment. On the other hand, the resistance proportion of both microbial groups in animals administered with enrofloxacin (RE in E) did not show significant differences with the control group (RE in C) until day 90 of experiment, maintaining such behavior until the end of the experiment (Fig. 6).

The detection method used in this study to determine the presence of antibiotic residues in meat samples showed no differences between controls and treatments. Thus, either the antimicrobials tested in this study do not accumulate in fish meat or the detection method is not sensitive enough to detect them.

4. Discussion

This work was aimed to evaluate the effect of antibiotics in subtherapeutic doses on biometrical and microbiological parameters in farmed fishes. With that purpose, juveniles of *Piaractus mesopotamicus* were fed with balanced feed added with two of the most used antibiotics in worldwide aquaculture -oxytetracycline and enrofloxacin-. After 120 days of experiment, survival and relative condition factor did not differ significantly between control and treatments. These results differ from those obtained by Vargas et al. (1993), after administering bacitracin-zinc in hybrid tilapia *(Oreochromis* sp.), concluded that only the control group presents a significantly higher survival. On the other hand, the higher values of mean weight and standard length found in this study in treated animals are consistent with Li et al. (2014) who, after analyzing the use of olaquindox on large yellow croaker (*Pseudosciaena crocea R.*), concluded that treated groups present a significantly higher weight gain when compared with control groups.

Before analyzing microbial resistance, it is important to highlight that in aquatic environments resistance trends correlate to the abundance and type of bacterial species present in the habitat (Esiobu et al., 2002). Thus, results should be clearly defined for species, type and conditions of culture herein evaluated.

Microbial analysis of intestinal content showed resistant bacteria in control group. This would indicate the presence of autochthonous strains with intrinsic resistance to antibiotics (Allen et al., 2010; Zhang and Feng, 2016; de Alcântara Rodrigues et al., 2020).

In treated groups there is a significant increase in the number of both E&NFB and AB resistant to antibiotics when compared with control. Such increase becomes significant after 30 days in treatment O and after 60 or 90 days in treatment E. These significant differences remain until the end of the experiment for both types of microorganisms in both treatments. These results could be explained as oxytetracycline being known to exert a sooner selection pressure than enrofloxacin (Samira and Guichard, 2009).

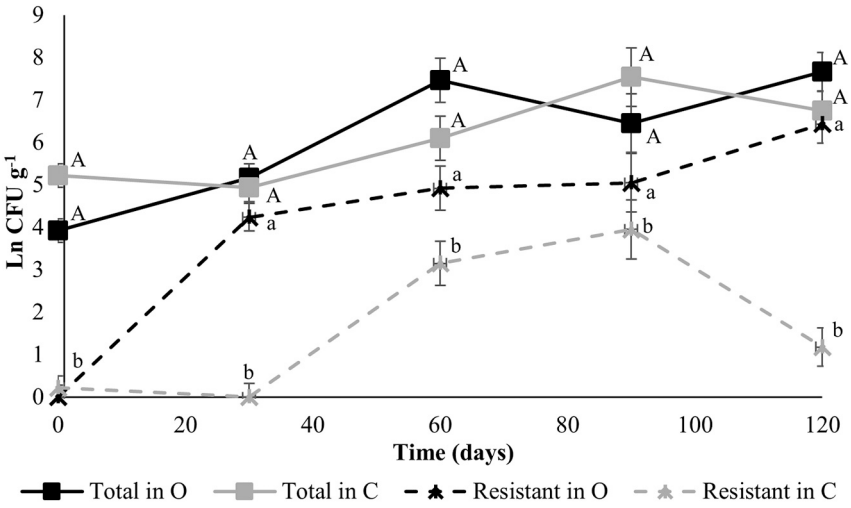

Fig. 2. Natural logarithm per gram of intestinal content of total and resistant *Enterobacteriaceae* and Gram-negative non-fermentative bacilli (E&NFB) in *Piaractus mesopotamicus* juveniles fed with balanced feed added with 75 mg kg-1 of fish of oxytetracycline (O) or without the addition of antibiotics (C) during 120 days. Vertical bars indicate standard error. Different lowercase letters indicate significant differences ($p <$ 0,05) in total counts in the same day of experiment. Different uppercase letters indicate significant differences ($p <$ 0,05) in resistant counts in the same day of experiment.

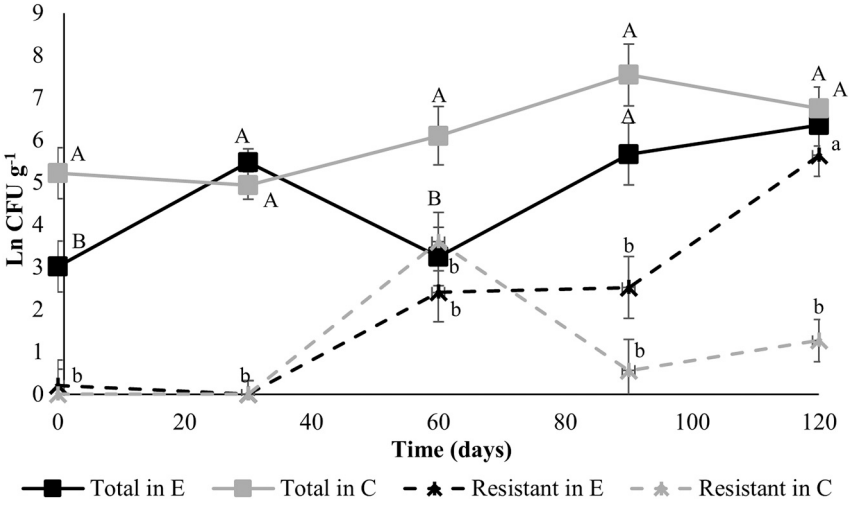

Fig. 3. Natural logarithm per gram of intestinal content of total and resistant *Enterobacteriaceae* and Gram-negative non-fermentative bacilli (E&NFB) in *Piaractus mesopotamicus* juveniles fed with balanced feed added with 10 mg kg^{-1} of fish of enrofloxacin (E) or without the addition of antibiotics (C) during 120 days. Vertical bars indicate standard error. Different lowercase letters indicate significant differences ($p < 0,05$) in total counts in the same day of experiment. Different uppercase letters indicate significant differences ($p < 0,05$) in resistant counts in the same day of experiment.

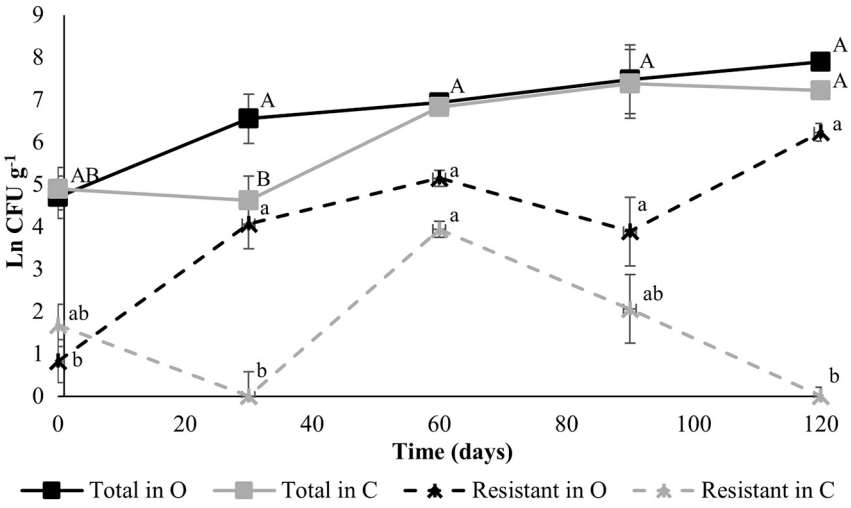

Fig. 4. Natural logarithm per gram of intestinal content of total and resistant aerobic bacteria (AB) in *Piaractus mesopotamicus* juveniles fed with balanced feed added with 75 mg kg-1 of fish of oxytetracycline (O) or without the addition of antibiotics (C) during 120 days. Vertical bars indicate standard error. Different lowercase letters indicate significant differences ($p < 0,05$) in total counts in the same day of experiment. Different uppercase letters indicate significant differences ($p < 0,05$) in.

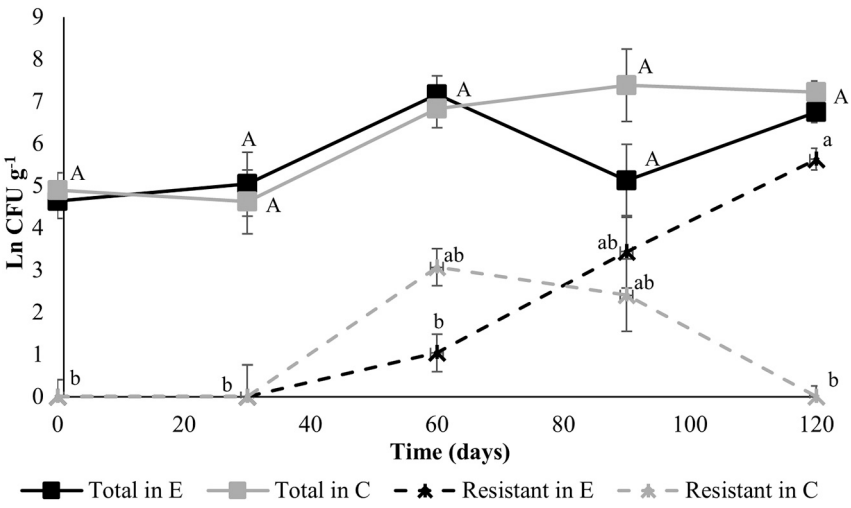

Fig. 5. Natural logarithm per gram of intestinal content of total and resistant aerobic bacteria (AB) in *Piaractus mesopotamicus* juveniles fed with balanced feed added with 10 mg kg^{-1} of fish of enrofloxacin (E) or without the addition of antibiotics (C) during 120 days. Vertical bars indicate standard error. Different lowercase letters indicate significant differences ($p < 0,05$) in total counts in the same day of experiment. Different uppercase letters indicate significant differences ($p < 0,05$) in resistant counts in the same day of experiment. Resistant counts in the same day of experiment.

The number of resistant microorganisms is an important parameter. However, in order to make evident that the presence of the antimicrobial drug is exerting a selection pressure, it is important to determine the proportion of resistant to total microorganisms.

These results show similar behavior to that observed in the number of resistant bacteria. Thus, by the end of the experiment, the proportion of E&NFB and AB resistant to each antibiotic in each treatment remained with high significant differences in comparison with the same parameter

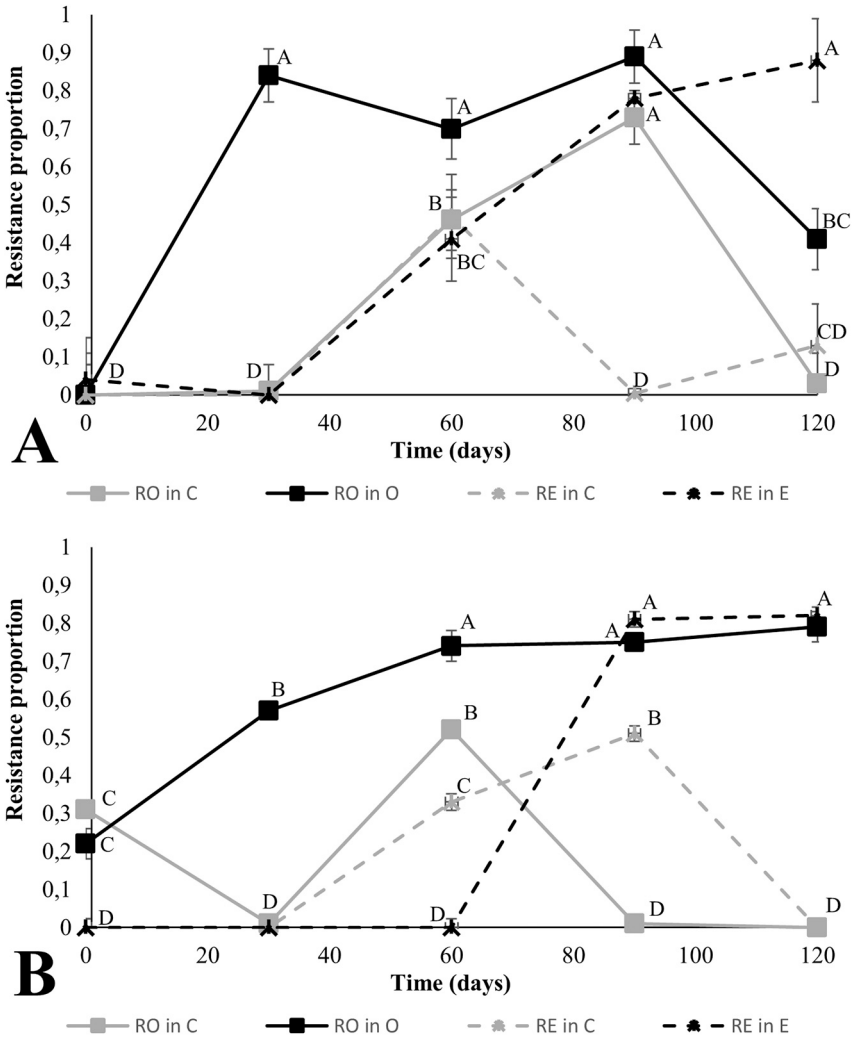

Fig. 6. Resistance proportion (resistant x total^{-1}) to oxytetracycline (RO) or enrofloxacin (RE) of *Enterobacteriaceae* and Gram-negative non-fermentative bacilli (A) and Aerobic bacteria (B) in *Piaractus mesopotamicus* juveniles fed with balanced feed added either with 75 mg kg-1 of fish of oxytetracycline (O), 10 mg kg^{-1} of fish of enrofloxacin (E) or without the addition of antibiotics (C) during 120 days. Vertical bars indicate standard error. Different letters indicate significant differences ($p < 0,05$).

in the control group.

Kondera et al. (2020) determined that the administration of therapeutic doses of oxytetracycline and gentamicin to *Cyprinus carpio* juveniles do not cause a considerable toxicity in hematological, biochemic and hematopoietic parameters. Being these doses much higher than those used in this study, it could be concluded that there were no toxic effects affecting animals in the present experiment.

On the other hand, Krupesha Sharma et al., 2021, demonstrated that the longer the administration period and higher the dose of oxytetracycline in *Trachinotus blochii*, the greater histomorphological damage in liver kidney and gills. Manna et al., 2021, also evaluated the biosafety of administrating oxytetracycline in doses ranging from 80 to 800 mg kg^{-1} in *Pangasianodon hypophthalmus* juveniles, determining that it does not cause significant toxic effects in behavior or mortality but diminishing feed intake and hepatotoxicity in the higher doses.

It is important to highlight that there are no references of studies evaluating neither non-subtherapeutic doses nor the selection pressure exerted by these antimicrobials.

The administration of antibiotics to animals destined for human consumption should be evaluated for the risk of the presence of residues in meat. Krupesha Sharma et al., 2021, administered *Trachinotus blochii* with 1, 3, 5 and 10 times the therapeutic dose and during 10, 20 and 30 days, detecting concentrations way too low from cut points accepted for muscle.

The method used did not detect antimicrobial residues, however, Mensah et al., (2019) stablished that the presence of such substances in muscle depends not only on the dose and time of exposure but also on the fish species and even their age. Thus, in following stages of these investigation line, we propose to evaluate longer periods of administration and in different species and ages.

5. Conclusions

All these results demonstrate that the administration of subtherapeutic doses of oxytetracycline increases significantly mean weight and standard length in *Piaractus mesopotamicus* juveniles, with no modifications in survival when compared with control. Besides, its administration in the doses and periods and using the method herein stablished does not induce the appearance of drug residues in meat samples, using the detection method used in this study. On the other hand, it was demonstrated that the use of feed added with subtherapeutic doses of oxytetracycline and enrofloxacin induces a significant increase in the numbers and proportions of resistant *Enterobacteriaceae* and non-fermentative Gram-negative bacilli and aerobic bacteria in the intestinal content. However, it is important to highlight that these significant increases are found sooner in animals treated with oxytetracycline, indicating that this drug exerts a selection pressure sooner than enrofloxacin.

Thus, it is stablished that the use of oxytetracycline as growth promoter has a benefit over some biometrical parameters. Though, as stated by the one health principles, it represents a potential risk, not only for human health but also for animals and environmental welfare, for

increasing the number of antibiotic resistant bacteria that are going to be either liberated into water courses or, even worst, delivered to consumers.

Funding

This work was supported by the Fondo para la Investigación Científica y Tecnológica [grant number PICT-2016-3202]; and the Secretaría General de Ciencia y Técnica de la Universidad Nacional del Nordeste [grant number PI17B004].

CRediT authorship contribution statement

Valeria I. Amable: Validation, Investigation. **María J. Valdéz Amarilla:** Validation, Investigation. **Paula L. Salas:** Validation, Investigation. **Jorge A. Mendoza:** Resources. **Sofía Lizardo Falcón:** Resources. **Silvia I. Boehringer:** Writing – review & editing, Project administration, Funding acquisition. **Sebastián Sánchez:** Methodology, Software, Formal analysis. **Marcos G. Guidoli:** Conceptualization, Writing – original draft, Visualization, Supervision.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

References

Allen, H.K., Donato, J., Huimi Wang, H., Cloud-Hansen, K.A., Davies, J., Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. Nat. Rev. Microbiol. 8, 251–259. https://doi.org/10.1038/nrmicro2312.

de Alcântara Rodrigues, I., Gomes Ferrari, R., Nunes Panzenhagen, P.H., Borges Mano, S., Conte-Junior, C.A., 2020. Antimicrobial resistance genes in bacteria from animal-based foods. Adv. Appl. Microbiol. 112, 143–183. https://doi.org/10.1016/bs.aambs.2020.03.001.

Esiobu, N., Armenta, L., Ike, J., 2002. Antibiotic resistance in soil and water environments. Int. J. Environ. Health Res. 12 (2), 133–144. https://doi.org/10.1080/09603120220129292.

Food and Agriculture Organization (FAO), 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action, Rome. https://doi.org/10.4060/ca9229en (accessed 26 August 2021).

Griboff, J., Carrizo, J.C., Bonansea, R.I., Valdés, M.E., Wunderlin, D.A., Amé, M.B., 2020. Multiantibiotic residues in commercial fish from Argentina. The presence of mixtures of antibiotics in edible fish, a challenge to health risk assessment. Food Chem. 332, 127380. https://doi.org/10.1016/j.foodchem.2020.127380.

Kondera, E., Bojarski, B., Lugowska, K., Kot, B., Witeska, M., 2020. Effects of oxytetracycline and gentamicin therapeutic doses on hematological, biochemical and hematopoietic parameters in *Cyprinus carpio* juveniles. Anim. 10 (12), 278. https://doi.org/10.3390/ani10122278.

Krupesha Sharma, S.R., Sumithra, T.G., Gangadharan, S., Shahansha, A., Sanil, N.K., Ashok Kumar, K., Patil, P.K., 2021. Evaluation of biosafety and tissue residue of oxytetracycline in juvenile snubnose pompano, *Trachinotus blochii* along with in vitro efficacy against fish pathogens. Aquac. 533, 736184 https://doi.org/10.1016/j.aquaculture.2020.736184.

Li, H., Wang, W., Mai, K., Ai, Q., Zhang, C., Zhang, L., 2014. Effect of dietary olaquindox on the growth of large yellow croaker (*Pseudosciaena crocea R.*) and the distribution of its residues in fish tissues. J. Ocean Univ. China 13, 820–824. https://doi.org/10.1007/s11802-014-2256-7.

Li, D., Wang, G., Du, L., Zheng, Y., Wang, Z., 2022. Recent advances in intelligent recognition methods for fish stress behavior. Aquac. Eng. 96, 102222 https://doi.org/10.1016/j.aquaeng.2021.102222.

Limbu, S.M., Chen, L., Zhang, M., Du, Z., 2021. A global analysis on the systemic effects of antibiotics in cultured fish and their potential human health risk: a review. Rev. Aquac. 13, 1015–1059. https://doi.org/10.1111/raq.12511.

Luchini, L., 2008. Piscicultura rural en estanques. Conocimientos básicos y prácticos para técnicos y productores de baja y mediana producción. In: Subsecretaría de Pesca y Acuicultura, Secretaría de Agricultura, Ganadería, Pesca y Alimentos.

Manna, S.K., Bera, A.K., Das, N., Bandopadhyay, C., Baitha, R., Ghadei, S.S., Patil, K., 2021. Determination of biosafety of the antibiotic oxytetracycline hydrochloride in *Pangasianodon hypophthalmus*. Aquac. Res. 52 (6), 2470–2480. https://doi.org/10.1111/are.15096.

Mensah, S.E.P., Dakpogan, H., Aboh, A.B., Chabi Sika, K., Ableto, M., Adjahoutonon, K.Y. K.B., Mensah, G.A., 2019. Occurrence of antibiotic residues in raw fish *Clarias gariepinus* and *Oreochromis niloticus* from intensive rearing system in Benin. Veterinaria Vet. Fac. Sarajevo. 68 (2), 91–94. https://hal-anses.archives-ouvertes.fr/anses-02336074.

Navarrete, P., Mardones, P., Opazo, R., Espejo, R., Romero, J., 2008. Oxytetracycline treatment reduces bacterial diversity of intestinal microbiota of Atlantic salmon. J. Aquat. Anim. Health 20 (3), 177–183. https://doi.org/10.1577/H07-043.1.

Panné Huidobro, S., 2020. Producción de Acuicultura Destinada al Consumo Humano Durante el año 2019. Ministerio de Agricultura, Ganadería y Pesca Argentina. https://www.magyp.gob.ar/sitio/areas/acuicultura/estadisticas/_archivos/000000_Producci%C3%B3n/190000_Producci%C3%B3n%20de%20Acuicultura%20destinada%20al%20consumo%20en%20Argentina%20-%202019.pdf.

Samira, S., Guichard, B., 2009. Bacterial antibiotic resistance in aquaculture. Aquac. Microbiol. Biotechnol. 1, 133–159.

Sapkota, A., Sapkota, A.R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., Lawrence, R., 2008. Aquaculture practices and potential human health risks: current knowledge and future priorities. Environ. Int. 34, 1215–1226. https://doi.org/10.1016/j.envint.2008.04.009.

Vargas, J., Auro, A., Fragroso, M., Ocampo, L., 1993. Evaluación de bacitracina zinc en tilapia hibrida (*Oreochromis sp.*). Vet. Mex. 24 (1), 31–35. https://www.medigraphic.com/pdfs/vetmex/vm-1993/vm931f.pdf.

Zhang, G., Feng, J., 2016. The intrinsic resistance of bacteria. Yi Chuan. 38 (10), 872–880. https://doi.org/10.16288/j.yczz.16-159.