Original Article

Anaesthetic Effect of Propofol on Rainbow Trout (Oncorhynchus Mykiss) in Two Different Concentrations

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Abstract

Objective- The study aims to determine efficacy of propofol as an immersion agent to induce anesthesia in rainbow trout (Oncorhynchus mykiss).

Design- Experimental study.

Animals- 36 healthy rainbow trout

Procedure- Trouts were sorted randomly in two groups, 18 fish each one. Both groups were anesthesized by bath, one of them with 2.5 mg/l, the other one at 5 mg/l concentration. During the experiment, basal respiratory rate, partial and total equilibrium loss, time to anesthesia, anaesthesia respiratory rate and manipulation response were recorded.

Results- Induction and recovery times as well as behavioural response were recorded, being significantly affected by propofol concentration (P <0.01). After exposure to 2,5 and 5 mg/l, fishes reached stage 3 anesthesia in 4,99 ± 1,07 and 2,81 ± 0,71 minutes respectively. Recovery time were 3,59 ± 1,44 for 2,5 mg/l and 7,49 ± 3,02 minutes for 5 mg/l. After the experiment, the fish remained for 48 hours in a pond attached to the unit, without any death. This study, showed the behavioural response of rainbow trout to anaesthesia as well effectiveness of propofol as anaesthetic. Propofol induce safe dose dependent anaesthesia, being useful for different tasks related to the management of culture trout, as it meets the criteria established in aquaculture use.

Conclusion and Clinical relevance- The results of the present work provide data to be used in surgical procedures and containment maneuvers in the different practices performed in fish farming.

Key words- Propofol, Rainbow trout, Anaesthesia

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Introduction

Intensification of aquaculture practices has led to increased levels of stress in fish. Handling, weighing, sorting by size, confinement, farming density, transportation and lower water quality acts as stressors. These stress factors induce changes in plasma cortisol, lactate, plasma chloride and sodium, glucose, lymphocyte count and feeding reduction, increasing susceptibility to diseases and mortality with significant losses of resources and productivity.1,2 In this context depressant drugs are considered as an advance in good management practices, balancing neuroendocrine and physiological changes that negatively affect the performance and the survival of fishes.3-7 Benzocaine, 2-phenoxyethanol, tricaine, eugenol, etomidate, ketamine, quinaldine, metomidate, xylazine and others are used. Their effects range from mild
sedation, reducing stress during handling and non-invasive procedures (artificial reproduction, induction of spawning, weight gain and body length, transport), to total anesthesia to abolish pain in surgical procedures and complex interventions (biopsies, reproductive techniques). In general they have demonstrated their effectiveness with advantages and limitations according to the species, however, there is no agent that is suitable for all species.

Therefore, there is a demand for new options combining effectiveness and safety. The first report on the anesthetic efficiency of propofol (2,6-diisopropylphenol) was published in 1973 in an experiment in rats and in 1977 it was used as an anesthetic agent in humans. It presents a brief onset of action, accelerated metabolism, rapid recovery after administration in bolus doses or by continuous infusion, and minimal side effects. In addition, it does not present a cumulative effect like thiopental.

Propofol depressant action involves a positive modulation of the gamma-aminobutyric acid neurotransmitter inhibitory function (GABA), through GABAA receptors.

Although propofol is not frequently used in fish, there are references in shark (Chiloscyllium plagiosum), dolphin (Tursiops truncatus), Sturgeon (Acipenser oxyrinchus), herbivorous carp (Ctenopharyngodon idella), catfish (Rhamdia quelen), tilapia (Oreochromis niloticus), ornamental fish Carassius auratus, benny (Barbus sharpeyi), and zebrafish (Danio rerio). In relation to rainbow trout (Oncorhynchus mykiss) only one pharmacokinetic study is reported.

The efficiency and safety of any anesthetic agent may vary according to species, stage of life and environmental conditions. This implies the need for further studies to establish the appropriate operating conditions and comparative advantages of propofol respect to other anesthetics. The context where the drug seems promising for sedation of fish is before transport, since there is evidence that it prevents peak of cortisol levels and preserves hematological, morphological and biochemical stability. Moreover it has a rapid metabolism, an extremely useful factor in the control of anesthesia. This characteristic has been demonstrated in rainbow trout, where absorption and elimination rates were high, with a half-life of 1.1 h at 17 °C.

On these premises, the objective of the work was to evaluate anesthesiological and physiological variables after propofol bath administration in two concentrations in rainbow trout, a species for which no information is available.

Materials and methods

Animals

The study was carried out in a fish farming establishment located in Las Tapias, Córdoba (Argentina). Juvenile trout (O. mykiss) (n = 36) were randomly extracted from an intensive culture unit, clinically healthy, of both sexes, with an approximate weight of 300 g and total length of approximately 28 cm.

Drug and Equipment

Anesthetic used was Propofol 1% (Abbott®, Argentina). Water pH was recorded with pH meter AltroniXTPA II, dissolved oxygen and temperature with an Oximeter Lutron DO / 5510. For weight register was used an electronic scale OHAUS Explorer®, 0.001gr sensitivity and an ichthyometer to obtain lengths of each fish.

Experimental design

Fish were divided in two groups of 18 animals each randomly. Group A and B were anesthetized by bath method with concentrations of 2.5 and 5 mg/l of propofol, respectively. Since there are no previous anesthesiological studies in rainbow trout, an intermediate dose was used in other species.

Three plastic containers of 30 liters each one, were placed in order to facilitate fish handling to minimize stress due to manipulation and the time spent outside the water. Each container was loaded with water from the supplying canal of the establishment, to maintain water conditions such as temperature and oxygenation, parameters of importance for fish metabolism and duration of anesthesia.

Containers number 1 and 3 were drug free. In container number 2, propofol was added directly into the water, without addition of other substances in order to obtain the established concentrations for each experimental group.

Anesthesia evaluation

The study sequence consisted extracting each fish from the culture pond and depositing it in container number 1 to record the basal respiratory rate, when the animal adopts normal swimming activity. Then it was transferred to container number 2 with propofol in the concentration to be evaluated, recording absence or presence of excitation and partial equilibrium loss and anesthesia times, according to the protocol proposed by Ross and Ross, 2008, Treves-Brown, 2000 and Velisek et al., 2007:

1. Light sedation - Slight loss of reactivity to external stimuli, equilibrium.
2. Deep sedation - Loss of reactivity to external stimuli except strong pressure; slight increase in opercular ventilation rate; normal equilibrium.
3. Partial loss of equilibrium - Partial loss of muscle tone, erratic swimming; reaction only to strong tactile and vibrational stimuli.
4. Total loss of normal balance - Total loss of muscle tone and equilibrium; rapid opercular ventilation (slow with some agents) reaction only to deep pressure stimuli.

When anesthesia was achieved, the respiratory rate was recorded and the animal was weighed and measured and then introduced to the container 3 to record the recovery time (recovery of normal swimming activity).

In order to maintain stable experimental conditions, the water in the containers and the anesthetic preparation were renewed after the passage of 6 fish.

Results

Average fish weight was 274.1 ± 28.7 g for group A and 304.5 ± 51.1 g for group B with no significantly differences (P > 0.05) between groups.

Physico-chemical characteristics of the water in the establishment were within the limits required for production, according to Blanco Cachafeiro, 1984, Mendoza-Bojorquez and Palomino-Ramos, 2004; temperature, pH and oxygenation remained stable throughout the course of work (Table 1).

There were significant differences (P < 0.01) in partial equilibrium losses of 1.03 ± 0.32 and 0.42 ± 0.22 minutes in 2.5 and 5 mg/l, respectively (Table 2, Figures 1 and 2). Time to anesthesia (stage of anesthesia 4) was significantly higher (P < 0.05) in the fish of group A, compared to the fish of group B and the average were 4.98 ± 1.06 and 2.81 ± 0.81 minutes, respectively. Regarding recovery time and the influence on respiratory activity (Table 2, Figure 3 and 4), the differences were also significant between both groups (P < 0.01). In 5 mg/l concentration, three fish exhibited slight initial excitation of short duration, characterized by rapid and erratic swimming.

At the end of the experiment, fishes were housed for 48 hours in a pond, with no changes in behavior or morality.

Statistical analysis

Table 1 data (water physico-chemical characteristics and anesthesiological parameters) are reported as mean (± SD). Levene’s test was used to test variance homogeneity and normality of data was tested using Shapiro–Wilk test. A non-parametric analysis was performed using the Mann-Whitney and U-test to verify the existence of significant differences between groups weight, partial and total time to equilibrium lose, time to anesthesia, recovery time and respiratory rate in anesthesia.

Table 1. Water physico-chemical characteristics for O. mykiss optimal development and growth.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean ± SD</th>
<th>Optimal value</th>
<th>Permissible range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>15.30</td>
<td>15.20</td>
<td>15.10</td>
<td>15.50</td>
<td>15.27 ± 0.17</td>
<td>15</td>
<td>9-17</td>
</tr>
<tr>
<td>pH</td>
<td>6.68</td>
<td>6.65</td>
<td>6.63</td>
<td>6.42</td>
<td>6.59 ± 0.11</td>
<td>7</td>
<td>6.5-9.5</td>
</tr>
<tr>
<td>Dissolved O2 (mg/l)</td>
<td>9.30</td>
<td>9.27</td>
<td>9.27</td>
<td>9.08</td>
<td>9.23 ± 0.10</td>
<td>8</td>
<td>6-10</td>
</tr>
</tbody>
</table>

References: 1,2,3,4 records of temperature, pH and oxygen concentration during the experiment

Table 2. Anesthesiological parameters evaluated in O. mykiss at different concentrations of propofol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2.5 mg/l</th>
<th>5 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (± S.D.) (grs)**</td>
<td>274.1 ± 28.7</td>
<td>304.5 ± 51.1</td>
</tr>
<tr>
<td>Partial equilibrium loss (minutes) *</td>
<td>1.03 ± 0.32</td>
<td>0.42 ± 0.22</td>
</tr>
<tr>
<td>Total equilibrium loss (minutes) *</td>
<td>3.19 ± 1.11</td>
<td>1.28 ± 0.45</td>
</tr>
<tr>
<td>Time to anesthesia (minutes)*</td>
<td>4.99 ± 1.07</td>
<td>2.81 ± 0.71</td>
</tr>
<tr>
<td>Recovery time (minutes)*</td>
<td>3.59 ± 1.44</td>
<td>7.49 ± 2.54</td>
</tr>
<tr>
<td>Basal respiratory rate (mov / min)</td>
<td>128.88 ±14.36</td>
<td>123.77 ± 9.62</td>
</tr>
<tr>
<td>Anesthesia respiratory rate (mov / min)*</td>
<td>74.00 ± 9.62</td>
<td>57.11 ± 9.48</td>
</tr>
<tr>
<td>Reached stage</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

* Significantly differences P < 0.01 between groups. ** No significantly differences (P >0.05) between groups (mov / min)
Discussion

Different authors propose that the ideal anesthetic should fulfill requirements such as rapid induction without hyperactivity, gradual recovery, absence of residues and toxicity, low cost and rapid metabolism and excretion of the organism. From these Llanos and Scotto, 2010, proposed three criteria for an anesthetic to be used in aquaculture: Effective, safe and economical. Efficacy is defined as the ability to produce a state of anesthesia in a period less than or equal to three minutes and recovery of normal swimming excitation in less than 10 minutes. If the latter criterion was considered, in 2.5 mg/l anesthesia it was achieved in 4.98 ± 1.06 minutes, higher than suggested but much less than the 13.4 ± 3.3 minutes reported in Koi carp in the same concentration. In Grass carp 2, 4 and 6 mg/l baths, had recovery times of 5, 16 and 10 minutes respectively and in goldfish were 8.52 ± 0.82 minutes in 7 mg/l. In rainbow trout, results showed a significant decrease in the rate of opercular movements in both concentrations, in contrast to koi carp, where the respiratory rate in anesthesia was not significantly modified. In relation to other studies in rainbow trout with other anesthetics (eugenol) it was observed that propofol maintained the ideal anesthetic properties just like eugenol, only requiring a lower dose, but the induction and recovery times were kept within of what was required in the aquatic systems, besides being safe without adverse effects after 48 hours post administration, and no dead fish. It was shown that propofol was an effective agent to
achieve anesthesia, regardless of the concentration used, although the higher the concentration, the shorter the induction time for anesthesia, and the longer the recovery time. For its possible use in aquatic species, doses lower than 2.5 mg/L must be considered, in order not to reach anesthesia conditions, simply reassuring the fish to optimize handling. Finally, the recorded results determined, as well as its pharmacokinetics\textsuperscript{15}, that propofol was a useful, safe and effective depressant drug for different tasks related to the management of farmed trout, since it met the established criteria for use in aquaculture.

**Acknowledgments**

We appreciate the staff of the Boca de Río Fish Farm, Córdoba, República Argentina. for their help

**Conflicts of interest**

None.

**References**

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چکیده
تأثیر بیهوشی پروپوفول در ماهی قزل‌الا (Oncorhynchus mykiss) در دو غلظت متفاوت

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هدف
هدف از این مطالعه تعیین تأثیر پروپوفول به عنوان داروی محلول در آب به منظور القای بی‌هوشی در ماهی قزل‌الا بود.

طرح
مطالعه تجربی

محیوت
روش کار- ماهی‌های قزل‌الا به طور تصادفی به دو گروه 18 تاپی تقسیم شدند. گروه 1 از ماهی‌های با استفاده از همام، دارویی به ترتیب با غلظت‌های 0/21 و 5/5 میلی گرم در لتر به‌طور تصادفی رسیده و ریسپرسیون میزان تیره‌سازی ایجاد گردید. زمان بی‌هوشی، زمان تنفس در خلاء بی‌هوشی و بازگشت بی‌هوشی برای غلظت‌های 25/5 میکروفریل در لتر برنامه‌ریزی 7 دقیقه و 74 ساعت در انتهای مطالعه ماهی‌ها به مدت 48 ساعت مورد استخر انجام و به هفته رونمایی می‌شود.

نتایج- زمان‌های القای و بازگشت از بی‌هوشی به‌روز گردید و پس از گرار گیری در معرض دارو با غلظت‌های 0/21 و 5/5 میلی گرم در لتر، ماهی‌ها به ترتیب بعد از 44/44 ± 1/29 دقیقه وارد مرحله 3 بی‌هوشی شدند. زمان بی‌هوشی 38/42 ± 1/47 دقیقه بود. پس از انتهای مطالعه ماهی‌ها به مدت 74 ساعت در رونق مناسب به اکوسیستم انباشته شدند.

نتیجه‌گیری و کلیدی- این مطالعه نشان داد که ماهی‌های قزل‌الا به بی‌هوشی با پروپوفول نسبت ویژه به دست سبب القای می‌شود و به‌طور کلی، پروپوفول به‌عنوان داروی محلول در آب مورد استخر استفاده می‌شود.