RESUMO.- [Teste lacrimal de Schirmer modificado e tonometria de rebote em papagaio-verdadeiro (Amazona aestiva)]. O objetivo deste estudo foi descrever o teste lacrimal de Schirmer modificado (TLSm), a pressão intraocular (PIO) pela tonometria de rebote e o comprimento da fissura palpebral (FP) do papagaio-verdadeiro (Amazona aestiva). Foram avaliados 35 papagaios adultos e saudáveis, provenientes de um Criadouro Conservacionista do Brasil. Apojo avaliação clínica e laboratorial, as aves foram fisicamente contidas para aferição, em ambos os olhos, do TLSm, da PIO pela tonometria de rebote e do comprimento da FP utilizando-se um paquímetro digital. Valor médio do TLSm foi 6.2±0.1mm/min e da PIO foi 6.4±0.1 mmHg, enquanto a aferição da FP foi 10.1±0.1 mm. Uma correlação moderada foi observada entre TLSm e a FP para olho direito (OD) (ρ=0.14) e olho esquerdo (OE) (ρ=0.20). Os resultados podem servir como valores de referência para testes oftalmicos para A. aestiva.

TERMOS DE INDEXAÇÃO: Schirmer tear test, tonometry, Amazon parrot, Amazona aestiva, birds, lacrimal production, intraocular pressure, palpebral fissure length, wild animals.

INTRODUCTION

The blue-fronted Amazon parrot (Amazona aestiva) measures 35-37 cm in length and weighs approximately 400 g, reaching sexual maturity of age at five years. Individuals of this species have a predominant green color, yellow head with blue forehead, and black feet and beak (Forshaw 2010). Because of their vocalization skills similar to speech and interaction with humans, it is bred as a pet (Godoy 2007). Birds are considered animals with great visual acuity, which are important factors for breeding, feeding and flight (Hart 2002, Reuter et al. 2011, Williams 2012, Rauscher et al. 2013). The ophthalmic reference values used for the mSTT and rebound tonometry in these animals lack inter- and intraspecies information.

Due to the lack of specific data for A. aestiva, we aimed to establish normal parameters for modified Schirmer tear test (mSTT), intraocular pressure (IOP), and palpebral fissure length (PFL). Also, to verify any correlation among mSTT and PFL.
MATERIALS AND METHODS

Ethics statements. The study was approved by the Ethics Committee for Animal Use of the University of Brasilia (44763/2014). In addition, it was conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Animals. Thirty-five blue-fronted Amazon parrots (70 eyes), adults of undetermined sex, from a conservation breeding center accredited by IBAMA (Brazilian Institute of the Environment and Renewable Natural Resources), in the Central-West region of Brazil, were used in this research. The animals were phenotypically similar, with an average weight of 400 g (300-420 g).

The birds were captured with the help of nets and kept under physical restraint. The evaluations were performed on different animals per week, between 08:30 and 16:30 h, with an average temperature of 28°C (18-29°C), and relative humidity of 62% (source: Clima Tempo). The parrots underwent clinical and laboratory evaluation (hematology and serum chemistry), followed by ophthalmic examination. Animals with visually detectable ocular abnormalities or clinical changes were not included in the study. The animals were subjected to inspection of the ocular surface and anterior segment structures by slit-lamp biomicroscopy (Kowa-SL®; Kowa, Tokyo, Japan).

Ophthalmic parameters. All ophthalmic parameters were determined by the same investigator, in both eyes. The lacrimal production was measured by modified STT (mSTT) (Schirmer Tear Test®, Ophthalmics Fórmulas, São Paulo, SP, Brazil) (Fig.1A), IOP was determined by rebound tonometry (TonoVET®, Jorgensen Labs, Loveland, CO) (Fig.1B), and PFL was measured with a digital caliper (150 mm capacity with precision/accuracy: ±0.01 mm) (Fig.1C).

Modified STT was carried out using standardized strips cut in half, reducing the width of the 5mm strip to 2.5 mm. The modified strips were placed into the lower conjunctival sac and kept for 60 seconds. The wetted length was immediately recorded. Tonometry was performed with the bird held in a vertical position, avoiding excessive pressure to the cervical region, to prevent iatrogenic IOP changes. Tono Vet® measurements were obtained once in each bird with the P setting, and only readings with no error bar were accepted.

A digital caliper was used to measure the horizontal length of the eyelid from lateral to medial commissure (palpebral fissure length), and the lids were kept closed during measurement.

Statistical analysis. Statistical analysis was performed with commercially available software (GraphPad Prism®). Paired t-test was used for mSTT, IOP and PFL analyses. P-value ≤ 0.05 was considered to be statistically significant. Correlation between PFL and mSTT was evaluated by Pearson correlation test (p≤0.5).

RESULTS

Statistics results of mSTT, IOP and PFL are summarized in Table 1. The Pearson correlation test showed a moderate correlation between mSTT and PFL for OS (p=0.20) and OD (p=0.14). No significant differences were observed comparing results obtained for mSTT, IOP and PFL between right and left eyes (p>0.05).

Table 1. Modified Schirmer Tear test (mSTT), intraocular pressure (IOP) and palpebral fissure length (PFL) values of Amazona aestiva (*Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>mSTT (mm/min)</th>
<th>IOP (mmHg)</th>
<th>PFL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td>6.4 ± 3.8 (OD)</td>
<td>6.3 ± 1.8 (OD)</td>
<td>10.1 ± 1.0 (OD)</td>
</tr>
<tr>
<td></td>
<td>6.5 ± 4.3 (OS)</td>
<td>6.1 ± 2.0 (OS)</td>
<td>10.2 ± 0.8 (OS)</td>
</tr>
<tr>
<td></td>
<td>6.2 ± 0.1*</td>
<td>6.4 ± 0.1*</td>
<td>10.1 ± 0.1*</td>
</tr>
</tbody>
</table>

DISCUSSION

Prior to conducting this research, a pilot study was performed. It was not feasible to use the standard STT strip in the conjunctival sac of the lower eyelid, although Storey et al. (2009) have been successful using this method in assessing the parrot species A. ventralis. Even after inserting the strip into the conjunctival sac, the birds did not allow the strip to remain for the time required for tear measurement, probably due to the discomfort caused by the standard-sized test strip. It was found that the modified strip, as previously described (da Silva et al. 2013), was tolerated by the animals, without causing excessive stress. The STT has shown restrictions when used in small eyes (Willis & Wilkie 1999, Müller et al. 2010, Beckwith-Cohen et al. 2015) which also was observed in the present study (Lange et al. 2012, Da Silva et al. 2013). According to Williams (2012), the use of a modified strip (cut in half with width of 2.5 mm) is a viable option in species of birds with small eyes.

Meekins et al. (2015) utilized the mSTT strip in flamingo eyes, inserting it in the temporal canthus with the help of palpebral forceps, because they had difficulties placing the standard strip in the bottom of the conjunctival sac. The values found by these authors were higher (12.3±4.5 mm/ min) compared to parrots.

Storey et al. (2009) described values of 7.9±2.6 and 5.1±3.3 mm/min for STT-1 and STT-2, respectively, evaluating A. ventralis parrots, where the values obtained for STT-1 were above those measured for A. aestiva. Methodological modifications certainly affect the results obtained and should be considered in the interpretation of the findings, besides anatomic and physiologic characteristics of the species studied. One can also consider that using a narrower strip means a smaller contact surface between the filter paper and the ocular surface (Meekins et al. 2015), resulting in less moistening. It should be noted that the A. aestiva sample studied was phenotypically similar, and a substantial number of animals were assessed for this species, enhancing the reliability of the results.

Fluctuations in tear production are documented to be influenced by age, time of year, time of day and environmen-
tual conditions (Wojick et al. 2013). In this study, tests were performed under the same conditions and time of year. It is also noteworthy that anatomic characteristics such as the position of the lacrimal gland and Harderian gland, which are closer to the nasolacrimal drainage system, can interfere with the moistening of the strip in these animals.

Given the difficulties described for the use of the STT strip (Willis & Wilkie 1999, Müller et al. 2010, Beckwith-Cohen et al. 2015) or even mSTT strip (Williams 2012, Kern & Colitz 2013, Somma et al. 2015, Meekins et al. 2015), some authors suggest the use of the phenol red test (Willis & Wilkie 1999, Kern & Colitz 2013, Beckwith-Cohen et al. 2015). Although this test is well tolerated by birds (Bliss et al. 2015), low reproducibility has been reported in large Psittaciformes (Holt et al. 2006), and better PRT reproducibility on A. ventralis (Storey et al. 2009).

Although application tonometry is considered an accurate and reliable method to measure IOP, it cannot be performed in eyes of animals with small corneal diameter (Rusansen et al. 2010, Barsotti et al. 2013) or it is not comfortable, as it was observed with the Amazon parrot. Previous tonometry studies using the Goldmann tonometer have been reported for birds of prey (11-16 mmHg) and parrots (20-25 mmHg). A study of 31 birds of prey using the rebound tonometer obtained an average of 9 mmHg, and in large birds, values close to 40 mmHg (Bayon et al. 2007).

Rebound tonometry was introduced in the past decade in veterinary medicine, and has been used in poultry. According to Meekins et al. (2015), interference by the movement of the nictitating membrane in birds is less using rebound compared to application tonometry. Studies comparing rebound tonometry and a manometer showed that, in some birds, IOP was overestimated, while it was underestimated in others. However, even with these variations, the values obtained are considered useful for clinical application (Jeong et al. 2007, Reuter et al. 2010, Wojick et al. 2013). To minimize such oscillations, it is suggested that a single method be used for a particular species (Meekins et al. 2015).

In view of the regularity of the values obtained and the number of animals evaluated, under the conditions in which the study was conducted, the resulting data can be used for reference values for tear production and IOP in the blue-fronted Amazon parrot.

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Conflict of interest statement.- The authors have no competing interests.

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