B-1342
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RESEARCH ARTICLE Indian Journal of Animal Research, Volume Issue : ()

Obtaining Ejaculates through Operant Conditioning of the Golden Eagle (*Aquila chrysaetos*)

J.A. Herrera-Barragán[1], S.S. Landa-García[2], J.J. Pérez-Rivero[1], J.M. Huitrón-Rodríguez[3], M.Á. León-Galván[4], A. Guzmán-Sánchez[1], C.S. Reyes-Mendoza[5]

10.18805/IJAR.B-1342

ABSTRACT

Background: The golden eagle, *Aquila chrysaetos* is one of the 65 species of birds of prey found in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora. It has been estimated that the world population is 250 thousand specimens. The operant conditioning to obtain ejaculates of *A. chrysaetos* is described and to contribute to assisted reproduction protocols in captivity.

Methods: In the reproductive season, the procedure was performed twice a week on 5 adult birds, in order to obtain ejaculates.

Result: The study lasted 17 weeks, in which 160 contacts were made in total. It was possible to obtain 20 ejaculates, which were obtained from 3 birds. 4% success was achieved in obtaining ejaculates. Additionally, behaviors of clinical utility for diagnosis, care and well-being were conditioned in birds. The above should be considered in assisted reproduction programs, since, as demonstrated by the results of this study, not all the birds provide sufficient ejaculates, for which reason, the evaluation of the individuals that present the greatest advance in operant conditioning to include them in assisted reproduction programs.

Key words: Captivity, Conservation, Falconiforms, Spermiogram.

INTRODUCTION

Currently at least 10% of the 300 species of the falconiform order are threatened worldwide (Dogliero *et al.* 2016). Mexico is a very diverse country, in which there are 1096 species of birds, 88 of them are birds of prey, among which is the golden eagle, *Aquila chrysaetos* considered one of the 65 species of birds of prey that are in category of risk at national level according to NOM-059-SEMARNAT-2010, being classified in appendix II of CITES (CITES 2015). The world population of *A. chrysaetos* is estimated at 250 thousand individuals. The number of individuals residing in Mexico is unknown to date; however, approximately 50 nests have been documented in the wild and more than 60 specimens in captivity (CONABIO 2011). Birds play a crucial role in food webs of ecosystem and are known as 'bio-indicators' a sensitive to minor environmental changes (Debnath *et al*, 2018).

Due to the limited knowledge of the species, breeding in captivity is far from being successful (Blanco *et al*. 2009; Dogliero *et al.* 2016), in addition to possible consequences that can be generated by stress in captivity, such as incompatibility between couples and aggressiveness, situations that cause inability to copulate naturally or asynchronous copulations (Blanco *et al.* 2009; Dogliero *et al.* 2016). Therefore, efforts have been made to incorporate artificial insemination (AI) in bird conservation programs; however, problems have been found to carry it out, derived from inefficiency in the training of birds for the semen collection (Blanco *et al.* 2009; Dogliero *et al.* 2016). In wild animals, unlike domestic species, simple contact with humans is not sufficient for imprinting and/or training to carry out diagnostic and therapeutic procedures (McGreevy *et al.* 2014; Beulah *et al*, 2020). Operant conditioning (OC) explains that the consequences of a behavior are what determine whether or not it will be reissued. If a behavior has positive consequences, there will be a greater probability that it will happen again and/or its frequency will increase (Huston *et al,* 2013). An example of the above has been described in Rhesus monkeys, where the use of OC with

[1]Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-Xochimilco. Calzada del Hueso 1100, col. Villa Quietud, Coyoacán, 04960, CDMX, México.
[2]Maestría en Biología de la Reproducción Animal, Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco 186, Leyes de Reforma, 09340 CDMX, México.
[3]Maestría en Ciencias Agropecuarias, Universidad Autónoma Metropolitana-Xochimilco. Calzada del Hueso 1100, col. Villa Quietud, Coyoacán, 04960, CDMX, México.
[4]Departamento de Biología. Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco 186, Leyes de Reforma, 09340 CDMX, México.
[5]Parque Reino Animal. Camino a Belén, Santa María, Oxtotipac, Otumba, C.P. 55908, Edo, Méx., México.

Corresponding Author: J.J. Pérez-Rivero, Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-Xochimilco. Calzada del Hueso 1100, col. Villa Quietud, Coyoacán, 04960, CDMX, México.
Email: kgobebg@unisa.ac.za

How to cite this article: Herrera-Barragán, J.A., Landa-García, S.S., Pérez-Rivero, J.J., Huitrón-Rodríguez, J.M., León-Galván, M.Á., Guzmán-Sánchez, A. and Reyes-Mendoza, C.S. (2021). Obtaining Ejaculates through Operant Conditioning of the Golden Eagle (*Aquila chrysaetos*). Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-1342.

Submitted: 27-12-2020 **Accepted:** 19-02-2021 **Online:** 22-04-2021

positive reinforcement was successfully used to reduce aggressive behaviors against other animals and personnel who handle them (Miniera *et al.* 2011); it has also been used for these animals to allow clinical maneuvers and to safely obtain ejaculates from the specimens (Clay *et al.* 2009; O´Brien *et al.* 2009; Schapiro *et al.* 2003).

Therefore, the objective of this work is to incorporate OC as a tool to facilitate the handling *A. chrysaetus* specimens in order to obtain ejaculates safely for the animals and the personnel that handle them.

MATERIALS AND METHODS

The work was carried out in the Neotropical region of Mexico, during breeding season 2020, from January to April (Espinosa *et al.* 2001). Five adult males in captivity, 4 to 12 years old, were included. The birds were housed in individual enclosures, with exposure to natural events such as rain, heat, cold and winds of different intensities, as well as the presence of a female of the same species per male, which was separated from the male by a barrier according to the enclosure of each one. The diet of the birds was integrated by rabbit, mouse and chicken meat, providing a total of 109 kcal/kg (Hand *et al.* 2000).

Birds collections locations

Parque Reino Animal: DGVS-ZOO-P-0074-03 -MEX
Club Futbol América / Aguilario Club América: DGVS-PIMVS-CR-IN-1847-CDMX/18
Aviario DILAJESH: DGVS -CR-IN-917-MEX/06

Conditioning

In each location, a specific physical area was defined to carry out the conditioning management of each birds. The five birds had previous training in behaviors used for falconry, such as staying on a glove on a person's arm and vision covering. Once this behavior was confirmed in all the birds during 17 weeks, they proceeded to carry out their conditioning to obtain ejaculates.

Tactile stimuli were performed using the dorso-ventral massage, which consisted of applying a gentle massage on the back in the head-cloaca direction, until it touched the pelvic bones, repeating it until the bird showed signs of relaxation (Fig 1), followed by a massage from the ventral area to the cloaca (Fig 2A), to finally apply light pressure, evert the cloaca (Fig 2B) and achieve ejaculation, which was collected with a graduated micro pipette (Fig 2C and D).

The conditioning sessions were carried out twice a week with a maximum of three stimuli per day per individual, without establishing schedules, so that the training was not related by the specimens to any other concurrent activity or casual event. Table 1 shows the protocol followed for the training of the specimens, establishing criteria to evaluate the progress in behavior. The conditioning of behaviors was reinforced in a positive way after each session and according to the criteria of progress, using the food that was part of their daily diet. In the case of obtaining an ejaculate, it was evaluated in a conventional way using spermatobioscopy.

Fig 1: Dorsal contact showing wing relaxation.

Fig 2: Conditioning carried out to obtain ejaculates. Cloacal contact (A), Rudder lift and cloacal pressure (B) Ejaculate collection (C and D).

(McGreevy *et al.* 2014; Villaverde-Morcillo *et al.* 2015; Herrera *et al*. 2013). Semen was discarded if contaminated with urine or fecal material (Blanco *et al.* 2009).

RESULTS AND DISCUSSION

Obtaining ejaculates

During 32 sessions in which 160 contacts were made with the specimens, a total of 480 stimuli were achieved through the dorso-ventral massage.

The progress criteria for training and obtaining semen from *A. chrysaetos* are shown in Table 1. On the other hand, general information on the golden eagles used is shown in Table 2.

Seminal indicators

The seminal parameters are shown in Table 3.

Regarding age, there was no evidence of a relationship between the age of the specimens and the number of ejaculates obtained from each one. This study demonstrates that the OC procedure allows obtaining and collecting adequate ejaculates, which can be used in assisted reproduction programs in captivity. Just as those obtained in the study carried out by Villaverde-Morcillo *et al.* (2015), in which the semen characteristics of a single specimen of golden eagle trained to allow semen recovery through cooperative copulation are described, in this study, azoospermic ejaculates were observed at the beginning and end of the reproductive season; similarly, a variation in ejaculate volume, concentration and sperm motility was observed, indicating the high variability of ejaculates of the species; In which rescued specimens that cannot be returned to the wild can be integrated (Aslan *et al,* 2018). According to Starling (2013), the effectiveness of operant conditioning is linked to the learning capacity of each subject, the ability of the trainer to apply that type of training and the routine interaction with the subject.

With the above, the importance of this work is evident, for with the correct and efficient training the selection and evaluation of the specimens that are intended to be used as breeders is allowed, without exposing at any time the integrity of the specimens or trainers, in addition of obtaining ejaculates; behaviors of clinical interest were achieved, including techniques that can help ejaculate collection more

Table 1: Progress criteria for training and obtaining *Aquila chrysaetos* semen.

Behavior	Behavioral criteria	Physiological Indicator	Advance
Desensitization /Tolerance of 3 people present	Quiet bird with no movements	Claws in gloves accommodation	10%
Tolerance/Pressure contact in the dorsal region	Bird without movement	Relaxation of wings	20%
Stimulus tolerance 1/Back massage (Fig 1)	Permanence of claws in glove	Relaxation of wings	30%
Cloacal-contact tolerance/Contact with pressure in the cloacal region.	Permanence of claws in glove	Slight front tilt	40%
Stimulus 2 tolerance/Massage in cloacal region	Permanence of claws in glove	Copulation movement	60%
Stimulation by dorso-ventral massage	Wing extension and relaxation	Wing extension and relaxation with vocalization	80%
Stimulus or arousal/Cloacal contact (Fig 2A)	Eversion of cloaca	Repeated eversion of cloaca	90%
Rudder lift and cloacal pressure(Fig 2B)	Wing extension and relaxation; Cloacal contractions	Cloacal contractions and vocalization	95%
Ejaculation	Ejaculation	Presence of ejaculate	100%

Table 2: General information on the golden eagle (*A chrysaetos)* used.

Subject number	Age(years)	Behavioral advancement(%)	No of obtained ejaculates	Obtained ejaculates(%)
1	12	100	12	60
2	8	100	5	25
3	10	100	3	15
4	10	60	0	0
5	4	40	0	0

Table 3: Seminal parameters obtained by conditioning of golden eagle (*Aquila chrysaetos*).

Parameters	Mean ± EE	Interquartile range(Q1-Q3)
Ejaculate volume (µl)	12 ± 0.36	10 to 13
Motility (%)*	75 ± 1.58	70 to 80
Sperm concentration** (30µl)	116704 ± 2569.05	112390.20 to 122901.70
Viability (%)	75 ± 1.74	67 to 79
Abnormalities (%)	2 ± 0.34	2 to 3

*Progressive motility.

**(Spermatozoids/ejaculate).

efficiently (McGreevy *et al,* 2014; Callealta *et al*, 2019). Additionally with assisted reproduction in birds, it contributes to the conservation of many species (Sami, 2015).

CONCLUSION

According to the obtained results, the degree of progress in OC is different among different animals; a specimen can be an excellent donor from which sufficient ejaculates can be obtained, which makes it ideal within an assisted reproduction program. However, there are other specimens that may have a lower capacity and require more OC sessions to donate their genetic material and even find individuals from whom no progress is obtained; therefore, it is recommended to include the largest number of birds in the OC programs to select those with the highest progress to consider them for assisted reproduction programs.

ACKNOWLEDGMENT

Omar Álvarez Jiménez/ Animal trainer / Club-América

Etics

The handling of each specimen was carried out according to the official management plan authorized by the Dirección General de Vida Silvestre (DGVS) –SEMARNAT-México.

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