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André Lucas Amaral Monteiro Rodrigues

Development of a protocol for assessing psittacine welfare: first steps

Juiz de Fora
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Dissertação apresentada ao Programa de Pós-Graduação em Biodiversidade e Conservação da Natureza da Universidade Federal de Juiz de Fora como requisito parcial à obtenção do título de Mestre. Área de concentração: Comportamento.

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RESUMO

Protocolos de avaliação de bem-estar são ferramentas práticas que visam padronizar e facilitar a avaliação do bem-estar de animais em diferentes contextos, a partir de indicadores que sejam validados cientificamente. O desenvolvimento de protocolos específicos que avaliem o comportamento natural de diferentes espécies e suas relações com o ambiente são de extrema importância para o aprimoramento da qualidade de vida de animais em cativeiro. Portanto, o objetivo do presente estudo foi elaborar um protocolo de avaliação de bem-estar de psitacídeos em cativeiro, utilizando indicadores de bem-estar validados cientificamente, tendo como base os princípios e critérios do Protocolo Welfare Quality®. Como objetivos específicos buscou-se: a) reunir uma série de indicadores (medidas) de bem-estar de psitacídeos em um instrumento de avaliação; b) avaliar a exequibilidade do protocolo por meio de sua aplicação em dois diferentes locais. O protocolo foi finalizado com um total de 71 questões, onde 19 fazem parte do questionário de manejo, 32 são medidas do recinto e 20 são medidas obtidas dos animais. As medidas englobaram manejo, gerenciamento, alimentação, saúde, condição física e comportamento. Após as aplicações nas duas localidades o protocolo foi avaliado criticamente e modificado de acordo com a necessidade. O protocolo se apresenta exequível e de fácil interpretação, podendo ser utilizado como uma ferramenta para a avaliação do bem-estar de psitacídeos cativos, avaliando diferentes aspectos do bem-estar. No presente estudo foi realizada a avaliação da exequibilidade de diferentes medidas de bem-estar. A validação do protocolo e a confiabilidade das medidas devem ser realizadas em estudos futuros.

Palavras-chave: bem-estar animal, comportamento, nutrição, Psittacidae, saúde.

RESUMO PARA DIVULGAÇÃO CIENTÍFICA

Os psitacídeos são um grupo de aves composto por papagaios, periquitos e araras. Muitos desses animais são criados em cativeiro sob condições que não os permitem ter uma boa qualidade de vida. Dessa forma, a utilização de metodologias para avaliar o bem-estar desses animais em cativeiro é de extrema importância para instituições como zoológicos e criadouros conservacionistas. Protocolos de avaliação de bem-estar são ferramentas práticas que visam padronizar e facilitar a avaliação do bem-estar de animais em diferentes contextos, a partir de indicadores que sejam validados cientificamente. O desenvolvimento de protocolos específicos que avaliem o comportamento natural de diferentes espécies e suas relações com o ambiente são de extrema importância para o aprimoramento da qualidade de vida de animais em cativeiro. Portanto, o objetivo desse estudo foi elaborar um protocolo de avaliação de bem-estar de psitacídeos em cativeiro, utilizando indicadores de bem-estar validados cientificamente, tendo como base os princípios e critérios de protocolos já desenvolvidos para outras espécies. Além disso, este estudo buscou reunir uma série de indicadores (medidas) de bem-estar de psitacídeos para formular um instrumento de avaliação e também avaliar a capacidade de aplicação do protocolo por meio de sua aplicação em dois diferentes locais. O protocolo foi finalizado com um total de 71 questões, onde 19 fazem parte do questionário de manejo da unidade avaliada, 32 são medidas obtidas através da avaliação do recinto onde esses animais estão inseridos e 20 são medidas obtidas diretament dos animais. As medidas englobaram manejo, gerenciamento, alimentação, saúde, condição física e comportamento. Após as aplicações nas duas localidades o protocolo foi avaliado criticamente e modificado de acordo com a necessidade de ajustes. O protocolo se apresenta exequível e de fácil interpretação, podendo ser utilizado como uma ferramenta para a avaliação do bem-estar de psitacídeos mantidos em cativeiro, avaliando diferentes aspectos do bem-estar.

ABSTRACT

Welfare assessment protocols are practical tools that aim to standardize and facilitate the assessment of animal welfare in different contexts, based on scientifically validated indicators. The development of specific protocols that assess the natural behavior of different species and their relationship with the environment is important for improving the quality of life of wild animals in captivity. Therefore, the aim of this study was to develop a protocol for assessing the welfare of psittacines in captivity based on the principles and criteria of the Welfare Quality® Protocol. The specific objectives were to: a) compile a series of psittacine welfare indicators into an assessment tool; b) evaluate the feasibility of the protocol by applying it in two different locations. The protocol was finalized with a total of 71 questions, 19 of which are part of the management questionnaire, 32 are environmental-based measurements and 20 are animal-based. The measurements cover management, feeding, health, physical condition and behavior. After the applications in both locations, the protocol was critically evaluated and modified. The protocol was regarded as feasible and easy to interpret, and can be used as a tool for assessing the welfare of captive psittacines, evaluating different aspects of welfare. The validation of the protocol and the reliability of the measures should be carried out in future studies.

Keywords: animal welfare, behavior, nutrition, Psittacidae, health.

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INTRODUCTION

Psittacines are one of the groups with the highest number of endangered species among birds (COLLAR *et al.*, 1994; OLAH *et al.,* 2016), with approximately one-third of their species threatened (SNYDER *et al.*, 2000; OLAHR *et al*., 2016). Habitat loss, low reproductive rates and the capture of individuals for the pet market are the main causes of parrot population decline in the wild (WRIGHT *et al*., 2001).

The beauty and vocal capacity of the various parrot species have led to a great demand for these animals on the pet market (ENGEBRETSON, 2006; WESTON & MEMON, 2009), and they are often obtained illegally (KUHNEN & KANAAN, 2014). From 1990 to 1994 alone, more than 2 million parrots were traded on the international market (SNYDER *et al*., 2000). Most of the time, the conditions in which these animals are transported are extremely cruel and unsanitary, with several individuals crammed into inadequate transport boxes, and there are records of animals being doused with water to reduce agitation and vocalization (GONZALEZ, 2003).

These animals arrive at their final destination with compromised health and are kept in captivity in an equally inadequate manner, leading to a high mortality rate and reduced lifespan (DREWS, 2000). In this way, many parrot owners periodically end up looking for new individuals to replace those that have died, increasing the demand for these animals in the illegal trade (DREWS, 2002). This type of behavior can be observed in different Latin American countries, where these animals are often exposed to precarious conditions and traded illegally (WESTON & MEMON, 2009).

In recent years, the greater demand for Psittaciformes in the pet market has led to an increase in the number of commercial breeders, which makes it difficult to monitor and verify the real origin of the animals and their herds, facilitating the sale of illegally obtained individuals within legalized institutions (KUHNEN & KANAAN, 2014). However, many of the trafficked animals are seized by inspection services, thanks to reports of animal abuse and inspection operations. These seized animals are taken to Wild Animal Screening Centers (CETAS) and can remain there for a long time until they are disposed of. However, the delay in disposal, overcrowding, and the state in which these birds arrive at these institutions end up reflecting a low level of welfare for these birds (KUHNEN & KANAAN, 2014).

Some of the animals that are unable to be released can be incorporated into the populations of zoos or legal commercial breeders. However, inadequate captivity conditions can lead to a worsening of the animals' quality of life, which results in various behavioral

changes, such as loss of flight capacity, aggressive behavior, apathy, self-mutilation or even stereotypy (VAN HOEK & TEN CATE, 1998). Even individuals that are companion animals and have affectionate owners can live in unsuitable conditions, being kept in small cages that limit locomotion, inadequate diets, causing nutritional problems and neglecting the need for veterinary care, resulting in high mortality rates (DREWS, 2002; RODRÍGUEZ *et al*., 2020). This reinforces the need to understand the behavior of these animals and how captivity affects their quality of life.

Thus, we can notice that there is a demand for information on the welfare of animals kept under human care (MELLOR *et al*., 2015) and, with this, there is a need for studies on the welfare of various species of which scientific knowledge is still insufficient (MANTECA *et al*., 2016; SHERWEN *et al*., 2018). The development of different welfare assessment methodologies for different species has therefore been encouraged (MELLOR *et al*., 2015; MANTECA *et al*., 2016; SHERWEN *et al*., 2018), to improve their quality of life.

Welfare assessment protocols are an important tool for animals kept under human care, such as in zoos and breeding facilities, as they allow different welfare indicators to be assessed in a standardized and reproducible way by different applicators (HENRIKSEN & MØLLER, 2015; BARNARD *et al*., 2016; MANTECA *et al*., 2016). These protocols are easy to use and, in general, require little training to be used by the technicians and managers of the establishments themselves (BARNARD *et al*., 2016). It is, therefore, necessary to develop protocols that take into account the natural behavior of these animals and their relationship with the environment (SHERWEN *et al*., 2018), making the protocols increasingly specific to the various taxonomic groups of vertebrate animals, especially those that are most often sent to captivity for different reasons, as is the case with psittacines.

Welfare principles and criteria determined by the international Welfare Quality project (BLOKHUIS, 2008) have been used in various welfare assessment studies (HENRIKSEN & MØLLER, 2015; BENN *et al*., 2019; KHATTAK *et al*., 2019) and are a suitable framework for formulating specific protocols for species that do not yet have a defined welfare assessment methodology, as is the case with psittacines.

In this way, the development of a protocol for psittacines, based on the principles and criteria already established in previous protocols (e.g. BLOKHUIS, 2008; HENRIKSEN & MØLLER, 2015; BARNARD *et al*., 2016; MANTECA *et al*., 2016, BARRY *et al*., 2019) and which includes scientifically validated welfare indicators, has the potential to facilitate the assessment of the welfare of these animals in captive environments. In this way, it becomes possible to identify and correct critical points in these establishments, in order to enable a better

quality of life in zoos, breeding and domestic environments, reinforcing the need for the development of the protocol. In the Welfare Quality® protocol, welfare indicators are grouped according to the principles of 'good feeding, ‘good housing’, 'good health' and 'appropriate behavior' (BLOKHUIS, 2008), as presented below.

Good feeding

According to Blokhuis (2008), good feeding consists of ensuring that the animal has enough food and water to be free from hunger and prolonged thirst. However, Péron and Grosset (2014) stress the importance of an adequate nutritional balance and the expression of natural behavior as important factors for good feeding. Therefore, in order to promote a good diet for captive psittacines, one must first understand the feeding behavior of these species and their nutritional requirements.

Rozek and collaborators (2010) noted that larger food sizes, in which the parrots could use both their feet and their beaks to manipulate the food, allowed for a longer foraging time than that normally found in captivity and closer to the time budget spent feeding in the wild. This increase in feeding time can lead to more positive welfare, as it allows the animal to behave in a way similar to that found in wild parrots. However, the commercial feed found on the market has a very small pellet size compared to the food offered in the study, many of which are too small even to be handled with the feet. This may be a relevant aspect of feeding these animals in captivity and could be an opportunity to improve the welfare of parrots.

Similarly, Rozek & Millam (2011) tested the preference of parrots for different sizes of food and confirmed that the orange-winged amazon (*Amazona amazonica*) used in the study mostly preferred much larger sizes of food than those available commercially, even if they had to overcome some obstacles to obtain it. This reinforces the importance of using larger food sizes, because, in addition to allowing a foraging time close to natural and the use of food handling behavior similar to that used in the wild, these larger foods are also more desired by these animals.

Psittacines feed on a variety of foods, including roots, leaves, shoots, fruit, seeds and invertebrates (BLANCO *et al*., 2017). However, there is a certain perception that these animals only feed on seeds, as these are foods for which they show a great preference (KALMAR *et al*., 2007, 2010). Because of this, under human care, these animals are often offered only seed mixtures, which leads to nutritional problems and very high food selectivity (KALMAR *et al*.,

2010), with malnutrition being one of the biggest causes of clinical problems in captive parrots (HARRISON, 1998).

Kalmar and colleagues (2010) found that parrots have a preference for oilseeds, such as sunflower seeds. When these foods are offered with others that are less palatable, they are consumed to the detriment of the others. This selectivity for these seeds ends up deregulating the diet formulated by the technical team and often leads to nutritional problems, since most of these seeds are deficient in protein and have an inadequate proportion of calcium and phosphorus (ULLREY *et al*., 1991). It is therefore necessary to use different feeding strategies to ensure that the birds receive adequate nutrition.

Some feeding strategies include the use of fruit to reduce the parrots' consumption of seeds, thus reducing the total energy ingested by these birds (KALMAR *et al*., 2010). However, offering seeds and fruit alone is not enough to provide adequate nutrition, and it is necessary to use commercially available feed as the basis of these animals' diet, together with a supplement of fruit, seeds and vegetables, thus guaranteeing a nutritionally balanced diet (ULLREY *et al*., 1991; KALMAR *et al*., 2007; PÉRON & GROSSET., 2013).

As for the criterion of absence of prolonged thirst, it is worth highlighting the importance of the quality of the water offered and the container in which the water is offered, since many psittaciformes have a habit of dirtying the water very quickly (MCCLUGGAGE, 1996). Therefore, the water should be changed and the containers should be cleaned and dried every day, as the proliferation of microorganisms can be very rapid in these containers (MCCLUGGAGE, 1996; KALMAR *et al*., 2007).

Good housing

Parrots living in the wild always need to be on the lookout for predators, so they use high tree branches to hide and watch for predators (LUESCHER & WILSON, 2006). However, in captivity, these animals often do not have the opportunity to perch on tree branches, so their enclosure must have perches at a sufficient height to promote this feeling of safety and comfort (LUESCHER & WILSON, 2006). Another way in which it is also possible to provide the animal with a sense of security is through the availability of places to hide, such as wooden boxes or visual barriers, allowing the parrot not to be seen (KALMAR, 2011).

In addition, parrots use tree branches to climb, play and forage, and it is extremely important to use perches and bars to simulate this characteristic of their natural environment (FORSHAW & COOPER, 1989; KALMAR, 2011). Perches should also be used to stimulate

flight. They should be arranged in the enclosure with enough space between them so that the birds are stimulated to fly from one perch to another (KALMAR *et al*., 2007). Another factor that stimulates flight is the shape of the enclosure, where rectangular enclosures will provide a better flight environment, allowing them to travel greater distances than in other enclosures with different shapes but the same volume (LUESCHER & WILSON, 2006; KALMAR, 2011).

Another important factor is the quality of the perches. These should be of varying sizes, so that parrots of different sizes can adjust to the diameter of the perch best suited to their feet. In this way, tree branches are often the best options as perches, as they have varying diameters, natural curvatures and are softer than most artificial perches, ensuring better blood circulation and maintaining the health of these birds' feet (KALMAR *et al*., 2007, STANFORD, 2010). In addition, to prevent parrots from pecking each other's feet, it is important that adjacent enclosures are separated by double wire mesh, or that there is enough space between them to avoid exposing their feet when climbing over the enclosure's wire mesh (KALMAR *et al*., 2007). Photoperiod is also an important factor to consider, with exposure to light occurring between 12 and 14 hours a day (MCCLUGGAGE, 1996; RUPLEY & SIMONE-FREILICHER, 2015).

Good health

Physical restraint is known to be one of the necessary practices in animal handling. It is a major cause of stress and can lead to injuries, such as bone fractures, if carried out incorrectly. Therefore, proper restraint can result in reduced stress, capture time and risk of injury for the animal (KALMAR *et al*., 2007). Proper capture and restraint can be achieved by using a towel and capturing the animal from the front, immobilizing the wings, legs and lower jaw by pressing lightly with the thumb (KALMAR *et al*., 2007). In this way, the bird's movements are restricted, preventing it from injuring itself by struggling or hurting whoever is handling it.

Some infectious diseases of psittaciformes are of medical and sanitary importance, and it is necessary to assess the birds for these diseases. Ibama (2021) has determined that, for birds housed in triage centers, laboratory tests must be carried out to detect the following diseases: salmonellosis, aspergillosis, trichomoniasis, chlamydiosis, Newcastle disease, Pacheco's disease, avian influenza, circovirus, polyomavirus, papillomatosis, proventricular dilatation disease, megabacteriosis, adenovirosis, poxivirosis and mycoplasmosis. Psittacines are susceptible to all of these diseases (SPENSER, 1991; MORRISEY, 1999; KOSKI, 2002; LIERZ, 2005; GIRLING, 2005; MONKS, 2005, CHITTY, 2005), and it is extremely important

to carry out a clinical assessment of the birds before they are placed with other individuals, in order to prevent the spread of diseases to the other birds in the enclosure. These diseases have different symptoms, but most of them include respiratory, gastrointestinal and feather and skin disorders (SPENSER, 1991; MORRISEY, 1999; KOSKI, 2002; LIERZ, 2005; GIRLING, 2005; MONKS, 2005, CHITTY, 2005). Therefore, evaluations of these characteristics are essential for a correct clinical assessment of the birds.

One way of assessing the bird's health is by inspecting and analyzing its feces (RUPLEY & SIMONE-FREILICHER, 2015). It is important to check for changes in volume, color, frequency and any changes that occur in the feces. Even though this is not a specific indicator for a particular disease, changes in the feces can indicate the onset of some disease in the animal (RUPLEY & SIMONE-FREILICHER, 2015). In addition, it is important to select the enclosure substrate appropriately, as some substrates, such as sawdust, can cause respiratory problems, others can contain fungal spores or even be ingested and cause gastrointestinal problems (RUPLEY & SIMONE-FREILICHER, 2015).

Dermatological problems are one of the most common ailments in psittacines, and the health of the feathers and integument of these birds is a representation of their general clinical condition and the environment in which the animal is housed (KOSKI, 2002). Even so, despite the visibility of the lesions and the ease of observing their appearance and development, assigning an etiology to these ailments is a difficult task due to their multifactorial nature (KOSKI, 2002). They can originate from viral, bacterial and fungal infections, as well as infestations by lice and mites, affecting the appearance of the bird's feathers, skin and beak (GILL, 2001; KOSKI, 2002).

Birds housed in unsuitable enclosures can often have abrasion of their flight and tail feathers (GILL, 2001). As a consequence of an unbalanced diet, birds can suffer from malnutrition due to deficiencies in vitamins, minerals, proteins and fatty acids, resulting in uneven feathers, thickening and/or dryness of the skin, especially on the face, feet and cloaca (KOSKI, 2002). The most common cause of a generalized change in the color of a bird's feathers is malnutrition (MACWHIRTER, 1994; KOSKI, 2002). Malnutrition can also generate stress marks, which are dark, horizontal lines caused by the release of corticosteroid hormone during feather development, often due to methionine deficiency (MACWHIRTER, 1994).

In addition to malnutrition, an unbalanced diet can lead to obesity, one of the most common problems in captive bird medicine, with excessive consumption of seeds and lack of exercise being the main causes (MACWHIRTER, 1994; HARRISON, 1998; KOSKI, 2002). Obesity can lead to the loss of feathers on the bird's chest and is quite common in parakeets

(PERRY *et al*., 1991). The exacerbated weight of the animal can also lead to the development of pododermatitis, an inflammatory condition characterized by the presence of erythema, swelling and abscesses on the soles of the birds' feet (PERRY *et al*., 1991). Unsuitable perches can also facilitate the development of pododermatitis, as an unsuitable foot position, pressure from the bird's weight and irritation of the skin surface are all factors that predispose to its development (PERRY *et al*., 1991).

Appropriate behavior

In recent years there has been an increase in understanding about the welfare of psittacines in captivity, but there is still much to be developed in this regard. We know that the absence of mental and physical stimuli in a stable and safe environment can lead to the development of behavioral problems (LIGHTFOOT & NACEWICZ, 2006). Thus, there is a need to provide varied stimuli that lead to the performance of appropriate behaviors and avoid the performance of undesired behaviors. In this sense, Meehan *et al*. (2002) showed how social enrichment can improve the behavior of parrots, reducing the frequency of stereotypies and significantly improving the welfare of young parrots when they are grouped with individuals of the same sex. In addition, other studies have also shown that environmental enrichment is capable of reducing fear of new objects or humans with which they are unfamiliar, as well as reducing the appearance of stereotypies (MEEHAN & MENCH, 2002; MEEHAN *et al*., 2004).

As mentioned before, feeding behavior is also extremely important for the well-being of parrots. The food offered should be large enough for the animal to take it to its beak with the help of its feet (ROZEK *et al*., 2010). In this way, the animal can handle the food as it wishes, and it is also possible to transport it to other parts of the enclosure, avoiding competition (ROZEK *et al*., 2010). In addition, the animals need to have the power of choice and the feeling of control over their environment, having the opportunity to choose between different foods being offered, even if they need to perform tasks to obtain them, as in the study carried out by Rozek & Millam (2011), where the animals chose the larger foods, despite the obstacles to obtaining them.

Another very common behavior that is easily seen in enclosures with open areas is rainbathing. Murphy and colleagues (2011) simulated tropical rains in enclosures for orange-winged amazon (*Amazona amazonica*) and observed that they voluntarily took baths and exhibited a specific behavior while doing so, allowing rainwater to penetrate all their feathers. Offering frequent baths, just as they would in the wild, is an interesting behavioral enrichment

for these animals, allowing them to clean their feathers and reduce the time spent preening them.

Restricting or preventing the performance of natural and healthy behaviors can lead to the development of undesired behaviors, which affect the welfare of birds kept under human care (PENG & BROOM, 2021). A relevant behavioral problem for psittacines is feather plucking syndrome, where exaggerated feather preening behavior leads to the plucking, cutting or destruction of feathers (SEIBERT, 2006). This behavior can lead to skin lesions that prevent normal feather growth, resulting in animals unable temporarily or permanently to recover lost feathers (SEIBERT, 2006). The plucking behavior is usually directed at the individual itself, but it can also occur in association with an enclosure mate, where the affected areas are the target of preening (VAN ZEELAND *et al*. 2009). According to Grindringler (1991), by consensus, 10% of birds kept in captivity suffer from feather plucking syndrome. Although it is a relatively common behavioral problem, its consequences may not be merely aesthetic and may cause medical problems for the animal, as it removes protection from the skin and makes it easier for injuries to appear, as well as making it more difficult to maintain temperature (VAN ZEELAND *et al*. 2009).

In addition, the majority of psittacine species are gregarious, seeking safety from predators, greater success in finding food and greater ease in finding breeding partners (SEIBERT, 2006). Within groups formed by psittacines, it is common to observe dominance behaviors between individuals, which leads to a reduction in competition events within the flock, and also affiliative behaviors, which allow for greater social cohesion between individuals (SEIBERT, 2006). Within this context, it is possible that birds, in the presence of humans, show these behaviors directed towards their guardians or caretakers, since hand-fed individuals from a young age exhibit a preference for socializing with humans rather than with conspecifics (FOX, 2006).

Final considerations

The assessment of different environmental, physiological and behavioral characteristics provides a deeper insight into the welfare of animals kept under human care (MANTECA *et al*., 2016). Thus, the use of parameters involving health, environment, nutrition and behavior aims to facilitate the assessment of welfare (SHERWEN *et al*., 2018). Thus, the development of a protocol aims to organize a set of scientifically validated measures in a simple and

systematic way to provide a tool for assessing welfare in all its complexity (WEMELSFELDER & MULLAN, 2014; MANTECA *et al*., 2016, SHERWEN *et al*., 2018). To be able to provide good housing, good feeding, good health and appropriate behavior, a variety of environmental and physiological characteristics of the birds need to be considered. The development of a welfare assessment protocol requires an understanding of the behavior, nutrition, physiology, ailments and psychology of different species of psittacines. This requires the selection of scientifically validated parameters that can encompass different characteristics related to the animal and its environment, in order to cover welfare from different perspectives.

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CHAPTER 2

Development of a protocol for assessing psittacine welfare: first steps

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Introduction

Psittacines (parrots, macaws and parrotlets) are a diverse group of birds with a strong, curved beak and zygodactyl feet (COLES, 2005). They can weigh up to 3 kg, like some species of macaw, or only about 10 grams, like some species of the genus *Micropsitta* (COLES, 2005). The beauty and vocal capacity of various psittacine species has led to a great demand for these animals on the pet market (ENGEBRETSON, 2006; WESTON & MEMON, 2009), often illegally (KUHNEN & KANAAN, 2014). Illegal possession of these animals results in apprehention by environmental inspectors. These apprehended animals are taken to Wildlife Rehabilitation Center (CETAS) and can remain there for a long period of time until they receive proper destination.

Psittacines kept as pets normally live in inadequate conditions, even though they are the object of affectionate feelings by their caretakers (DREWS, 2002; RODRÍGUEZ *et al*., 2020). As a result, psittacines can show various behavioral changes as a consequence of captivity, such as loss of flight ability, feather plucking, aggressive behavior, apathy, self-mutilation and stereotypies (VAN HOEK & TEN CATE, 1998).

Some of the seized animals that are unable to be released into the wild can be incorporated into the collections of breeding centers and zoos. In such facilities, there is growing concern about animal welfare (MELLOR *et al*., 2015) and, as a result, there is a need

for studies on the welfare of various species of which scientific knowledge is still insufficient (MANTECA *et al*., 2016; SHERWEN *et al*., 2018). The development of various welfare assessment methodologies for different species has therefore been encouraged (MELLOR *et al*., 2015; MANTECA *et al*., 2016; SHERWEN et al., 2018), with the aim of better evaluating and improving their quality of life.

Welfare Assessment Protocols (WAP) are an important tools for animals kept under human care, such as zoos and breeding facilities, as they enable to assess different welfare indicators in a standardized way that can be replicated by different assessors (HENRIKSEN & MØLLER, 2015; BARNARD *et al*., 2016; MANTECA *et al*., 2016). It is suggested to the WAPs to be easy to use and to require little training, in a way that they can be applied by technicians and managers (BARNARD *et al*., 2016). This raises the need to develop protocols that consider the animals' natural behaviors, their relationships with the environment and specie-specific behavioral needs (SHERWEN *et al*., 2018). Therefore, the welfare assessment tools should be specific to the various taxonomic groups of vertebrates, especially those that are most often sent into captivity, as is the case of the psittacines.

The development of a WAP consists of bringing together various welfare assessment measures separated into principles and criteria that encompass different perspectives on welfare (BARNARD *et al*. 2016; MANTECA *et al*. 2016; BARRY *et al*., 2019). To this end, the selected measures should be field-tested to ascertain how feasible their application is (feasibility), the ability of the same and/or different assessors to equally assess the same situation (reliability) and how good this measure is at assessing the welfare characteristic for which it was selected (validation) (HENRIKSEN & MØLLER, 2015; YON *et al*., 2019; MAHER *et al*., 2021). Finally, the WAP must provide the assessor with a welfare score through a weighted sum of the values obtained from each measure (BOTREAU *et al*., 2007).

Welfare principles and criteria determined by the international Welfare Quality® Project (BLOKHUIS, 2008) have been used in various welfare assessment studies (HENRIKSEN & MØLLER, 2015; BENN *et al*., 2019; KHATTAK *et al*., 2019) and are an adequate framework for formulating specific protocols for species that do not yet have a defined standardization of specific welfare assessment methodologies, as is the case of psittacines. Welfare indicators are generally grouped according to the principles of 'good feeding', 'good housing', 'good health' and 'appropriate behavior' (BLOKHUI,S 2008). In this way, developing a WAP for psittacines based on the principles and criteria previously established in protocols for other species (e.g. BLOKHUIS, 2008; HENRIKSEN & MØLLER, 2015; BARNARD *et al*., 2016; Manteca *et al*., 2016; Barry *et al*. 2019) has the potential to facilitate the assessment of the welfare of psittacines

kept under human care. Thus, the aim of this study was to take the first step towards the develop a WAP for psittacines kept under human care, based on the principles and criteria of the Welfare Quality® Protocol (BLOKHUIS, 2008). In this study we: a) compiled a series of indicators (measures) of psittacine welfare into an assessment tool; and b) assessed the feasibility of the protocol by applying it in two different locations.

Material and Methods

A qualitative and observational study was developed. As it involved the application of a protocol, with the consequent capture and handling of the animals, it was approved by the Ethics Committee for the Use of Animals at the Federal University of Juiz de Fora (CEUA - UFJF, protocol 011/2022). It was also approved by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) through authorization protocol 02555.000006/2022-50, and by the Instituto Estadual de Florestas (IEF), through authorization number 41541198.

Developing the assessment protocol

To structure the assessment tool, we used welfare protocols already published for other species, such as: domestic mammals (BARNARD *et al*., 2016; VIKSTEN *et al*., 2017; DA SILVA & SANT'ANNA 2018; BARRY *et al.,* 2019), poultry (WELFARE QUALITY®, 2009), foxes and ferrets (HENRIKSEN & MØLLER, 2015), urial (*Ovis vignei*, a wild sheep) (KHATTAK *et al*., 2019), and zoo animals in general (SHERWEN *et al*., 2018). The protocol included parameters related to the enclosure in which the animals are kept and parameters related to the animals themselves, using the four principles and 12 criteria defined by Welfare Quality® Project (WELFARE QUALITY, 2009) (Table 1).

Table 1: List of welfare principles and criteria developed by the Welfare Quality® Project (WELFARE QUALITY, 2009).

Principles	**Criteria**		**Examples of indicators**
Good feeding	1	Absence of prolonged hunger	Body condition score
	2	Absence of prolonged thirst	Availability of water fountains
Good housing	3	Confort around resting	Spacing of perches

	4	Thermal confort	Availability of shade
	5	Ease of movement	Availability of space
Good health	6	Absence of injuries	Clinical inspection
	7	Absence of disease	Clinical signs of disease
	8	Absence of pain induced by management procedures	Use of analgesia and anesthesia
Appropriate behavior	9	Expression of social behaviors	Negative/positive interactions
	10	Expression of other behaviors	Occurrence of self-mutilation
	11	Good human-animal relationship	Escape distance
	12	Positive emotional state	Qualitative Behavioral Assessment (QBA)

The principles have 2-4 criteria and were based on behavioral, health and environmental parameters (enclosure characteristics), allowing to assess characteristics related to the welfare of psittacines (BLOKHUIS, 2008). Some parameters were not included in the proposed protocol due to its difficulty of practical application, such as: i) physiological parameters, which require laboratory analysis and are therefore not suitable for an audit scheme; ii) Qualitative Behavioral Assessment (QBA), which requires the assessor to be familiar with the method, the species, and the individuals in order to obtain the desired information (TRAVNIK *et al*., 2020). Thus, the proposed protocol presented these parameters in order to qualify and quantify characteristics relevant to animal welfare through objective evaluation by the assessors.

Assessing the feasibility of the WAP

The practical feasibility of the WAP was assessed in two different locations. The first was the Wildlife Rehabilitation Center in Juiz de Fora (Figure 1A), Minas Gerais state, southeastern Brazil. In the enclosure (2.30m x 3.00m x 10.46m), 44 birds were housed at the time of the assessment, two of which were *Pionus maximiliani*, one *Diopsittaca nobilis*, four *Primolius maracana*, one *Ara ararauna,* 19 *Psittacara leucophthalmus*, 11 *Amazona aestiva*, five *Amazona amazonica* and one *Pyrrhura* sp.

Figure 1: Enclosures used in this study. A: Multi-psittacine-species enclosure of the Wildlife Rehabilitation Center of Juiz de Fora (CETAS-JF), Brazil. Authored by André Monteiro. B: Wild Animal Release Area enclosure where individuals of six psittacine species were kept. Authored by Maria Eduarda Caçador Branco. C: Internal area of the enclosure at the CETAS-JF. Authored by André Monteiro. D: Internal area of the enclosure of the ASAS area (Wild Animal Release Area). Authored by Maria Eduarda Caçador Branco.

The second area is located in Santana do Deserto, also in Minas Gerais State, Brazil. This is a Wild Animal Release Area (ASAS Project - IBAMA/IEF), where the birds spent a period in captivity for pre-release acclimatization, in order to gradually adapt to the wild environment. It consisted of a single enclosure (3.24m x 7.06m x 12.81m; Figure 1B and D), which housed 43 birds, 25 of which were *Psittacara leucophthalmus*, 10 *Amazona aestiva*, two *Ara ararauna*, three *Primolius maracana*, one *Pyrrhura* sp. and two *Forpus xanthopterygius*.

The AWP was applied by two observers, who carried out all the stages of the assessments at both sites. Parameters of the environment and of the animals were assessed, obtaining information on the quality of the environment, health, behavior, social characteristics and for all the animals in the environment as a whole. The general level of ease/difficulty of applying the measures, the length of time used to apply the measures and possible challenges

in carrying out the protocol by a single evaluator were assessed in order to analyze its feasibility.

Results

At CETAS-JF, the AWP assessment lasted in total 5h33min, while at the ASAS area it lasted 4h40min. The instructions for the application of the protocol follow on Appendix 1 to 6.

Management Questionnaire

The questionnaire for the manager was initially developed with 17 questions, which were discussed between the authors based on previous knowledge in the area of the protocol and a previous review of the literature (BLOKHUIS, 2008; HENRIKSEN & MØLLER, 2015; BARNARD *et al*., 2016; MANTECA *et al*., 2016, BARRY *et al*., 2019). The questions were related to management methods, enclosure maintenance, the number of animals housed, food storage and other issues related to the management of the unit (APPENDIX 1).

Throughout the protocol, the multiple-choice questions were designed to be simple and straightforward, and the answers were pre-established on a scale in which as higher the escores, more inadequate the conditions assessed are, with score 1 attributed to the most adequate answer. The questionnaire also included open questions which were answered discursively, and which were noted down for later evaluation.

The first version of the questionnaire was applied at CETAS-JF, where an interview was held with the Coordinator of the State Forestry Institute (IEF), responsible for managing CETAS-JF, lasting 1h03min. After the interview, some questions had to be modified to improve the flow of the survey. Thus, the questions related to environmental enrichment were removed from the "measures of the environment" section and moved to the "management questionnaire" section. In addition, a question relating to the number of animals undergoing veterinary treatment was included. After the adjustments, the questionnaire had a total of 19 questions (APPENDIX 1). The questionnaire was reapplied at the ASAS area, where the interview was conducted with the employee responsible for managing the birds, lasting 17min. After reapplication, the questionnaire proved to be adequate, and no further changes were performed.

The questions sought to gather information on how the staff responsible for the birds maintain and manage the drinkers and troughs (number, cleanliness, and food storage) and their animals (number of animals housed, disease control, morbidity and mortality rates), in an

attempt to assess the unit's ability to care for injured and sick animals, as well as looking after the specific needs of these birds in terms of feeding times, lighting and hygiene.

At CETAS-JF, fruits were stored in refrigerators, while feed and seeds were stored in closed containers and in feed dispensers for daily use. The food provided was extruded feed, a mixture of seeds and grains, minced fruit and vegetables and whole fruit. The proportion offered is 50% feed, 40% fruit and vegetables and 10% mixed seeds and grains. Feeding times were 8h00min, when the feed was offered, and 14h00min, when the fruit and seeds were offered. The water of drinkers was changed daily. The aviaries are swept daily and washed with high-pressure water jets every two days, with quaternary ammonia applied throughout the enclosure and the cages heat-treated with fire. In order to control infectious diseases, deworming, feces and blood collection and quarantine of individuals with suspected diseases were carried out. The psittacines are divided into two enclosures, a module with five compartments and nine cages. Of all the animals housed, only three were alone at the time of the visit. The CETAS-JF had an outpatient clinic for veterinary procedures and two bird treatment units, as well as a quarantine area with six modules. The enclosures were naturally lit and had access to rain for bathing. Individuals kept in cages and modules were exposed to the sun at least once a day, but were not bathed as they are undergoing treatment. The staff was properly trained to handle the animals and analgesia or anesthesia was used for invasive veterinary procedures.

In the ASAS area, the seeds were stored in closed recipients and the fruit was kept in the fridge. The food offered was a variety of fruits (papaya, banana, apple, watermelon), sunflower and corn seeds. No extruded feed was offered, and water was provided through a fountain with constant water change. The feeding times were 7h30min and 3h30min. The floor was swept every morning and afternoon. The aviary was washed once a week with water, without the use of cleaning products. In cases of sick o injured animals, CETAS-JF was notified to remove the birds. So, there were no facilities for medical treatment and no veterinary procedures were carried out in the area. The institution had only one enclosure in use for the birds, with natural lighting and access to uncovered area where birds could be exposed to rain.. The animals could bathe both in the rain and in the water fountain (the same used for drinking). Environmental enrichment was carried out by providing sand with stones every 15 days and leafy branches three times a week.

Environment-based Measures

After applying the questionnaire, the environment was assessed. This was the section with the highest number of items to be assessed by the observers, totaling 32 questions about the cleanliness, size, quality and safety of the enclosure (APPENDIX 2). These items sought to confirm some of the information obtained through the questionnaire and to qualitatively assess the characteristics of the enclosures (Figures 1C and D). In this way, we tried to assess different aspects of the environment in which these animals are housed, such as the structure, size, cleanliness, material of the perches, floor and platforms, as well as features that allow the animals to protect themselves from the weather condition. The questions were designed to be answered qualitatively and on a binomial scale ("yes" or "no" / "adequate" or "inadequate").

During the first application of the protocol at CETAS-JF, the complexity of the environment and its cleanliness meant that the answers "yes" and "no" or "adequate" and "inadequate" corresponded to the situation observed by the evaluators. However, in the ASAS area, where the protocol was applied for the second time, situations were observed in which the answer could be an intermediate value. It was therefore decided that for some questions there would be more answer options. An example of this is the assessment of the cleanliness of the feeders as adequate and inadequate, since this separation would not distinguish between feeders that were somewhat dirty but still fit for use and feeders that were completely clean. In this way, by adding an intermediate answer, the form presented a better representation of the conditions observed. In addition, some health measures were obtained at this stage of the protocol, observing how the birds behave in terms of thermal comfort and rest, as these characteristics are directly linked to an environment criterion (thermal comfort).

At CETAS-JF, the feeders were clean, free of dirt and easily accessible to all the housed animals and were removed in the end of the day to be cleaned. The water was supplied by metal recipients arranged around the enclosure, which were clean, had enough water and were easily accessible to all the birds (Figure 5A). The enclosure was clean and had a masonry floor and there were no changes to the droppings in the enclosure. The perches were made of wood or branches, and all were of adequate thickness and did not overlap. It is well known that overlapping perches can lead to one bird defecating on the other and, consequently, to soil the feathers, which is undesirable. The aviary also had six platforms, no hiding places and no structures capable of trapping or injuring the birds, such as strings, nails, wire ends or bars. The volume and area available were one bird every 1.64m^3 and 0.71m^2, respectively. There was no source of constant noise pollution and half of the enclosure was covered. The birds were able

to move around the warmer or cooler areas of the enclosure and none of the birds were panting, had their wings and beaks open or were hunched over with their feathers ruffled. The perches did not allow vertical or horizontal exploration of the enclosure and the animals unable to fly were unable to explore the entire enclosure. The distance between the perches encouraged flight and the housing allowed vertical and horizontal flight. There was double railing between the pens.

Figure 2: Water and food provision. A: Water supply through metal recipients arranged around the enclosure at CETAS-JF. Authored by Authored by André Monteiro. B: Water supply through a constantly replenished water fountain in the ASAS area. Authored by Maria Eduarda Caçador Branco. C: Dirty feeder in the ASAS area (Wild Animal Release Area). Authored by Maria Eduarda Caçador Branco. D: Clean feeder at CETAS-JF. Authored by Polonia Nunes.

In the ASAS area, the water supply was provided by a fountain that constantly replaced the water. The water was clean and plentiful, allowing all the birds to use the drinker without difficulty (Figure 2B). The feeders were fixed, had inadequate access and dirt was apparent (Figure 2D), compared to CETAS were the feeders were clean (Figure 2D). The enclosure was

clean and had a masonry floor, and there were no droppings in the enclosure. All the perches were made of wood or tree branches, and more than half were regarded as having a suitable diameter, with less than half of the perches overlapping each other. The perches did not allow exploration of the vertical and horizontal environment, but they did encourage flight. The aviary had four platforms and hiding places where the birds could take shelter. There were also some pointed structures, which could injure the birds. The birds were visually exposed to predators, as there were not enough hiding places for all the birds. There was no source of noise pollution and less than half of the enclosure was covered. The birds were able to move between the warmer or cooler areas of the aviary and no animals were panting or shrinking. The available volume and area were one bird every 6.81m³ and 2.1m², respectively. Animals unable to fly were able to explore most of the enclosure and the housing allowed vertical and horizontal flight. The enclosure had enrichment in the form of branches with hanging fruit.

Animal-based Measures

a) Health and physical condition

Animal measurements included health, nutrition and hygiene assessments (Table 2), which were obtained by capturing and individually assessing a sample of the animals in the enclosure. Initially, the weight, body condition score, presence of injury due to improper ringing, plumage condition (Figure 7), changes to the integument, scabs around the beak/eyes, and any injuries present on the bird were assessed in order to answer eight questions (APPENDIX 3).

Both in the first and second application of the protocol, it was possible to evaluate each animal for the physical alterations, most of which were easy to identify. The only measure that was removed from the evaluation was the weighing of the animals in order to reduce the birds stress responses during handling, and reduce the evaluation time.

In the first assessment, the body condition score was evaluated from 1 to 3, with 1 being underweight and 3 being overweight, but with the removal of the weighing of the animals, the measurement of the scores was modified in the second assessment, becoming from 1 to 5 (Table 2), to be more comprehensive. Finally, an assessment of the dirtiness of the animals' feathers was added, indicating the birds' ability to perform self-care functions within the enclosure.

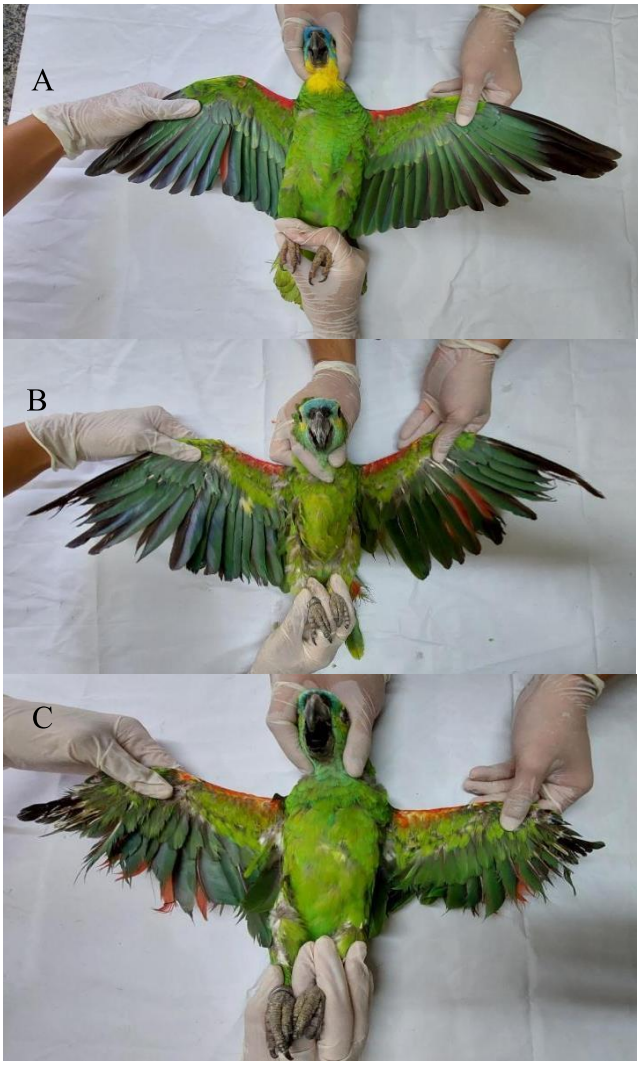

Figure 3: A: Plumage condition score. A score 1, defined as complete plumage, with no missing or broken feathers B: Flight feathers' score 2: Partially complete plumage, with some missing and/or broken feathers. C: Flight feathers' score 3: Plumage with several missing and/or broken feathers.

Sampling was carried out based on the sampling suggestions proposed in the Welfare Quality® (2009) WAP, where for enclosures with up to 40 individuals, the the sampling number is 30 individuals. For pens with 50 individuals, the suggested number is 32, but if this is not possible, 30 individuals should be sampled. For enclosures with 100 individuals, sampling should be 49, and if this is not possible, 40. In enclosures with 200 individuals, the values are 65 and 51. And finally, for 300 individuals, the values are 73 and 55 (WELFARE QUALITY®, 2009).

The assessment was carried out by physically restraining the birds. They were captured using a bird catching net and immobilized for the assessment. At CETAS-JF, 23 (52.27%) birds were assessed, although the suggested number of animals present in the enclosure would be 30

birds (WELFARE QUALITY®, 2009). Of these, only one bird (4.35%) did not reach a good score (2), being emaciated (score 1). None of the animals had ringing injury or scabs around the eyes or beak. However, of the 23 animals evaluated, three (13.04%) had wounds and six (26.09%) had altered skin.

In the ASAS area, 32 (74.42%) birds were evaluated, in line with the suggested sampling value of 30 birds (WELFARE QUALITY®, 2009). Of these, one (3.13%) bird had a injury due to incorrect ringing, five (15.66%) had dirty feathers, five (15.66%) had altered integument and two (6.25%) had injuries. In addition, another six (18.75%) animals had a pelvic limb amputated or rotated. Thus, of the 32 animals in the enclosure, 19 (59.38%) had some altered physical condition.

b) Behavior

The bird's behavior was assessed using an ethogram adapted from Ramos *et al*. (2021) and the assessment was carried out using a scan (MARTIN & BATESON, 2021) with a sampling interval of five minutes and a duration of 60 minutes according to Barry *et al*. (2019). In the first evaluation of the ethogram, 14 behaviors were recorded (Table 3). The number of animals and the ease of movement around the enclosures made it difficult to observe the behaviors and evaluate the ethogram, so some changes were made for the second evaluation. Of the 14 behaviors initially suggested, seven behavioral categories were kept in the final version of the protocol (Table 3), and the others (alertness, preening, allopreening, locomotion, interaction with the environment, imitation of human speech, sleeping)) were excluded from the evaluation because they were difficult to observe, due to the large number of animals in the enclosures. The allopreening behavior was incorporated into the positive social interaction behavior to avoid redundancy. Of the six behaviors kept in the ethogram, four were recorded using instantaneous scan sampling and the other three were recorded using a continuous sampling (Table 3).

The modified version of the ethogram was applied in the ASAS area. The behaviors observed during the scan were distributed as follows: 10% of the events recorded were positive social interaction, 28.75% feeding, 0.63% interaction with environmental enrichment, and 60.62% inactivity, while continuous observation showed a frequency of 0.28 negative social interactions/min and no abnormal behavior or loud vocalization during the observation time.

Initially, nine behavioral questions were designed to be answered using the data collected with the ethogram. After reducing the behavioral categories to improve the feasibility of behavioral recording, the number of questions reduced to seven (APPENDIX 4)

Four behavioral tests were also carried out: the voluntary approach test (adapted from KALMAR, 2007), the escape distance test (adapted from KALMAR, 2007), the food offer test (adapted from Ramos *et al*. 2020) and the flight capacity test (adapted from Ramos *et al*. 2020). The tests were carried out both at CETAS-JF (n = 27) and at the ASAS release area (n = 43). There was no need to modify the tests, as they could be carried out without any major difficulties.

The voluntary approach test was carried out with the assessor dividing the enclosure into three quadrants and standing still for 3 minutes in each quadrant. The animals' behavioral responses were categorized based on the approach to the observer within an arm's length, being considered a voluntary approach (APPENDIX 5). We tried to avoid animals that were on the ground or in the feeders. At CETAS-JF, of the 27 animals tested, four (14.81%) approached the evaluator. In the ASAS area, of the 43 animals tested, one (2.33%) approached the evaluator.

Table 2: List of measurements obtained from the animals during the physical assessment.

Indicator	Definition	Score	Score level
Body score	Chest muscle volume assessment	Scale from 1 to 5.	1 - Very thin. 2 - Thin 3 - Good 4 - Overweight 5 - Obese
Ringing injury	Presence of overtightened rings.	Present or absent	Present - Ring is tight and causing injury. Absent - Ring not tight and not causing any injury.
Plumage condition	Plumages are broken, cut, worn or torn.	Present or absent.	Present - The plumage is broken, cut, frayed or torn. Absent - The plumage is not damaged in any way
Tegument alteration	Presence of alterations to the integument, such as texture, color and size	Present or absent	Present - Presence of changes in color, texture, size of beak, feet and nails. Absent - No changes to the beak, feet or nails.
Dirtiness	Dirt from the environment on the animal, showing a lack of hygiene.	Present or absent	Presence - Presence of dirt such as feces, blood, soil and other products. Absence - Animal with a clean appearance and no dirt from the environment.

Eye/beak scabs	Presence of crusts indicating disease on beaks and eyes.	Present or absent	Presence - The animal has scabs around its eyes or beaks that could be associated with disease. Absence - The animal does not have any scabs around its eyes or beaks.
Wounds	Presence of lesions and injuries that could affect the animal's health.	Present or absent	Present - The animal has skin wounds or lesions on its body. Absence - The animal is healthy and shows no signs of injury.

Table 3: List of behavioral categories used in the ethogram.

Behavioral category	Description
Inactive (number of inactive animals during observation time)	The psittacine remains in a neutral posture, standing on the ground, on the screen or on the perches, with its eyes open or closed;
Positive social interaction (occurrences)	Close approach and perching, less than 1 cm of distance between two or more individuals.
Negative social interaction (occurrences)	Agonistic interactions (when the bird opens its beak, vocalizes and pecks at some part of another bird's body); Threatening to peck or hit another bird with its feet.
Feeding (number of animals feeding)	Feeding on the food available in the feeder.
Interaction with environmental enrichment (number of animals interacting)	The bird approaches and touches the food or physical enrichment items with its beak or feet.
Loud vocalization (number of animals vocalizing during the observation period)	Loud, shrill and/or repeated vocalizations in a short space of time, showing stress.
Abnormal behaviors (number of animals displaying abnormal behavior during the observation period)	Repetitive behaviors; bird repetitively pecking at the screen or walking from side to side; bird pulling its feathers;

The escape distance test was carried out by touching each animal in the enclosure individually. If the birds did not allow the touch and moved away, it was considered an escape (APPENDIX 5). If the animals allowed the touch or did not move away, it was counted as an escape distance of zero. Animals out of reach and animals on the floor of the enclosure were also counted. Some animals didn't run away and didn't allow to be touched, but showed aggression towards the assessor by trying to peck his hand. These animals were also discriminated against and counted as attempted aggression. At CETAS-JF, 19 (70.04%) animals allowed to be touched, while four (14.81%) showed signs of aggression. In the ASAS area, 14 (32.56%) allowed to be touched, five (11.63%) were out of reach, 10 (23.26%) were on the ground and one (2.33%) showed signs of aggression.

The food offer test was carried out in a similar way to the escape distance test, where the assessor offered a handful of sunflower seeds to all the animals in the enclosure, except for the animals out of reach and the animals on the floor. All the animals that accepted the food and approached the assessor's hand were counted. Animals on the floor and animals out of reach were also counted. At CETAS-JF, 14 (51.85%) animals accepted the food. In the ASAS area, 14 (32.56%) accepted the food, five (11.63%) were out of reach and six (13.93%) were on the ground.

The flight capacity test aimed to identify the birds' difficulties in locomotion. It was carried out using a bird-catching net in which the assessor threatened each bird individually with a supposed attempt to catch it. Animals that could not fly or had difficulty walking around perches, bars and platforms or taking flight from the ground were characterized as having impaired locomotion. As the bird-catching net was big enough to reach all the animals in the enclosure, there were no animals out of reach. Animals that were on the ground were included in the test. At CETAS-JF, 22 (81.48%) of the birds evaluated had impaired locomotion, while at the ASAS area, 28 (65.12%) birds had impaired locomotion.

Discussion

Management Questionnaire

The first welfare principle included was absence of prolonged hunger. For psittacines, feeding should be based on extruded feed, fruit, vegetables and seeds, in that order of importance (ULLREY *et al*., 1991; KALMAR *et al*., 2007; PÉRON & GROSSET, 2013), which was observed at CETAS-JF, but the ASAS area does not offer extruded feed to the birds.

This is due to the fact that these birds are being prepared for release and the food offered is intended to naturalize their diet and facilitate their adaptation to the wild.

As for food storage, seeds and extruded feed are stored in closed containers and fruit is kept in the fridge at both sites. The improper storage of seeds should be evaluated, as it can lead to a nutritional imbalance, where previously formulated seed diets, mixed and stored, will segregate by size and weight over time, modifying the idealized formulation (KALMAR *et al*., 2010). Feed, fruit and vegetables should be stored in a well-ventilated place, off the ground and in a low-humidity area, and it is important to cool these foods when possible to avoid microbiological growth as much as possible (ROUDYBUSH, 1996).

Feeding times should be at moments consistent with peak feeding periods in the wild, so that the food is as fresh as possible, preferably in the early morning and late afternoon (ROZEK *et al*., 2010). At both sites, food is offered in the early morning and mid-afternoon, with the feeding times at CETAS-JF being 8:00 and 14:00 and at the ASAS area being 7:30 and 15:30. Both at CETAS-JF and in the ASAS area we witnessed the mid-afternoon feeding time and, even though the mid-afternoon feeding time is not recommended in the literature, the animals showed a lot of interest in the food.

With regard to lighting, photoperiod schedules must be respected, and it is recommended that the birds either have access to natural lighting or that the controlled photoperiod is kept below 14 hours (MCCLUGGAGE, 1996). In both locations, the enclosure allowed natural lighting, which was the best option considering the most natural condition.

Control of infectious diseases should be carried out by properly cleaning and sanitizing the environment and the utensils used, as well as quarantining individuals who have spots, plaques, nasal and eye discharges or are suspected of having a disease (LIERZ, 2005). As for the clinical assessment, both locations have the veterinarian in charge of the State Forestry Institute responsible for CETAS-JF to carry these tasks out. However, as the ASAS area is located in another municipality, the general assessment of the birds is carried out by visualization by the workers and, if any bird shows any noticeable physical or behavioral changes, the CETAS-JF veterinarian is called in to check. In this way, disease control at CETAS-JF is expected to be more efficient because there is a veterinarian on hand every day, whereas in the ASAS area this is not the case.

Finally, capturing and handling must be done carefully so as not to injure the animals. This can be done using bird catching nets in aviaries and large enclosures, or towels when they are in cages (KALMAR *et al*., 2007). The immobilization of Psittaciformes should be carried out with the aim of preventing the head from moving while the wings and legs are immobilized

using a towel that covers and wraps around the bird, taking care that the pressure exerted does not prevent the air sacs from expanding and that the bird does not suffer from hyperthermia or excessive stress (BEST, 2005; KALMAR *et al*., 2007). In this way, the risk of injury to the handler and the bird is minimized. The entire CETAS-JF staff responsible for handling the birds is made up of trained biologists, veterinarians and handlers, while the ASAS area staff receive prior training by CETAS-JF staff in how to handle the birds. Thus, capture and immobilization methods was regarded as adequate and safe based on the manager reports at both sites.

Environment-based Measures

After applying the protocol, it was noted that both locations were physically very similar, with the enclosures consisting of a masonry floor and a wall, through which double grilles were fixed at CETAS-JF, and single grilles in the ASAS area. While the masonry floor allows for better cleaning, prevents the proliferation of parasite eggs and microorganism spores (JONES, 2005), it can also cause injuries to birds that are unable to fly properly. Injuries caused by falls and wing flapping while the birds are on the ground are common, as well as feather abrasion, especially of the remiges and rectrices (GILL, 2001). In addition, the presence of walls where the bars of the enclosure are inserted is important to prevent the entry of rodents (JONES, 2005) or predators that manage to force open the bars.

Although masonry flooring has positive points in terms of ease of cleaning and disease control (JONES, 2005), in the protocol, masonry flooring was classified as the worst option for an enclosure. This is due to the poor condition of the feathers, due to the abrasion caused by the floor and railings (GILL, 2001), and the increased chance of injuries to the plantar surface of the feet (PERRY *et al*., 1991; GILL, 2001; NIELSEN *et al*., 2012). Floors with vegetation or soil cover were determined to result in the best welfare conditions, since Brazilian legislation determines that for small, medium and large Psittaciformes the floor should be sand, earth or grass (IBAMA, 2015). In addition, the greater difficulty of sanitizing non-concrete floors is offset by the fact that they generate less abrasion, which results in healthier feathers and feet (PERRY *et al*., 1991; GILL, 2001; NIELSEN *et al*., 2012). The floor should be cleaned daily and food and droppings should not accumulate anywhere in the enclosure, especially in enclosures with a daily influx of new birds (MCCLUGAGGE, 1996).

The enclosures at both sites are partly made of wire mesh and partly covered, allowing sunlight and rain to pass through, but also allowing the animals to rest in the shade and avoid getting wet. This type of environment, which allows the animal to choose different conditions,

is ideal (IBAMA, 2015), since access to the open air, sunlight and rain significantly improve the condition of the feathers and skin of Psittaciformes (JONES, 2005), and the birds are also able to protect themselves from the sun at the hottest times of the day (IBAMA, 2015). On the other hand, covering with bars allows contaminants carried by free-living birds to enter (JONES, 2005). In addition to making it easier for predators to see the birds inside the pen, the presence of wire mesh can also pose a risk in terms of the presence of piercing surfaces, which can lead to poorer skin conditions (BARNARD *et al*., 2016).

Both at CETAS-JF and in the ASAS area, it was easy to see and assess the perches. The perches at both sites were made of wood or tree branches. Overlapping perches allow birds to defecate on each other, which can lead to dirty feathers and should be avoided. Tree branches are the best options for perches, as they prevent injuries to the birds' feet, and the different thicknesses of the branches allow animals of different sizes to perch on the thickness that best accommodates their feet, without their toes overlapping (KALMAR *et al*., 2007). The proper accommodation of the birds' feet on the perch allows for greater comfort and prevents injuries to the plantar surface of the feet, avoiding the appearance of pododermatitis (PERRY *et al*., 1991; GILL, 2001; NIELSEN *et al*., 2012).

As for the feeders and drinkers, they were assessed for cleanliness, access and whether the containers allowed the birds to feed or drink without difficulty. All drinkers and feeders should be washed and dried daily, as microbiological growth occurs within a few hours (MCCLUGGAGE, 1996). In addition, the material of the feeders and drinkers is also important, and it is necessary to use materials that the birds cannot destroy (MCCLUGGAGE, 1996). In both locations, the cleanliness levels of the feeders and drinkers were regarded as adequate and they were clean. However, in terms of accessibility, in the ASAS area, access to the water source was somewhat limited for birds that were unable to fly properly and was characterized as intermediate.

In addition, while the availability of food and water can be interpreted as indicators of the absence of hunger and thirst, it is also necessary to ensure that the food available provides the necessary nutritional requirements (ENGEBRETSON, 2006) and is of a size that promotes proper manipulation through the beak, tongue and feet (ROZEK & MILLAM, 2011). In order to assess these aspects of nutrition, questions were added to the questionnaire about the food and how it is offered to the birds housed in the enclosures. In this way, we investigated not only the availability and hygiene of the food being offered, but also its nutritional quality and presentation. A nutritionally balanced diet for Psittaciformes should consist of a combination of extruded feed, fruit, seeds and vegetables (PÉRON & GROSSET, 2014; KALMAR *et al*.,

2010) and allow manipulation through the Psittaciformes' normal behavior (ROZEK *et al*., 2010; ROZEK & MILLAM, 2011).

Another important issue that has been assessed in the environment is the extent to which it allows birds to explore it both horizontally and vertically (PENG *et al*., 2013; IBAMA, 2015), either by flight or by moving around perches, bars and ropes, since the greater the physical complexity of the environment, the less stereotypies develop (KEIPER, 1969; MEEHAN *et al*., 2004). Locomotion is one of the most restricted behaviors in captivity (MEEHAN & MENCH, 2006). Flight should be encouraged through adequate spacing between perches (MCCLUGAGGE, 1996; KALMAR, 2011), as physical activity in captive animals can be an important factor in reducing the effects of oxidative stress (LARCOMBE, 2015), and flight is the main method of locomotion for birds and its impediment can have negative consequences for their welfare (MAPLE & PERDUE, 2013).

In addition to flying, the animals must be able to move around the environment, having access to water and feeders via perches, ropes, wire mesh and platforms, as most of the animals in the study areas evaluated are unable to fly due to their lack of feathers or the poor conditions they were in before being received at CETAS. As such, the environment needs hiding places that allow these birds to hide from predators and where they can rest without being seen (BEST, 2005), as well as enough individual space for their freedom of movement. Ibama (2015) states that, for breeding centers and zoos, for small birds (< 25cm), the maximum density should be two individuals per m². For medium-sized birds (≥ 25 cm or ≤ 55 cm), the maximum density should be two birds per 5m². For large birds (> 55 cm), the maximum density is two individuals per 10 m². The two sites therefore have insufficient area for adequate freedom of movement considering this Ibama's resolution (Ibama, 2015), although this resolution does not limit the space for rehabilitation centers.

The protocol allowed to assess whether all the birds present in the enclosure were able to explore the environment in its entirety, which was observed in both locations, where branches, ropes and platforms allowed the birds to move around the enclosure and stimulated flight. At CETAS-JF, the branches offered to the birds were freshly cut and had a a lots of leaves, allowing the birds to shelter and hide. In the ASAS area, there were not many leafy branches, but the enclosure had bamboo hiding places and other wooden structures.

Increasing environmental complexity together with providing opportunities for foraging and social interaction are forms of enrichment for Psittaciformes that positively influence welfare (MEEHAN & MENCH, 2006). Environmental enrichments can reduce fear of new objects and of humans (MEEHAN & MENCH, 2002; MEEHAN *et al*., 2003; FOX &

MILLAM, 2007), result in increased foraging time (Coulton *et al*. 1997), reduce the expression of abnormal behaviors (MEEHAN *et al*., 2002, 2003, 2004; CLYVIA *et al.,* 2015; AZEVEDO *et al.,* 2016), increase the diversity of behaviors (MEEHAN *et al.,* 2003; ROZEK *et al.,* 2010; CLYVIA *et al.,* 2015), increase feeding time (ROZEK *et al.,* 2010) and allow the opportunity for choices and preferences (ROZEK & MILLAM, 2011).). In this way, environmental and social complexity and the use of environmental enrichments were assessed through the questionnaire and the behavioral animal-based measures.

Animal-based Measures

a) Health and physical condition

Some of the physical alterations found were not related to the quality of the enclosure analyzed, but rather to the fact that the animals present in the ASAS area come from CETAS-JF and, in turn, are trafficked or rescued animals and often arrive at the site with these physical alterations. As such, the possibility of including a parameter to assess the length of time the bird has been kept in on that environment was raised, as it could reveal whether the state of the feathers may be a result of the current enclosure or the period prior to arrival at the facility. However, as the sites may have several enclosures containing a very large number of birds, individualizing them to assess the length of time each bird has been in the facility and its consequent influence on feather quality is unfeasible due to the excessive effort and time involved. We therefore decided not to make any further changes to this part of the protocol.

The body condition of birds can be assessed by palpating the pectoral muscle and the sternum bone (GREGORY & ROBINS, 1998; ROSSKOPF & WOERPEL, 1998; RAFTERY, 2005), which is the best way of assessing body condition in the absence of weighing (RAFTERY, 2005). The pectoral musculature is the largest muscle group in birds (EVANS, 1998) and, although there is variation between species, the keel of the sternum should never be clearly prominent in relation to the musculature (ROSSKOPF & WOERPEL, 1998). In addition, species with a tendency to obesity end up depositing fat in this region, which is easily seen over the skin (RAFTERY, 2005). Poor body condition can be indicative of disease and/or malnutrition (KHATTAK *et al.,* 2019). Thus, the use of a body condition score allows for a better assessment of the bird's current state of health (GREGORY & ROBINS, 1998; CLEMENTS & SANCHEZ, 2015). Scores from 1 to 5 are commonly used in the literature for different species (AUDIGE *et al.,* 1998; HICKMAN & SWAN, 2010; CLEMENTS & SANCHEZ, 2015) and provide less inter-observer variability (HICKMAN & SWAN, 2010).

In terms of clinical assessment, the main clinical signs of infectious systemic diseases in Psittaciformes are apathy, changes to the feathers, beak and skin, difficulty breathing, nasal and ocular secretions (LIERZ, 2005). Therefore, the observer should pay attention to any visible changes in the physiognomy of the birds being assessed, as they may be indicative of the presence of infectious diseases. In addition, another important factor to assess is the integrity of the feathers on the wings and rectrices, which when cut, torn or broken prevent the birds from taking flight and affect their welfare as a whole (MAPLE & PERDUE, 2013). The appearance of these birds should also be assessed in terms of their ability to clean and care for their own feathers, as a lack of self-care can lead to new feathers becoming trapped in their sheaths and also leaving the feathers looking untidy (KOSKI, 2002).

Another factor that can lead to a health risk for birds in breeders, zoos and rescue centers is ringing. Ringing is carried out with the aim of identifying the bird, but improper ringing can lead to Psittaciformes having their legs garroted (JONES, 2005), because the rings used are often half-open metal rings, which, when attached incorrectly, allow birds with stronger beaks to force the ring open towards their leg, which leads to compression and consequent injury or even loss of the leg (JONES, 2005).

b) Behavior

Behavioral assessment is necessary because psittacines kept in captivity can exhibit a range of undesirable behaviors resulting from inadequate conditions in their environment (MEEHAN *et al.,* 2003, 2004; KALMAR *et al.,* 2007; RUPLEY & SIMONE-FREILICHER 2015; WILLIANS *et al.,* 2017). A large, physically and socially complex environment that exposes the bird to different stimuli can reduce the occurrence of unwanted behaviors by offering the bird opportunities to exercise its natural behavior (MEEHAN *et al.,* 2004; MANTECA *et al.,* 2016; WILLIANS *et al.,* 2017). In our evaluation, the birds showed little interaction with the environmental enrichment items available during the measurements, compared to other studies that showed a higher proportion of records of this behavior (MEEHAN *et al.,* 2003, 2004; AZEVEDO *et al.,* 2016; RAMOS *et al.,* 2020). Andrade and Azevedo (2011) and Azevedo *et al.* (2016) observed that the introduction of environmental enrichments reduced the presence of abnormal behaviors and increased the frequency of natural behaviors, such as enclosure exploration and foraging in psittacines. Clyvia et al. (2015) observed that the feather plucking behavior of a pair of macaws (*Guaruba guarouba*) decreased in the presence of environmental enrichment, while social interaction increased during enrichment and after its removal. Thus, interaction with enrichment can be an important factor

in reducing the occurrence of unwanted behaviors. . The birds' reduced interaction with the enrichement items provided could be due to the behavioral observation time and duration (60 minutes), that was defined to priorize the feasibility of the protocol, as discussed by Barry *et al* (2019). It can also be due to the lack of interest in the enrichments offered during our evaluation and may suggest that they have become accustomed to the enrichments that had been available for days, reducing their effectiveness and making. It is recommended to change the environmental enrichment items used over time to maintain the animals' interest and motivation to explore.

Assessing feeding time also influences the welfare of Psittaciformes, as animals in the wild spend most of their day searching for food, and increasing this activity in captivity can result in a reduction of abnormal behaviors by improving the welfare of these birds (ROZEK *et al.,* 2010). In addition, evaluating negative social interactions is extremely important, because even though there are behaviors normally performed by both free-living and captive animals, when the intensity is too high it can become an indicator of poor welfare (MANTECA *et al.,* 2016). In addition, both negative and positive social interaction events can result in a higher quality environment, more diverse behaviors and a more positive general welfare, with a reduction in abnormal behaviors (MEEHAN *et al.,* 2003; WILLIANS *et al.,* 2017). Loud vocalization events are also more common in psittacines with restricted social relationships (MEEHAN *et al.,* 2003), which may indicate a negative welfare of individuals who vocalize for long periods over the days.

Therefore, the categories kept in the final version of the ethogram are the ones we consider most important for a quick and applicable welfare assessment in any environment, whether of greater or lesser complexity and with a few or many animals to be sampled. The presence of these behaviors in the final ethogram reinforces the assessor's general perception of the suitability of the environment being assessed, allowing them to determine more consistently how good or bad is the welfare of the animals being assessed. Thus, the final version of the ethogram proved to be adequate and allows the evaluator to easily answer the questions in the protocol.

As for the behavioral tests, they all proved to be feasible and easy to apply. The voluntary approach, flight distance and food offer tests allow us to assess how close the birds are to humans, which, depending on the purpose of the bird's breeding site, can be positive or negative (FRANZONE *et al.,* 2022). Since the objective of CETAS-JF and the ASAS area is to release these animals to live in the wild, the closer the birds get to humans, the worse for their welfare, since this closeness can lead to their capture and negative relations with human

populations (BERKUNSKY *et al.,* 2017; LOPES *et al.,* 2018). However, for commercial breeders and birds kept in captivity as pets, proximity would be considered a positive factor, as it would indicate a more positive human-animal relationship (AENGUS & MILLAM, 1999, MEEHAN & MENCH, 2006). That said, it's up to the protocol assessor to determine whether the test result is positive or negative in view of the objective of keeping the birds under human care (whether they will be temporary or permanent).

The flight capacity test allowed to assess how well birds could use the entire environment in which they are kept, and if the structures present allowed animals that are unable to fly to explore the enclosure. In addition, animals that are unable to fly have a reduced quality of welfare in the enclosure, since flight is the main method of locomotion for birds and its impediment can have negative consequences for their welfare (MAPLE & PERDUE, 2013). In the enclosures of CETAS-JF and projects such as the ASAS area, these precautions must be doubled, as many of the animals of their groups come from trafficking and many are missing pelvic limbs and/or flight feathers, making their locomotion much more difficult than that of healthy animals. Another relevant factor for these sites is the number of chicks that grow up at CETAS-JF in enclosures without flight stimulation, due to its limited size. Thus, these birds that have not been properly stimulated may have difficulties maintaining flight and may therefore need flight training, which in addition to improving their flight skills, may result in a greater variety of behaviors reproduced after their release (LOPES *et al.,* 2017). In this way, the difficulty of flight is a factor that reinforces the need for the physical complexity of the environment to be sufficient for these animals, which are unable to fly, to be able to access all parts of the enclosure, reducing the development of unwanted behaviors (KEIPER, 1969; MEEHAN *et al.,* 2004).

The aim of drawing up a WAP for psittacines is to be able to provide a score based on the welfare quality of the site being assessed (BLOKHUIS, 2008; HENRIKSEN & MØLLER, 2015). Quantifying the welfare score is a long process and requires the protocol to be tested in different locations that allow variable recording of a wide range of situations in relation to the welfare score (CLEGG, *et al.,* 2015; BARRY, *et al.,* 2019). The WAP proposed here is an initial stage, where the parameters for assessing welfare were selected and tested for their feasibility. It should be noted that one of the Welfare Quality® criteria, the emotional state, measured using the qualitative behavioral assessment - QBA, was not included in the protocol due to the difficulty in obtaining reliability for this measure, as in other similar studies (CLEGG, *et al.,* 2015; KHATTAK, *et al.,* 2019). Future studies should assess the reliability and repeatability of the selected parameters, followed by the possible inclusion of QBA. In addition, the application

of the protocol in various locations will allow the development of a scoring standard in order to quantify how positive or negative welfare is in the study areas and to objectively and numerically rank locations in terms of psittacines welfare.

Conclusion

The proposed WAP for psittacines is feasible and easy to interpret, and can be used as a practical tool for assessing the welfare of psittacines kept under human care in the short and long term, evaluating different aspects of welfare. This can provide an alternative for rescue centers, breeding facilities, zoos and other institutions that kept psittacines. The validation and repeatability of the protocol and the reliability of the measurements should be assessed in future studies.

Animal Welfare Implications

The development of a welfare assessment protocol has the potential to facilitate the assessment of the welfare of Psittaciformes kept under human care. This allows to identify and correct management critical points, in order to allow for a better quality of life in zoos, breeding facilities and domestic environments.

Competing interests

None.

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Appendix 1

CAPTIVE PSITTACINE WELFARE ASSESSMENT PROTOCOL

MANAGEMENT QUESTIONNAIRE

How is the food stored?

How is the animal health management?

How are infectious diseases controlled?

How many psittacines are housed in the establishment?

The animals are divided into how many enclosures?

How many animals are housed alone? Why?

How many animals are received at the establishment each year?

What is the annual mortality rate?

Are there facilities where animals can receive medical treatment?

Are the animals undergoing treatment housed separately from the others?

How many animals are currently being treated?

What kind of food do you feed the animals? In what proportions? What is the size? (pellets and fruit cuts)

What are the feeding times?

Is the water changed daily?

What is the type of lighting?

1 - Natural lighting
2 - Artificial lighting for at least 12h.
3 - Artificial lighting for less than 12 hours.

Are animals allowed to bathe regularly?	1 – yes	2 – no.

Are environmental enrichments provided? What kind? How many times a week? Is there monitoring?

Is analgesia or anesthesia used in invasive veterinary procedures?	1 – yes	2 – no
Is the animal properly captured and handled?	1 – yes	2 – no

Appendix 2

Measurements of the environment

Evaluate the hygiene of the feeders in scores:	1- clean	2 - dirty	3- very dirty	
Assess the availability and accessibility of feeders for all the animals housed.	1- accessible	2 - not very accessible	3- inaccessible	
Is the food big enough for the birds to handle with their beaks and feet?	1- yes	2- no		
What is the water supply like?				
Can the animals use the drinker without difficulty?	1 - yes	2 - no		
Assess the hygiene of the drinking bowls:	1- clean	2 - dirty	3- very dirty	
Is there enough water for all the animals housed?	1 - yes	2 - no		
Assess the availability and accessibility of drinking bowls for all the animals housed.	1- accessible	2 - not very accessible	3- inaccessible	
Assess the cleanliness of the housing:	1- clean	2 - dirty	3- very dirty	
What type of floor?	1 - soil with vegetation cover.	2 - soil	3 - masonry	
Where are the animals perched (in %)?	1 - perches	2 - wire mesh	3 - platforms	4 - floors.

What material are the perches made of?	1 - wood or tree branches	2 - plastic	3 - metal.	
How many perches are of adequate thickness?	1 - all	2 - more than half	3 - less than half	4 - None
How many perches overlap partially or completely?				
How many platforms does the enclosure have?				
Are there any sharp structures, nails, string, wires or structures on which the animal could get stuck or hurt itself in the housing?	1 - yes	2 - no		
Does the enclosure have any hiding places if the animal doesn't want to be seen?	1 - yes	2 - no		
Is the animal visually exposed to possible predators?	1 - no	2 - yes		
Is there a constant source of noise pollution?	1 - no	2 - yes		
Is the ratio of covered to uncovered area ideal?	1 - yes	2 - Insufficient coverage.	3 - fully covered	4 - fully uncovered
Is the animal able to move between warmer or cooler areas of the enclosure?	1 - yes	2 - no		
How many animals are panting, with their wings and beaks open?	1 - none	2 - less than half	3 - more than half	4 - all.
How many animals are curled up with their feathers ruffled?	1 - none	2 - less than half	3 - more than half	4 - all.
What is the available area and volume per animal?				

Do perches allow exploration of the vertical and horizontal environment?	1 - sim	2 - no
Are animals unable to fly able to explore most of the environment?	1 - sim	2 – no
Does the distance between perches encourage flight?	1 - sim	2 – no
Does the housing allow vertical and horizontal flight?	1 - sim	2 – no
Are there environmental enrichment items in the enclosures?	1 - sim	2 - no
What are they? (describe).		
Is there a double wire mesh or sufficient distance between the pens to prevent the animals from pecking each other's feet?	1 – yes	2 – no
Is there any change in the feces in the enclosure?	1 – yes	2 – no

Appendix 3

Measurements obtained from animals - Health

How many animals have dirt or feces on their feathers and/or other parts of their body?	1 - none	2 - less than half	3 - more than half	4 - all.
How many animals in general appear to be in good health, with no obvious signs of injuries and/or fractures?	1 - all	2 - more than half	3 - less than half	4 - all.
Do any animals have ringing injury?	1 - none	2 - less than half	3 - more than half	4 - all.
Are any animals listless or prostrate?	1 - none	2 - less than half	3 - more than half	4 - all.
Do any animals have breathing difficulties?	1 - none	2 - less than half	3 - more than half	4 - all.
Do any animals have scabs around their eyes or beaks?	1 - none	2 - less than half	3 - more than half	4 - all.
Do any animals have paws, feathers, beaks or nails that are abnormal in size, color or texture?	1 - none	2 - less than half	3 - more than half	4 - all.
Do any animals have clipped wing feathers?	1 - none	2 - less than half	3 - more than half	4 - all.

Appendix 4

Measurements obtained from the animals - Behavior

How many animals are using environmental enrichment?	1 - all	2 - more than half	3 - less than half	4 - none.
How many animals perform positive social behavior?	1 - all	2 - more than half	3 - less than half	4 - none.
How many animals vocalize continuously and loudly?	1 - none	2 - less than half	3 - more than half	4 - all.
How many animals make human-like vocalizations?	1 - none	2 - less than half	3 - more than half	4 - all.
How many animals perform negative social behavior?	1 - none	2 - less than half	3 - more than half	4 - all.
Do any animals pluck feathers?	1 - none	2 - less than half	3 - more than half	4 - all.
Do any animals make repetitive movements for long periods of time, such as pecking, gnawing, manipulating objects or moving around?	1 - none	2 - less than half	3 - more than half	4 - all.

Appendix 5

Behavioral tests

Proportion of animals that approach the observer.	1 - none	2 - less than half	3 - more than half	4 - all.
Proportion of animals that accept food from an unknown observer.	1 - none	2 - less than half	3 - more than half	4 - all.
Proportion of animals that accept being touched (less than 10 cm close) by an unknown observer	1 - none	2 - less than half	3 - more than half	4 - all.
Are there any animals that can't fly?	1 - none	2 - less than half	3 - more than half	4 - all.
Do any animals have difficulty locomoting?	1 - none	2 - less than half	3 - more than half	4 - all.

Appendix 6

Protocol application guide

Management Questionnaire

-How is food stored?

Seeds and feed should be kept in closed compartments, off the ground. Fruit and vegetables should preferably be stored refrigerated. In colder environments, they can also be stored in ventilated areas and off the ground, as an intermediate storage condition.

1 - Adequate
2 - Partially adequate
3 - Inadequate

-How is the health management of the animals?

Sanitization of materials, feeders and drinkers and cleaning of enclosures and quarantine areas must be carried out daily. Medical prophylaxis must be carried out by a trained professional.

1 - Adequate
2 - Partially adequate
3 - Inadequate

-How is infectious disease control carried out?

Control of infectious diseases must be carried out through a control plan created by trained professionals, involving quarantine and treatment of infected animals, sanitization of facilities, observation of exposed animals and recurrent clinical evaluation of the herd.

1 - Adequate
2 - Partially adequate
3 - Inadequate

-How many psittaciformes are housed in the establishment?

Number of animals present in the establishment at the time of the assessment. For information purposes only, not assessed.

-How many nurseries do the animals live in?

Number of enclosures the institution has and the number of enclosures being used. For information purposes only, not assessed.

-How many animals are housed alone? Why?

Number of animals housed alone and the reasons why. For information purposes only, not evaluated.

-How many animals are received at the establishment each year?

Number of animals received over the course of a year. For information purposes only, not evaluated.

-What are the annual mortality rates?

The establishment's mortality rate per year. The percentage value will be used as a score and added to the other scores obtained in the protocol.

-Are there facilities for medical treatment?

Whether or not there are medical facilities for treating the establishment's birds.

1 - Present
2 - Absent

-Are the animals undergoing treatment housed separately from the others?

Whether or not there is a quarantine area for animals undergoing treatment.

1 - Present
2 - Absence

1. Nutrition

CRITERIA: ABSENCE OF PROLONGED HUNGER

- Evaluate the birds' body condition in scores

1 - severely underweight
2 - underweight
3 - adequate
4 - overweight
5 - severely overweight.

1 - Adequate (3)
2 - Partially adequate (2 and 4)

3 - Inadequate (1 and 5)

- What type of feed do you give the animals? In what proportions? What size (pellets and fruit cuts)?

The diet should be mostly extruded feed and supplemented with fruit, vegetables and seeds. The size of the food should be sufficient for the birds to be able to manipulate it with their feet and beak, which will vary depending on the species being assessed.

1 - Adequate
2 - Partially adequate
3 - Inadequate

- Evaluate the hygiene of the feeders in scores.

1 - Clean: clean feeders with no accumulation of dirt.
2 - Dirty: feeders are dirty, but these are few and/or recent.
3 - Very dirty: accumulation of deteriorated food material and dirt in large quantities and/or old.

- Assess the availability and accessibility of feeders for all the animals housed.

1 - Adequate. All birds are able to access the feeders.
2 - partially adequate. Most animals are able to access the feeders.
3 - inadequate. Few animals are able to access the feeder.

- What are the feeding times? Describe.

Feeding times should be early in the morning and late in the afternoon.

1 - Adequate. Early morning and late afternoon.
2 - Partially adequate. Morning and afternoon.
3 - Unsuitable. None of the above.

CRITERION: ABSENCE OF PROLONGED THIRST

- How is the water supply?

Evaluate whether the water supply is made of potable water, in a suitable and hygienic place.

1 - Adequate.
2 - Partially adequate.
3 - Inadequate.

- Can the animals use the drinking trough without difficulty?

1 - Yes. The drinkers are the right height and shape for the bird to be able to take the water without difficulty.
2 - No. The height or shape of the trough makes it difficult for the bird to collect water.

- Are the drinkers clean? Is the water changed daily?

1 - Clean. The water is clear and not dirty.
2 - Slightly dirty. The water is somewhat turbid and dirty.
3 - Very dirty. The water is cloudy with accumulated dirt.

-Is there enough water for all the animals housed?

1 - Yes, there is enough water for all the animals to quench their thirst.
2 - No. The amount of water is insufficient to quench the thirst of all the animals in the vivarium.

- Assess the availability and accessibility of drinking fountains for all the animals housed.

1- Adequate, all the animals are able to access the drinking troughs, even those unable to fly or with locomotion difficulties. There are enough drinkers for the number of birds.
2- Not very accessible. Some birds in the enclosure cannot access the drinker.
3- Not accessible. Most of the birds cannot access the drinker or there are not enough drinkers available for the number of birds.

2. Good housing

CRITERIA: COMFORT AROUND RESTING

-Assess the cleanliness of the accommodation:

1- Clean. The accommodation is clean and free of dirt.
2- Dirty. The accommodation has fresh and/or small amounts of organic matter.
3- Very dirty. The accommodation has a large accumulation of organic matter and/or is old.

-What type of floor?

1 - Ground with vegetation cover.
2 - Soil
3 - Masonry

Determine the type of floor in the vivarium.

-Where are the animals perched (in %)?

1 - perches
2 - side railings
3 - platforms
4 - floors.

Determine the percentage in which each type of support is being used by the birds.

-What material are the perches made of?

1 - wood or tree branches
2 - plastic
3 - metal.

Determine the material of the perches in the nursery.

-How many perches are of adequate thickness?

1 - None
2 - Less than half
3 - More than half
4 - All

The perches must be thick enough for the birds' feet to fit on without the back and front toes meeting.

-How many platforms does the aviary have?

Number of platforms in the vivarium

-How many perches partially or completely overlap?

Proportion of perches that partially or completely overlap. Only count perches that overlap in the same direction.

1 - None
2 - Less than half
3 - More than half
4 - All

-What type of lighting?

1 - Natural lighting.
2 - Artificial lighting for at least 12h.
3 - Artificial lighting for less than 12h.

-Are there any sharp structures, nails, string, wires or structures that the animal could get stuck in or hurt?
1 - yes. There are structures that could injure the animal.
2 - no. The number of structures is minimal or non-existent.

-Are the animals allowed to be bathed on a regular basis?
1 - Yes. The animals are able to bathe either in natural showers, artificial showers or natural or artificial bodies of water.
2 - No. The animals are not able to bathe in any of the above ways.

-Does the accommodation have any hiding places if the animal doesn't want to be seen?

1 - Yes. There are cavities, physical barriers or perches at heights that allow the animal to hide.
2 - No. There are no ways for the animal to hide.

-Is the animal physically and/or visually exposed to possible predators?

1 - No. The animal has no exposure to any predators.
2 - Yes. The animal is housed near predators that are able to see it, or it is housed in accommodation that allows potential predators access.

-Is there a constant source of noise pollution?

1 - No. The environment has low noise and the sounds of other animals.
2 - Yes. The environment has loud noises, sounds from radios, visitors, sounds from equipment or some other form of noise pollution.

CRITERIA: THERMAL COMFORT

-Is the ratio of covered to uncovered area ideal?

1 - Yes. All the birds can find shelter in both the covered and uncovered areas.
2 - Insufficient or too much cover.
3 - Fully covered
4 - Fully uncovered

The ideal environment is able to house all the birds in the enclosure in both its uncovered and covered areas, allowing them to enjoy the characteristics of each environment.

-Is the animal able to move between warmer or cooler areas of the enclosure?

1 - Yes. The animal is perfectly capable of selecting the area with the temperature it likes best.
2 - No. The vivarium does not allow the animal to move between cooler or warmer areas.

-How many animals are panting, with their wings and beaks open?

1 - None
2 - Less than half
3 - More than half
4 - All

-How many animals are curled up with their feathers ruffled?

1 - None
2 - Less than half
3 - More than half
4 – All

CRITERIA: EASE OF MOVEMENT

-What is the available area and volume per animal?

Calculate the available area of the ponds and divide by the number of animals. Ibama (2015) states that for small birds (< 25cm) the maximum density should be 2 individuals per m^2. For medium-sized birds (≥ 25 cm or ≤ 55 cm) the maximum density should be 2 birds per $5m^2$. For large birds (> 55 cm) the maximum density is 2 individuals per $10m^2$.

1 - Adequate.
2 - Inadequate.

-Do the perches allow exploration of the vertical and horizontal environment?

1 - Yes. The perches are arranged in such a way that the animal is able to move around and explore the whole environment, moving from one perch to another both vertically and horizontally.
2 - no. The perches are not connected in such a way as to facilitate the animal's movement vertically and/or horizontally.

-Are animals unable to fly able to explore most of the environment?
1 - yes . The animals are able to explore most of the environment through perches, platforms and bars.
2 - no. The animals are unable to use perches, platforms or bars to explore most of the environment.

-Does the distance between perches encourage flight?
1 - Yes. The distance between the perches is sufficient for the birds to fly from one to the other.
2 - No. The distance does not allow flight between perches.

-Does the house allow vertical and horizontal flight?
1 - Yes. The size of the house is sufficient for the birds to make short or long flights both vertically and horizontally.
2 - No. The size of the house does not allow for short or long vertical or horizontal flights.

-Is environmental enrichment available in the enclosures? What are they?

1 - Yes, there are environmental enrichments in the enclosure.
2 - No, there are no environmental enrichment items.

Describe the types of environmental enrichment present in the enclosure.

-Is the use of environmental enrichment monitored?

1 - Yes. Environmental enrichment is monitored to assess whether it is being used.
2 - No. Environmental enrichment is not monitored.

3. Good Health

CRITERIA: ABSENCE OF INJURIES

-How many animals, in general, appear to be in good health, with no obvious signs of injuries and/or fractures?

1 - All of them
2 - More than half
3 - Less than half
4 - None

Evaluate injuries, fractures and/or other visible alterations during the clinical inspection.

-Does any animal have difficulty moving?

1 - None
2 - Less than half
3 - More than half
4 - All

Evaluate during the locomotion difficulty test and clinical inspection whether the birds have any physical alterations that result in difficulty moving around on the floor, bars, platforms or perches.

-Does any animal have a ring injury?

1 - None
2 - Less than half
3 - More than half
4 - All

During the clinical inspection, assess the proportion of animals that have a ring garroting the leg.

- In side-by-side pens, is there a double railing or sufficient distance between them to prevent the animals from pecking each other's toes?

1 - Yes. The animals are not able to peck the animals in the next pen.
2 - No. The animals are able to peck the toes of the individuals in the next pen.

CRITERIA: ABSENCE OF DISEASE

-Are there any apathetic or prostrate animals?

1 - None
2 - Less than half
3 - More than half
4 - All

Assess whether there are any birds in the enclosure that are shrunken and have ruffled their feathers, show little responsiveness, are prostrate or apathetic.

-Is there any change in the droppings in the enclosure?

1 - No. The droppings are in their normal state of color and consistency.

2 - Yes. The feces are altered in color and/or consistency.

-Does any animal have breathing difficulties?

1 - None
2 - Less than half
3 - More than half
4 - All

Evaluate during the clinical inspection whether the bird is having difficulty breathing, obstruction of the air cavities or the presence of breathing noises.

-Does any animal have scabs around the eyes or beak?

1 - None
2 - Less than half
3 - More than half
4 - All

During the clinical inspection, assess the presence of scabs resulting from eye secretions or nasal discharges, wounds or pathological changes around the eyes and beak.

-Does any animal have paws, feathers, beak or nails with abnormal size, color or texture?

1 - None
2 - Less than half

3 - More than half
4 - All

During the clinical inspection, assess changes in the feet, feathers, beak and nails that indicate abnormal color, size or texture.

CRITERIA: ABSENCE OF PAIN CAUSED BY MANAGEMENT PROCEDURES.

-Is analgesia or anesthesia used in potentially painful veterinary procedures?

1 - Yes. Analgesics or anesthetics are used in procedures with the potential to cause pain to the animal.
2 - No. Procedures with the potential to cause pain are carried out without the use of analgesics or anesthetics.

-Are the animal captured and handled properly?

1 - Yes. Handling is carried out in a careful and non-violent manner, with the correct technique, which consists of immobilizing the bird's head with the hands while immobilizing the wings and legs with a towel that covers and wraps the bird, taking care that the pressure exerted does not prevent the expansion of the air sacs and that the bird does not suffer from hyperthermia or excessive stress. The bird must be caught using towels, pikes or nets.
2 - No. Handling is carried out with excessive force, lack of restraint skills or without the use of suitable equipment.

-Do any animals have their wing feathers clipped?

1 - None
2 - Less than half
3 - More than half
4 - All

Assess the integrity of the wing feathers during the clinical inspection.

4. **Comportamento Apropriado**

Behavioral category	Description
Inactive (number of inactive animals during observation time)	The psittacine remains in a neutral posture, standing on the ground, on the screen or on the perches, with its eyes open or closed;
Positive social interaction (occurrences)	Close approach and perching, less than 1 cm.
Negative social interaction (occurrences)	Agonistic interactions (when the bird opens its beak, vocalizes and pecks at some part of another bird's body); Threatening to peck or hit another bird with its feet.
Feeding (number of animals feeding)	Feeding on the food available in the feeder.
Interaction with environmental enrichment (number of animals interacting)	The bird approaches and touches the food or physical enrichment items with its beak or feet.
Loud vocalization (number of animals vocalizing during the observation period)	Loud, shrill and/or repeated vocalizations in a short space of time, showing stress.
Abnormal behavior (number of animals displaying abnormal behavior during the observation period)	Repetitive behaviors; bird repetitively pecking at the screen or walking from side to side; bird pulling its feathers; Imitation of human speech;

CRITERIA: EXPRESSION OF SOCIAL BEHAVIOR

-Are the animals using environmental enrichment?

1 - All
2 - More than half
3 - Less than half
4 - None

Use the ethogram to assess whether the birds are interacting with the environmental enrichment.

-Do the animals perform positive social behavior?

1 - All
2 - More than half
3 - Less than half
4 - None

Use the ethogram to assess whether the birds are performing positive social behavior, defined as perching at a distance of less than 1cm.

-How many animals vocalize continuously and loudly?

1 - None
2 - Less than half
3 - More than half
4 - All

Use the ethogram to assess whether the birds are vocalizing continuously and loudly.

Are the animals performing negative social behavior?

1 - None
2 - Less than half
3 - More than half
4 - All

Evaluate through the ethogram whether the birds are performing negative social behavior, through agonistic interactions or threats of aggression.

CRITERIA: EXPRESSION OF OTHER BEHAVIORS

- Are there animals that can't fly?

1 - None
2 - Less than half
3 - More than half
4 - All

During the locomotion difficulty test, assess whether the birds have difficulty taking flight.

-Does any animal pluck its feathers?

1 - None
2 - Less than half
3 - More than half
4 - All

Evaluate during the application of the ethogram how many birds show feather plucking behavior among the undesirable behaviors presented.

-Does any animal make repetitive movements for long periods of time, such as pecking, gnawing, manipulating an object or moving around?

1 - None
2 - Less than half
3 - More than half
4 - All

Evaluate during the application of the ethogram how many birds show repetitive behaviors for long periods of time, these movements being carried out by pecking, manipulating some object or moving around, within the undesired behaviors presented.

How many animals make human vocalizations?

1 - None
2 - Less than half
3 - More than half
4 - All

During the application of the ethogram, assess how many birds make human vocalizations, within the undesired behaviors presented.

CRITERIA: HUMAN ANIMAL RELATIONSHIP

While institutions such as CETAS and ASAS aim to release birds and human-animal relationships should be avoided, institutions such as commercial breeders and zoos need a closer human-animal relationship. Thus, the measures in this criterion will be scored based on the institution's objective and it is up to the assessor to determine the score in these cases, following the pattern that 1 represents the best score and 4 the worst.

-Proportion of animals that accept being touched by an unknown observer.

none
less than half
more than half
all.

Use the escape distance test to see how many animals accept being touched or approached closer than 10cm.

-Proportion of animals that accept food from an unknown observer.

none
less than half
more than half
all.

Assess how many animals accept food from a stranger using the food offer test.

-Proportion of animals that approach the observer.

none
less than half
more than half
all.

Use the voluntary approach test to see how many birds approach the observer.

ANIMAL MEASUREMENTS ASSESSMENT WORKSHEET

Species	Score	Garrote	Feather condition	Integument alteration	Eye/beak scabs	Injuries	Notes

BEHAVIORAL RECORDING WORKSHEET

Observer:		Date:		Start:		End:		Group:	
Hour	Pen	Inactive	Int-enr	Soc-pos	Aliment	Soc-neg	Vocaliz	Comp-anor	Notes

BEHAVIORAL TESTING WORKSHEET

Date		Observer		Period	
Pen	Voluntary approach	Escape distance	Food offer	Locomotion difficulties	Notes: