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A Welfare Protocol to Assess the Welfare of Huemul (*Hippocamelus bisulcus*) in Conservation Centers

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Simple Summary: Animal welfare is an important aspect of conservation programs for endangered species. Wild species can be bred and kept in captivity but, unlike domestic animals, there is a lack of welfare assessment protocols for most of these species. In this study, we have developed a protocol for the assessment of the welfare of Huemul (*hippocamelus bisulcus*) in conservation centers. We have gathered the existing literature on the behaviour, ecology, conservation plans and. captive management for this species. We used a welfare assessment protocol for cattle as the base and suggested 23 indicators that we considered useful to assess welfare in huemuls. This proposed protocol, which is the first existing protocol for assessing huemul welfare, is rigorous and systematic but also simple and practical. However, further research is needed to validate the protocol in conservation centers.

Abstract: Animal welfare assessment protocols are important for identifying welfare problems in conservation programs. This study aimed to develop a baseline welfare protocol for the assessment of welfare in huemul (*Hippocamelus bisulcus*) in conservation centers. This protocol is based on the Welfare Quality® (WQ) framework for cattle, considering the available literature on the behaviours, ecology, conservation plans, captive management of this species, and welfare assessment protocols for other ungulate species. As a result, the protocol was specifically developed for huemuls and included four principles, 12 criteria, and 23 animal- and resource-based indicators. The 12 criteria of the WQ protocol were reduced to nine, and three new criteria were added because they were both feasible and essential for welfare assessment in captive huemuls. This protocol is mainly intended to identify welfare problems in endangered species in the context of conservation centers (reproduction, rescue, rehabilitation, or treatment centers). Thus, aggregation of different measures to obtain a global score has not been proposed. However, a scoring system that assigns a value on a 0-2 scale (0= No welfare concern; 1= welfare concern; 2= urgent welfare concern) is proposed for each category. Although further research is still needed to fully validate the protocol, this is the first development of a completed protocol for huemul welfare assessment.

Keywords: behaviour; captivity; hippocamelus bisulcus; huemul; animal-based measures; welfare assessment; deer

1. Introduction

Animal welfare is an important aspect and is a priority of "ex-situ" conservation programs [1]. Guaranteeing the best welfare status of captive animals is important, as well as ensuring that captive breeding programs are robust and sustainable [2]. Well-being is an attribute of each individual and cannot be measured directly but must be evaluated using indicators, which are variables that can be measured objectively and provide information on the well-being of animals [1]. Welfare evaluation protocols combine several indicators and include a description of how to measure each using simple surveys, enclosure inspections, and animal observations [3]. Welfare Quality® (WQ) protocols are among the most used for evaluating the welfare of farm animals (bovines, pigs, and poultry) [4].

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The *Hippocamelus bisulcus* (huemul), an endemic species of Chile and Argentina, is part of the Cervidae family and the southernmost deer in the world [5]. Currently, it inhabits fragmented areas in the southern regions of both countries, ranging from sea level to 3,000 m above sea level, inhabiting areas covered mainly by Nothofagus forests, dwarf shrubs, rocky slopes, and meadows [6]. The diet of ruminants is strictly herbivorous. Although a preference for leaves, bush shoots, trees, and grasses has been described [6], the diet can vary substantially from one subpopulation to another [7].

This species has been declared endangered worldwide, with a declining population trend and an estimated current population of no more than 1,048–2,000 individuals [6–8]. In Chile and Argentina, there is currently only one private center for the conservation and reproduction of the huemul, located in the region of "Los Ríos," in southern Chile (39º51´S, 71º57´W), and it is expected that other centers will be opened in the future [6]. To date, there have been no published protocols to assess the welfare of this deer species, which could improve both the quality of life of captive individuals and their impact on conservation. Therefore, this study aimed to design and develop a welfare assessment protocol for captive huemuls in centers for conservation purposes.

2. Materials and Methods

The protocol is based on the WQ framework for cattle and has been adapted for use with huemuls because of its widely accepted structure and standards, comprehensive design, and potential application to other species in managed care [3,9]. Of the 12 WQ criteria, three were modified considering the current knowledge of the biology of the species, the difficulty of evaluation, and some new protocol proposals for other ungulate species [3,10]. The indicators in the assessment were developed predominantly using published literature, describing wild and captive huemul health, behaviour, and ecology as well as information on the welfare, health, and behaviour of other deer species. For each criterion and indicator, we used the following search words related to it: Hippocamelus bisulcus/huemul/deer/ungulate, which were used in the Google Scholar and Web of Science™ search engines. A total of 64 papers were reviewed and we found useful information to add to the protocol in seventeen of the papers, of which three papers provided useful detailed information on the behaviour and general biology [6,11,12] and eight for health of the species [13–20]. We also carried out searches in libraries of Institutions in Chile and Argentina, where different documents provided information on historical conservation plans for this species.

This protocol is mainly intended to be used to identify welfare problems in endangered species in the context of conservation centers. Thus, aggregation of different measures to obtain a global score has not been proposed. However, a scoring system that assigns a value on a 0-2 scale (0= no welfare concern, 1= welfare concern, 2= urgent welfare concern) was proposed for each category. This would facilitate the monitoring of welfare evolution over time and/or compare different conservation centers in the future to identify the best care practices.

For this protocol, "animal group" should be understood as all the animals that are in the same facility, as a family group, or as unrelated individuals.

3. Results

3.1. Development of the Welfare Protocol

The protocol developed for the welfare assessment of captive huemul included four principles, 12 criteria, and 23 indicators (Table 1). The 12 criteria of the WQ protocol were reduced to nine. One farm-related criterion was excluded (absence of pain induced by management procedures) since the handling and contact between humans and huemul tend to be absent or tiny for reintroduction purposes. Moreover, the type of handling performed on these animals did not include any practices addressed by this criterion in the WQ. The criterion of comfort around rest was removed because of the difficulty of its measurement given the field conditions. The criterion 'positive emotional state' was also removed because there was a lack of valid indicators in this species. In addition, the criteria "presence of minerals in the diet" and "adequate social environment" were added, as they were considered essential for welfare assessment in captive huemuls and measurable. A description of the

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12 criteria together with their proposed indicators and justifications is presented in the following sections.

Table 1. Principles, criteria, and indicators of the protocol to assess welfare in captive huemuls.

Principles	Criteria	Indicators	Indicator Type
Good Feeding	1. Absence of prolonged hunger	1.1 Body condition	Animal-based
	2. Absence of prolonged thirst	2.1 Availability of water	Resource-based
	3. Presence of minerals in the diet	3.1 Adequate mineral supplementation	Resource-based
Good Housing	4. Thermal comfort	4.1 Availability of shade	Resource-based
		4.2 Availability of shelter	Resource-based
	5. Ease of movement	5.1 Enclosure size	Resource-based
	6. Adequate Enclosure standards	6.1 Perimeter fence	Resource-based
		6.2 Quarantine zone	Resource-based
Good Health	7. Absence of disease	7.1 Nasal discharge	Animal-based
		7.2 Ocular discharge	Animal-based
		7.3 Hampered respiration	Animal-based
		7.4 Diarrhoea	Animal-based
	8. Absence of injuries	8.1 Lameness	Animal-based
		8.2 Integument alterations	Animal-based
Appropriate Behaviour	9. Expression of social behaviour	9.1 Affiliative behaviour	Animal-based
		9.2 Intra-specific aggression	Animal-based
	10. Expression of other behaviours	10.1 Stereotypies	Animal-based
		10.2 Environmental enrichment	Resource-based
	11. Good animal handling	11.1 Caretakers training program	Resource-based
		11.2 11.2 Capture, immobilization, and handling	Resource-based
	12. Adequate social environment	12.1 Number of huemuls	Resource-based
		12.2 Composition of the group	Resource-based
		12.3 Presence of animals (other species)	Resource-based

3.1.1. Absence of Prolonged Hunger

Both poor and excessive body conditions are indicative of a welfare problem and have been previously described as a management concern for captive deer [21]. To date, no body-condition scale has been developed for huemuls. Therefore, the guidelines for the evaluation of this indicator in another deer (*Cervus elaphus*), which uses a 5-point scale [22,23] were followed, but for this protocol, they were changed and reduced to a three-point scale.

Adult animals (older than one year) were visually evaluated from the lateral and posterior views. A scale of 0 to 2 was used, where 0 = adequate body condition; the pelvis, ribs, and spine were not easily distinguished or appeared rounded rather than sharp; the rump area was flat or slightly convex; 1 = leanness, pelvis, ribs, and spine prominent and concave area of the rump; 2 = animal with a destitute condition, cachexia (croup very concave, column visible), or fat condition (wings of the pelvis were concealed under a thick layer of fat; sacral spinous processes were well enveloped, and the rump areas were convex).

3.1.2. Absence of Prolonged Thirst

Access to high-quality water ad-libitum is one of the most important welfare requirements for most animals [10]. Ideally, huemuls must have access to watercourses, as seen in the wild [24,25]. The presence and availability of water sources throughout the year for each animal group should be visually evaluated. The indicator was scored as either 0 (presence of at least one permanent water source per animal group) or 2 (absence of at least one permanent water source per animal group).

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3.1.3. Presence of Minerals in the Diet

To maintain normal physiological functions, wild ungulates often seek a source of salt [10]. Due to the habitat conditions of the huemul, some studies described micronutrient deficiencies for this species affecting growth, skeletal development, reproduction, and immuno-competence [26–28]. In addition, it has been observed that huemuls that have accessed blocks of mineral salts have a better coat and antler condition, and females that have given birth to heavier offspring have been recorded [29]. The current protocol included one indicator (3.1) that was assessed for each animal group. At least two blocks of mineral salt (3 kg) were visually inspected. The scoring system was 0 when mineral salt blocks were present, and 2 when no blocks were present.

3.1.4. Thermal Comfort

In captivity, there may be long-term exposure to temperatures different from those in the natural habitat, which can eventually result in heat shock and stress in animals [3]. Due to its natural habitat, huemul is better adapted to tolerate low to medium temperatures than to high temperatures. In general, wild and captive deer require protection from direct sunlight [30,31]. In winter, it seeks refuge in mixed forest and grassland areas [6].

Two indicators were developed to assess thermal comfort: availability of shade (4.1) and shelter (4.2). To assess these indicators, it must be recorded whether all animals in each facility in the center have access at the same time to adequate shade and shelter simultaneously. The presence of natural shadows (e.g., trees), scrub areas, forests, and artificial structures in conservation centers must be visually evaluated. Satellite-type botanical records and geographical charts are required to determine the resource availability throughout the year. The score was 0 for the presence of resources and 2 for the absence of resources.

3.1.5. Ease of Movement

Limited information is available on the minimum space per animal in captivity for huemuls [24]. Recommendations suggest that areas of up to 50 ha are needed for semi-captivity, but densities or other parameters considering the number of individuals are not shown [32]. Other studies on national parks in Chile have reported 309 ha or 3 km2 per established family group of up to four individuals [12]. According to the experience of the only huemul conservation center in Chile (Huilo Huilo Reserve), it has 64 hectares allowing to have 7 adult animals (density 10/km2) with good results for the reproduction of huemuls [25].

An indicator was developed to assess ease of movement in each animal group. The area of the enclosure must be calculated, and the number of hectares must be determined. The enclosure was scored as follows:0 “size of the enclosure> 64 hectares”; 1 “size of the enclosure> 50 and <64 hectares”; 2 “size of the enclosure <50 hectares”.

3.1.6. Adequate Enclosure Standards

Enclosures designed and constructed for the rearing and conservation of wild animals must meet the highest criteria for any standard enclosure in which the animals are housed [10]. The perimeter fence of the huemul enclosure must be at least 3.5 meters high, have double wiring with material approved for deer, electrified wire in the upper part, single in the middle, and double in the lower part, working permanently [25]. It must have vegetation inside and not trees outside to allow predators to enter [32]. The availability of quarantine facilities must be ensured to ensure effective captive management and breeding programs [10]. The quarantine for all species must be under the supervision of a veterinarian and must consist of a minimum of 30 days unless otherwise directed by the center veterinarian [33].

Two indicators have been developed. The presence of a perimeter fence and an area exclusively dedicated to quarantine should be visually evaluated, as should conditions in terms of measurements and materiality. Satellite-type photographic and botanical records are required to determine the daily availability of vegetation in the internal part of the perimeter of the entire fence throughout the year.

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In addition, annual records of animal income, time spent in this area, and veterinary medical professionals responsible for inspection and handling should be requested. The enclosure was scored as follows:0 “presence of perimeter fence and quarantine area” and 2 “absence of the resources”.

3.1.7. Absence of Disease

WQ protocols for ruminants include indicators that can be used to assess gastrointestinal and respiratory conditions through remote observation [3]. There are many reports of findings of different pathogens present in populations of huemul, such as viral diseases like bovine viral diarrhoea [13] and parasitic [14,15], mainly due to the contact with domestic animals and the introduction of exotic herbivores. Huemul feces are small and rounded, black in colour like those of a sheep; however, depending on the diet they can be cylindrical in shape [34]. In wild deer including huemul, the presence of Mycobacterium avium subsp. paratuberculosis can cause the disease "Paratuberculosis," which can present among other clinical signs with diarrhoea [18,19].

Four indicators were proposed: "nasal discharge" (7.1), "ocular discharge" (7.2), "hampered respiration" (7.3), and "diarrhoea" (7.4). All these indicators were validated in the WQ [4] for the welfare assessment of cattle. However, in the case of the “ocular discharge indicator” given the body size of the huemul, and that the number of individuals to be evaluated is less than that used in cattle farms, we are interested in evaluating the health of each of the individuals, in this protocol we will evaluate both eyes and we use the indicator described for horses [35]. The diarrhoea indicator was developed based on the indicator described for sheep and goats [36,37]. If any indicators were observed, animals were assessed as 2 “presence of the sign” and 0 "when animals had no sign of these indicators”.

3.1.8. Absence of Injuries

Two indicators were developed, lameness (8.1) and integument alterations (8.2). Hoof problems are common in ruminants [3]. Some causes of lameness have been described in huemuls, which show clinical signs of variable severities [20]. Moving animals were visually observed from behind and from the side on a flat, non-slippery surface. The moment and rhythm of the steps will be evaluated, as well as the weight bearing on each limb [4]. Animals must be scored as 0 = “no lameness, correct timing, rhythm, and support; 1 = “moderate lameness, imperfect rhythm, only one leg affected”; 2 = “severe lameness, alteration when bearing weight on one limb, or more than one leg affected”.

Integument alterations such as patches and lesions without hair or swelling can be a consequence of disease, rough handling, intraspecific aggression, interspecific aggression, or an inappropriate physical environment [3]. However, caution must be exercised since the huemul is a species that presents "molting" or change of the coat, which molts 2 times a year, in autumn and spring [6,34]. Recently, infectious and parasitic diseases have caused alterations in integuments of different regions of the body [6,16,17].

Only skin changes with a minimum diameter of 2 cm to the greatest extent should be counted. A hairless patch includes an area of hair loss with undamaged skin and extensive thinning of the coat owing to parasites and hyperkeratosis. Injury or swelling included skin damage in the form of scabs or wounds, dermatitis due to ectoparasites, and ear injuries due to torn ear tags. Without touching the animals, three body regions on both sides of the tested animal were examined: body, hind leg, and front leg. These body regions were scanned from back to front, excluding the lower side of the abdomen and the inner side of the legs but including the inner side of the opposite hind leg. Animals were scored as follows:0 = “no alteration of the integument (no hairless area, no injury, no inflammation)"; 1 = "minor integument alterations: at least one patch without hair, but without injury/swelling"; 2 = "severe integument alterations: at least one lesion/swelling or a large hairless patch".

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3.1.9. Expression of Social Behaviours

Huemuls form small groups of two to four individuals, which can be permanent or transitory with little or no sexual segregation. The minor difference in absolute mass between males and females is atypical in medium-sized deer and could explain the difference in social behaviour between medium-sized deer and other deer species [6]. In wildlife, different affiliative behaviours have been described, highlighting that of males and females in the reproductive stage, between the mother and her offspring, and among juvenile individuals [12].

Antagonistic behaviours must be recorded to assess negative social interactions between members of a group. These behaviours include physical interactions, vocal communication, fighting, and/or chasing [12]. In family groups of huemuls in the wild, expulsion behavior has been observed, which consists of an adult chasing a young animal (older than one year of age) within the territory to initiate its independence [12].

Two indicators were developed: "affiliative behavior" (9.1) and "intraspecific aggression" (9.2). An ethogram (Table 2) was developed based on the social behaviour of huemul [6,11,12], and the information for behaviour patterns described for other species was also used [3,4,10]. The overall observation time was 180 min per group in continuous 20 min sessions [3].

Table 2. Description of the social behaviours (affiliative and aggressive) that are included in the welfare protocol for Huemul (Adapted from [3,4,6,10–12]).

Type of Behaviour	Behaviour Pattern	Description of Behaviour
Affiliative behaviour	Mutual grooming	The animal brushes with its muzzle any part of the body of another group mate except for the anal region or the prepuce. If the animal stops brushing the receiver for more than 10 s and then starts brushing the same receiver again, this is recorded as a new bout. It is also taken as a new bout if the actor starts brushing another receiver or if there is a role reversal between actor and receiver.
	Social smelling	The animal smells any part of the body of another group mate except for the anal region or the prepuce. If the animal stops smelling for more than 10 s and then starts smelling the same receiver again, this is recorded as a new bout. It is also taken as a new bout if the actor starts smelling another receiver or if there is a role reversal between actor and receiver.
	Licking	One animal licks any part of another animal with the tongue except for the anal region or urine. If the actor animal stops licking for 10 s and starts again, it is to be counted as a new bout, regardless of whether the actor licks the same receiver or another. If the actor receives reversal brushing from the receiver, it should also be counted as a new bout.
	Suckling	The behaviour of calves while consuming milk from the udder. A phase during which a calf is allowed to suckle milk from the dam.
	Horning	The head plays with the physical contact of two animals. The animals rub foreheads, horn bases, or horns against the head or neck of one another without obvious agonistic intention. Neither of the opponents takes advantage of the situation to become victorious. It is taken as a new bout if the same animals start horning after 10 s or more, or if the horning partner changes.
Aggressive behaviour	Displacement with physical contact	Interaction is where the actor is butting, hitting, thrusting, striking, pushing, or penetrating the receiver with the forehead, horns, horn base, or any other part of the body with a forceful movement resulting in the receiver giving up its position.
	Displacement without physical contact	The actor threatens or interacts with the receiver without making any physical contact resulting in the receiver giving up its position.
	Chasing	The actor makes an animal flee or give up its current position by following fast or running behind it, sometimes with added threats like jerky head movements. Chasing is recorded even if it is not followed by an interaction with physical contact.
	Fighting	Two contestants vigorously push their heads (foreheads, horn bases, and/or horns) against each other while planting their feet on the ground, both exerting force against each other. A new bout starts if the same

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	animals restart fighting after more than ten s or if the fighting partner changes.

Depending on the time of year in which the evaluation was conducted, the following classifications (Table 3) were used to evaluate the interactions between the animals.

Table 3. Description of the social interaction for Huemuls (Adapted from [6,11,12]).

Interaction	Scale = 0	Scale = 1
Adult male-female interactions	"Male and female rest together. There is vocal communication. Mating behaviours (flehmen, smelling, licking, mounting) are seen in the reproductive season.	"Absence of affiliative behaviour and/or mating behaviours in the reproductive season".
Fawn interactions	"Female maintenance recurrent contact with the fawn by smelling, touching, and licking. It can also be seen in vocal communication and/or playing. If the juvenile is still in the group with the fawn, occasionally play will also be observed".	"Absence the mother-fawn and/or fawn-yearling (juvenile) affiliative behaviour".
Interaction between juvenile or adult individuals in the group	"There is licking and/or smelling, and/or "horning" behaviours"	"Absence of affiliative behaviour".
Adult male-male interactions	"Absence of antagonistic behaviour: snorting, stomping, trashing bushes and/or fighting"	"Presence of antagonistic behaviour included snorting, stomping, trashing bushes and/or fighting"
Yearling interactions	"Absence of expulsion behaviour (adult chasing a yearling)".	"Presence of expulsion behaviour (adult chasing a yearling)".

3.1.10. Expression of Other Behaviours

A behavior that is invariable, repetitive, and without any clear purpose is called a stereotyped behavior [3]. To date, there has been no precedent for stereotypical behavior in captive huemuls. However, wild ungulates in captivity have been reported to be more prone to develop oral stereotypies [1,38]. In captive ungulates, object licking, dirt-eating, and tongue rolling [39] bite the fence [10]. This protocol proposes that all animals should be assessed for the presence or absence of "stereotypical behaviours" (10.1) during observation of the expression of social behavior. Animals were scored as 0 "stereotypes absent" and 1 "stereotypes present".

An enriched environment may be promising for species aimed at reintroduction into the wild [10]. The huemul prefers to live in mixed areas with the presence of forests and open spaces with shrubs and grasses [6]. Although there are few management guidelines for this species [24], guidelines for other ungulate species recommend providing opportunities for animals to perform natural browsing, grazing, fleeing, and/or hiding behaviours. To measure environmental enrichment (10.2), the presence of structural components, such as rocks and irregular terrain, vegetation, forests, and grasslands should be evaluated, and the use of these resources must be recorded through physical presence, grazing, and browsing. The enclosure is scored as follows:0 = "presence and use of resources"; 1 = "presence of resources but its use is not observed during the evaluation"; 2 = "lack of resources".

3.1.11. Good Animal Handling

Two indicators were developed to assess this criterion: caretaker training program (11.1), and capture, immobilization, transport, and handling (11.2). There should be a technical and veterinary team with experience and resources to check and, if necessary, treat sick animals. Avoiding stress is the main consideration, and thus, human contact with animals is kept to a minimum [40]. The training of the personnel that monitors and manages the huemul in a semi-confinement center is of utmost importance to meet the objectives set for the reproduction and subsequent reintroduction of this species [25] and for protecting individual welfare.

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The presence of evidence of training and education programs and protocols should be evaluated. Knowledge of the natural history of this species, the basis of animal welfare, monitoring strategies, and management of animals in semi-confinement, as well as the medicine of wild ungulates, must be accredited. The enclosure was scored as follows:0 = "evidence of knowledge of the species, monitoring strategies and management of animals in semi-confinement, as well as medicine of wild ungulates"; 2 = "absence of evidence of knowledge about one or several areas of training".

The methods used to capture, immobilize, transport, and manipulate animals must be reviewed and approved by the animal health authority of the country where the conservation center is located before performing the procedures [40,41]. In addition, they must consider the characteristics and age (juveniles or adults) of the huemuls, as well as a record of everything that has been done [12,42]. The presence of updated best-practice protocols for capture, immobilization, and handling must be recorded. These protocols should be refined to reduce the stress, fear, health effects, and other negative effects of these activities on animal welfare. The veterinarian responsible for the center must sign the protocol. The enclosure is scored as follows:0 = "presence of protocols and registers; 2 = "absence of protocols and/or records".

3.1.12. Adequate Social Environment

Three indicators were developed: the number of huemuls, composition of the group, and presence of animals of other species. Huemuls are gregarious deer characterized by the formation of small groups that can be permanent or transitory [12]. Permanent family groups are commonly shaped by two to four members [6], and transitory groups of one–11 individuals can be found, depending on the season of the year [43,44]. The number of Huemuls (12.1) per group was recorded. The enclosure was scored as follows:0 = "presence of records of the number of individuals per established animal group and an adequate number of individuals per family group (minimum 2 animals)"; 2 = "no registration of the number of individuals per animal group and/or an inappropriate number of individuals per family group (a solitary individual for more than 3 months)".

Group size is not the only factor to consider, as group composition and compatibility between animals are also important [3]. A permanent family group of huemul can include at least one male, one female, and one or two offspring of different ages [12]. The composition of the animal groups (12.2) in the center should be evaluated visually and utilizing records, and the sex and age of everyone must be recorded. The enclosure was scored as follows:0 = "presence of registration of adequate composition of the group (s) of the center (one male and one female, with or without offspring)"; 2 = "absence of records or inadequate group composition".

The presence of predatory animals such as pumas (*Puma concolor*), foxes (*Lycalopex culpaeus*/*Lycalopex griseus*), and feral dogs (*Canis familiaris*) can negatively affect the well-being and group dynamics of huemuls [25,42]. In addition, the presence of cattle [45] and other species of deer introduced (*Cervus elaphus*), can be a risk factor due to competition for food resources and the transmission of different diseases to the huemul [46]. When assessing the presence of animals of other species (12.3), the conservation center must ensure that the perimeter fence can prevent the entry of other animals (cows, other deer, and predators). The enclosure is scored as follows:0 = "absence of other species of deer, no cattle, and no predators"; 2 = "evidence of the temporary or permanent presence of deer, cattle or predators".

4. Discussion

Initially, interest in animal welfare focused on farm and laboratory animals. Recently, the welfare of pets and zoo animals [47] has gained considerable relevance. Rather, interest in wild animals has been directed primarily toward conservation [1]. However, in recent years, animal welfare has become an important aspect of captive wild animal populations and "ex-situ" conservation programs, not only for ethical and for legal reasons, but also to maintain healthy individuals and populations, ensure the success of the program, since many welfare problems harm reproduction [1,2]. The conservation approach based only on the protection of some sites and

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observational studies is not enough to prevent the huemul from becoming extinct. Existing information gaps can be narrowed by investigating huemul in semi-captivity [48].

Animal welfare comprises the emotional state, physical health, and behaviours of animals [3]. Multiple factors that may influence animal welfare create a holistic approach, with multiple indicators necessary to obtain a clear picture of an animal's welfare status [49]. Therefore, it is necessary to combine several indicators adapted to the biology and ecology of this species [50] to obtain a complete and appropriate welfare assessment for a particular species. This welfare protocol, developed for huemuls, is the first documented work toward developing a standardized welfare assessment tool for this species, which is in danger of extinction [8]. Other protocols have been developed specifically for other wild species in captivity based on WQ [3,51].

In protocols used to assess animal welfare, the criteria and indicators do not always have the same ease of application. Despite being an important criterion for inclusion in the welfare assessment protocol, positive emotional state was not included. This is due to the lack of current research in conservation centers or zoos concerning captive ungulates and thus validated indicators [3,10]. However, indicators to assess the emotional state of huemuls should be added in the future, as knowledge of the emotional states of ungulates increases and validated indicators appear.

Another criterion that was not included in this protocol was comfort while resting. Several behavioural indicators are used to evaluate comfort around rest, including rising and lying down movements (such as the time spent lying down, frequency of lying bouts, and duration of individual bouts) [4,50]. However, to evaluate these indicators, it is necessary to observe the animals when they perform resting behaviours, which is difficult case to evaluate in this species because visualization is often difficult owing to the presence of vegetation and observation at different times of the day. Despite this, it has been described that the huemuls when lying down, first drop one of their front knees, and then the other until it is in a "kneeling" position [34], and during the rest, the huemul observes, sleeps, and "ruminates" [44]. In addition, a seasonal resting pattern has been described for the huemul in the wild [44], with rest being variable throughout the seasons of the year, with three periods in spring-summer (total four to seven hours), while in autumn- winter is reduced to two shorter periods (between 4 and 5 3/4 hours), which has not been described in captive conditions, and for which observation will be required to create a new comfort around resting indicator in the future.

The goal of protocols aimed at assessing the welfare of wild animals kept in captivity is to use them regularly as a management tool in the centers where these animals are held [3]. Protocols developed using the WQ protocol for farm animals as a reference for different species differ in terms of the time required for implementation [10]. The protocol developed for minks and foxes requires three visits to each farm [51]; For bottlenose dolphins, the protocol requires two days for a complete welfare assessment of a dolphin pod, including up to 10 individuals [9]. In the case of protocols for Dorcas's gazelles, which require less than six h per herd (17 individuals) [3], protocols for Punjab urial require 5 h for complete assessment of 23 individuals [10]. The proposed welfare protocol was designed to be practical and could be executed in two days for every four huemuls. This is because the smallest period to evaluate social behavior (affiliative and aggressive) was 180 min in continuous sessions of at least 20 min per group of animals. During this time, it is also possible to evaluate the expression of other behaviours, such as stereotypies and the presence and use of resources in an enriched environment. In addition, we must consider in this protocol that some indicators may be difficult to evaluate with precise results and that the evaluation time may be longer, especially in animals of wild origin such as the huemul, when they are kept in enclosures with dense vegetation. For example, the measurement of body condition and health indicators can take longer than that of other species, given the difficulty in adequately observing each animal on a wide surface that may be covered with vegetation.

Measurements can be broadly separated from those carried out by visual observation while animals go about their daily activities, measurements assessed partly by consulting the person in charge of the conservation center and the records, and those measured opportunistically. Systematic behavioural monitoring is essential to achieve the highest standards of well-being, and, as such, behavioural assessments of huemuls can be applied regularly as a management tool, with or without

a full assessment, as described for other types of animals [9]. In principle, animal welfare assessments should be conducted by trained persons familiar with the methodology, metrics, and evaluation tools [9]. Therefore, those responsible for the conservation center should be trained to use this tool properly.

This protocol is simple, practical, and systematic. However, after conducting practical applications in the field, it must be accompanied by a complete instruction manual with information on technical sheets and photographic and video references. Although the huemul is the only south american deer in danger of extinction according to the IUCN [8], it is the species with the least known information [48]. Our protocol is presented as an initial step in assessing welfare in captivity huemuls; however, its validation has not yet been completed. Among the challenges for the application of this protocol is to evaluate each indicator under field conditions, establish and/or adjust evaluation times, and use powerful equipment, such as the latest generation of binoculars and high-resolution cameras.

5. Conclusions

We developed a protocol for the assessment of the welfare of huemul in conservation centers, using a protocol for the assessment of welfare in cattle as a base, and applying modifications and species-specific adaptations. The first specific protocol developed for huemuls comprised four basic principles, 12 criteria, and 23 animal- and resource-based indicators. This protocol requires validation in conservation centers (reproduction, rescue, rehabilitation, or treatment centers), which may be established in the future to preserve this species. In addition, this protocol can be used as a basis for developing welfare protocols, such as those for other captive deer species.

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