Universitat Autònoma de Barcelona

Assessment of Welfare in Captive Wild Ungulates

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Memòria presentada per **Marina Salas Coscollola**

Sota la direcció del **Dr. Xavier Manteca Vilanova** i del **Dr. Hugo Fernández Bellon**

Per a l'obtenció del grau de doctora dins del programa de doctorat de Producció Animal del Departament de Ciència Animal i dels Aliments de la Universitat Autònoma de Barcelona

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FACULTAT DE VETERINÀRIA

El Dr. Xavier Manteca Vilanova, catedràtic del departament de Ciència Animal i dels Aliments de la Universitat Autònoma de Barcelona, i el Dr. Hugo Fernández Bellon, veterinari del Parc Zoològic de Barcelona,

Certifiquen:

Que la memòria titulada '**Assessment of Welfare in Captive Wild Ungulates**', presentada per Marina Salas Coscollola amb la finalitat d'optar al grau de doctora en Veterinària, ha estat realitzada sota la seva direcció i, considerant-la acabada, autoritzen la seva presentació perquè sigui jutjada per la comissió corresponent.

I perquè consti als efectes oportuns, signen la present a Bellaterra el 15 de juny de 2017.

Dr. Xavier Manteca Vilanova

Dr. Hugo Fernández Bellon

"Only if we understand, can we care. Only if we care, we will help. Only if we help, shall they be saved"

Jane Goodall

AGRAÏMENTS

Escriure aquesta tesi no només m'ha ajudat a créixer a nivell professional, sinó que també m'ha donat l'oportunitat de créixer com a persona, la qual cosa considero que és encara més important. Durant aquests últims anys he conegut o he seguit caminant al costat de gent que m'ha empès a donar forma a aquest projecte i sense la qual no podria presentar aquesta memòria. Vull ressaltar el paper de certes persones en concret, però, tot i així, m'agradaria que tot aquell que llegeixi aquestes paraules (anomenat o no) senti una part d'aquesta tesi com a seva.

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ABSTRACT

Ensuring high standards of welfare in wild animals kept in captivity is essential for ethical and legal reasons, as well as for the establishment and maintenance of viable populations of animals in good health. The general aim of this thesis was the study and assessment of animal welfare in wild ungulates in captivity through the use of case studies in three different species of ungulates: dorcas gazelles (*Gazella dorcas*), fallow deer (*Dama dama*) and Spanish ibex (*Capra pyrenaica*). Wild animals kept in captivity face different situations that can cause chronic stress to the individuals. The concentration of cortisol or cortisol metabolites has been advanced as an indicator to quantify stress in many species. In this thesis, two different welfare issues (social stress and visitor effect) were studied using behavioural and physiological animal-based welfare indicators. High levels of aggressive behaviours can impair welfare by causing physical damage and chronic stress to the animals. The sensitivity of social behaviour and hair cortisol concentration was evaluated in four groups of dorcas gazelles. Significant differences between groups of gazelles were found in frequency of negative social behaviour and hair cortisol concentration, suggesting that hair cortisol levels are sensitive to differences in the social structure of dorcas gazelles. Visitor presence has been described on occasions as having a negative effect on the welfare of captive animals. Our study of the visitor effect in fallow deer and Spanish ibex used the expression of vigilance behaviours and the concentrations of faecal cortisol metabolites as welfare indicators. Conflicting results between these indicators suggested that a multidimensional approach is necessary in order to properly assess welfare. The visitor presence increased the expression of vigilance behaviours, but did not have a negative effect on the faecal cortisol metabolites concentration in Spanish ibex and fallow deer. Finally, a protocol for the assessment of welfare in captive dorcas gazelles was developed and applied to five different groups of this species. The protocol included 23 welfare indicators and it was found to be useful to detect areas for improvement in all groups assessed. The protocol presented in this thesis could be a useful tool for the centres that keep dorcas gazelles under their care and want to routinely check the welfare of the animals.

RESUM

És essencial assegurar alts estàndards de benestar en animals salvatges en captivitat, tant per motius ètics i legals, com per l'establiment i el manteniment de poblacions d'animals sanes i viables. L'objectiu general d'aquesta tesi fou l'estudi i l'avaluació del benestar en ungulats salvatges en captivitat, a partir de diferents estudis, en tres espècies d'ungulats: la gasela dorcas (*Gazella dorcas*), la daina (*Dama dama*) i la cabra salvatge (*Capra pyrenaica*). Els animals salvatges que es troben en captivitat afronten diferents situacions que els poden causar estrès crònic. La concentració de cortisol o de metabòlits de cortisol es considera un indicador per quantificar l'estrès en moltes espècies. En aquesta tesi, dos aspectes relacionats amb el benestar (l'estrès social i l'efecte dels visitants) s'han estudiat utilitzant indicadors de benestar basats en l'animal i relacionats amb el seu comportament i la seva fisiologia. Nivells elevats de comportaments agressius poden perjudicar el benestar causant dany físic i estrès crònic. Es va avaluar la sensibilitat del comportament social i de la concentració de cortisol en pèl en quatre grups de gaseles dorcas i es van trobar diferències significatives entre els grups pel que fa a la freqüència de comportaments socials negatius i a la concentració de cortisol en pèl. Això suggereix que els nivells de cortisol en pèl són sensibles a les diferències en l'estructura social de les gaseles dorcas. En ocasions, la presència dels visitants s'ha considerat causant d'un efecte negatiu en el benestar dels animals salvatges en captivitat. En un estudi sobre l'efecte dels visitants en daines i cabres salvatges, vam utilitzar com a indicadors de benestar l'expressió de comportaments de vigilància i les concentracions de metabòlits de cortisol en femta. Els resultats obtinguts a partir d'aquests indicadors eren contradictoris, suggerint que cal una aproximació multidimensional per tal d'assessorar el benestar correctament. Es va observar un augment en l'expressió de comportaments de vigilància quan hi havia més públic, però no es va veure un efecte negatiu en la concentració de metabòlits de cortisol en femta ni en cabra salvatge ni en daina. Finalment, es va desenvolupar i aplicar en cinc grups d'animals un protocol per l'avaluació del benestar en gaseles dorcas captives. El protocol incloïa 23 indicadors de benestar i va provar ser útil per detectar àrees de millora en tots els grups avaluats. El protocol presentat en aquesta tesi podria ser una eina pràctica per tots aquells centres que tenen gaseles dorcas i que volen controlar de manera rutinària el benestar dels animals sota el seu càrrec.

RESUMEN

Es esencial asegurar altos estándares de bienestar en animales salvajes en cautividad, tanto por motivos éticos y legales, como por el establecimiento y mantenimiento de poblaciones de animales sanas y viables. El objetivo general de esta tesis fue el estudio y la evaluación del bienestar en ungulados salvajes en cautividad, a partir de diferentes estudios, en tres especies de ungulados: la gacela dorcas (*Gazella dorcas*), el gamo (*Dama dama*) y la cabra salvaje (*Capra pyrenaica*). Los animales salvajes en cautividad afrontan diferentes situaciones que les pueden causar estrés crónico. La concentración de cortisol o de metabolitos de cortisol se considera un indicador para cuantificar el estrés en muchas especies. En esta tesis, dos aspectos relacionados con el bienestar (el estrés social y el efecto de los visitantes) se han estudiado utilizando indicadores de bienestar basados en el animal y relacionados con su comportamiento y su fisiología. Niveles elevados de comportamientos agresivos pueden perjudicar el bienestar causando daño físico y estrés crónico. Se evaluó la sensibilidad del comportamiento social y de la concentración de cortisol en pelo en cuatro grupos de gacelas dorcas y se encontraron diferencias significativas entre los grupos en la frecuencia de comportamientos sociales negativos y en la concentración de cortisol en pelo. Esto sugiere que los niveles de cortisol en pelo son sensibles a las diferencias en la estructura social de las gacelas dorcas. En ocasiones, la presencia de los visitantes se ha considerado causante de un efecto negativo en el bienestar de los animales salvajes en cautividad. En un estudio sobre el efecto de los visitantes en gamos y cabras salvajes, utilizamos como indicadores de bienestar la expresión de los comportamientos de vigilancia y las concentraciones de los metabolitos de cortisol en heces. Los resultados obtenidos a partir de estos indicadores eran contradictorios, sugiriendo que es necesaria una aproximación multidimensional para poder asesorar correctamente el bienestar. Se observó un aumento en la expresión de comportamientos de vigilancia cuando había más público, pero no se observó un efecto negativo en la concentración de metabolitos de cortisol en heces ni en cabra salvaje ni en gamo. Finalmente, se desarrolló y aplicó en cinco grupos de animales un protocolo para la evaluación del bienestar en gacelas dorcas cautivas. El protocolo incluía 23 indicadores de bienestar y probó ser útil para detectar áreas de mejora en todos los grupos evaluados. El protocolo presentado en esta tesis podría ser una herramienta práctica para todos aquellos centros que tienen gacelas dorcas y que quieren controlar de manera rutinaria el bienestar de los animales bajo su cuidado.

GENERAL INTRODUCTION

Humans have been concerned about animal well-being even before welfare was considered a science. Nevertheless, scientific interest in studying animal welfare did not ensue until the 1960's (Keeling et al 2011). The starting point was society's concern about contemporary farming techniques, especially after the publication of the book 'Animal Machines' by Ruth Harrison (1964).

A long debate about whether or not animals are able to experience emotions such as stress, fear, or pain has existed through the ages (Duncan 2006). However, the public's concern about the ethical caring for the animals' quality of life also implies the general acceptance that animals, or at least some species, are sentient beings capable of suffering.

Today, it is widely accepted that mammals and birds are capable to feel and experience emotions similar to ones of humans. However, amphibians, reptiles, fish and invertebrates do not benefit from this general acceptance. This could be due to the fact that it is easier for humans to empathize with species phylogenetically closer to ours, whereas the uncritical anthropomorphism might lead to false concepts and hinders the proper assessment of animal welfare. Animal ethics was the starting point of caring for the well-being of species apart from ours, but it is important to separate science and scientific objectivity from moral judgment (Broom and Fraser 2007).

1. Concept of animal welfare

1.1. Animal welfare as a measure of the animal's adaptation to its environment

Broom (1986) defined the welfare of an individual as 'its state as regards its attempts to cope with its environment'. Three situations could arise when observing an animal adapting to its surroundings.

The first situation is when an animal displays a clear failure to cope with its environment. The individual will achieve a poor welfare state which can lead to the appearances of disease or death.

The second situation is when the animal can cope with the environment without effort or any expenditure of resources. The animal can then achieve an optimal welfare state.

The third situation is found where the individual is able to somewhat cope with its environment with varying degrees of difficulty. That can lead to stress responses (such as increased heart-rate or adrenal activity) and to behavioural abnormalities (abnormal/excessive activity, inactivity, lack of responsiveness, stereotypies, and self-inflicted injury).

1.2. Three approaches to animal welfare

There are at least three overlapping ethical concerns related to animal well-being and three main approaches to define welfare are accepted (Fraser et al 1997).

The first approach includes the capability of an animal to express behaviours proper for its species. Within this view, the animal will have a poor welfare state if it is not able to perform its full repertoire of behaviours. Certainly, there are instances where the animal is forbidden to perform behaviours normal for its species (for example, the so-called behavioural needs) which can have negative consequences on its welfare. However, there are behaviours that only appear as a response to adverse situations. Therefore, the performance of these concrete behaviours is not indicative of a good welfare state, even if the behaviours are included in the repertoire of what is considered normal behaviours for a certain species.

The second approach includes the animal's emotional state and, therefore, considers well-being as the result of subjective experiences. Emotional welfare depends on the animal experiencing positive states (such as comfort or pleasure) and not experiencing negative emotions (such as pain, suffering, stress, fear, hunger or thirst). However, even when the subjective experiences are important for a positive welfare state, it is difficult to study the emotions of animals in a scientific way.

The third approach includes the normal biological functioning of the animals and it considers that welfare can be negatively affected by disturbances such as disease, malnutrition, injury or physical and thermal discomfort. In this approach, experiences like fear, pain and hunger would not be considered significant to welfare except if related to health problems. However, poor physical health is not the only reason for impaired welfare.

None of these approaches completely define animal welfare, yet they are overlapping constants between them. An integrative perspective that combines the three approaches, permits researchers to agree on a common definition of animal welfare (Fraser et al 1997). In other words, animal welfare is a multidimensional concept that includes the behaviour, emotional state, and physical health of the individual.

The principle of the Five Freedoms developed by the Farm Animal Welfare Council (FAWC 1992) was the first attempt to consider animal welfare as a multidimensional and integrative perspective of these three approaches. The Five Freedoms propose that animal welfare is optimal if the following statements are fulfilled:

- Freedom from hunger and thirst by providing access to fresh water and a diet that maintains full health and vigour.
- Freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area.
- Freedom from pain, injury or disease by prevention or rapid diagnosis and treatment.
- Freedom to express normal behaviour by providing sufficient space, proper facilities and company of the animal's own kind.
- Freedom from fear and distress by ensuring conditions and treatment which avoid mental suffering.

Even when the original aim of the Five Freedoms was the development of a tool to assess the welfare of farm animals, the requisites were general enough to be applied to other species including lab, companion, and zoo animals.

Today, animal welfare science tries to emphasise the importance of positive states. In order for the animals to experience a good welfare state, it is important to minimise their negative experiences while enhancing the opportunities for them to have positive ones (Mellor 2016).

In fact, it is biologically impossible to eliminate stress or to provide an environment that is free from negative experiences (such as thirst, hunger, discomfort, pain, fear, distress, malnutrition, disease, or injury) even during short periods of its life (Mellor 2016). Two types of negative subjective experiences have been described: survival-related or internally generated, and situation-related or externally generated negative effects (Mellor and Beausoleil 2015).

Survival-related negative effects are part of the homeostatic mechanisms that induce animals to engage in specific goal-directed behaviours essential for their survival (Mellor and Beausoleil 2015). In this case, being free of these negative effects can be detrimental for welfare, since their existence is important to motivate life-sustaining behaviours. For example, thirst elicits water seeking and drinking behaviour while hunger elicits behaviours for acquisition of food. However, animal management must find a way to keep the intensity of these emotions within tolerable limits. Some survival-related negative effects or experiences are thirst, hunger, pain, breathlessness, nausea, dizziness, debility, sickness and weakness (Mellor 2016).

Some situation-related negative effects are anxiety, panic, fear, frustration, anger, helplessness, loneliness, boredom, and depression (Mellor 2016). Additionally, negative environmental experiences include the effects associated with sensory inputs that contribute to an animal's cognitive evaluation of its external circumstances. A consequence of an impoverished environment is that an animal will not have the ability to perform certain environmentally focused tasks and interactions with other animals will be limited. (Mellor and Beausoleil 2015).

Survival-related experiences can be eliminated by appropriate interventions, but only temporarily. However, the situation-related affects can be replaced by positive experiences when the circumstances surrounding the animal improve, allowing it to engage in behaviours that are rewarding for the individual. Stimulating environments may enable the animals to experience comfort, pleasure, confidence, interest and a sense of control (Yeates and Main 2008).

In conclusion, good animal welfare management should ensure the reduction of survival-related negative experiences to tolerably low levels. Provision of an improved environment that allows the animal a greater chance to experience positive affects (Mellor 2015), such as comfort, pleasure, interest, confidence and a greater sense of control (Mellor 2016) is paramount.

2. Welfare in zoo animals

The aim of the earliest zoos was the collection and exhibition of wild or tropical animals. However, through time, the aims changed. Today zoos are meant to fulfil four roles: conservation, education, research and finally, the entertainment of the zoo patrons (Reade and Waran, 1996).

In 1999, The Council of the European Union approved a directive related to the keeping of wild animals in zoos (Council Directive 1999/22/EC 1999). This directive speaks to the important role zoos have in the conservation of biodiversity as well as indicates activities related with conservation that all zoos should implement. Therefore, according to the directive, zoos should participate in continued research, promoting education of zoo visitors, accommodating animals under conditions that satisfy the conservation and biological needs for each species. In addition, there is an ongoing need to supply animals with enrichment opportunities, establish veterinary programs, prevent the escape of animals (or the intrusion of outside pest and vermin) and keep records of the species in captivity. However, not even the word ‘welfare’ nor ‘well-being’ is mentioned once in this document.

Animal welfare in zoos was directly addressed after the publication of the World Association of Zoos and Aquarium (WAZA) Animal Welfare Strategy (Mellor et al 2015). This report aimed to be a guide for zoos and aquariums to achieve high standards of animal welfare in support of their goals as modern conservation organizations. The document contains material that provides information on welfare topics such as the assessment, monitoring and management of animal welfare, environmental enrichment, and exhibit design. It also includes views on how welfare can be applied in other circumstances such as breeding, conservation programmes, collection, planning, and research.

3. The importance of zoo animal welfare

Animal welfare is gaining attention regarding the conservation of species not only due to ethical and legal reasons, but also because optimal welfare can ensure stable and healthy populations, something very important for conservation purposes (Mellor et al 2015). Zoo animal welfare is important for ensuring optimal conditions for the possible future release of animals into the wild.

Modern zoos and centres that keep wildlife in captivity are making an effort to improve the quality of life of animals under their care. The current trend is to build larger and more naturalistic facilities and to provide enriched environments for the animals (Reade and Waran 1996; Shepherdson et al 1998; Young 2003). This affords animals the opportunity to express certain behaviours that otherwise would be unlikely to be seen in captivity (Swaisgood 2007). The aim of environmental enrichment is to increase the physical, social, and temporal complexity of captive settings (Shepherdson et al 1998; Young 2003). The physical complexity is related to providing animal enclosures with a variety of visual, auditory, gustatory and olfactory stimuli for the animal. Social complexity is very important for some species, and group size, composition, and mixing different species in the same enclosure, have to be especially considered. The temporal complexity is related with unpredictable changes into the fixed environment (Carlstead and Shepherdson 2000).

The enrichment provided in captive conditions can have different positive impacts on the welfare of animals. The health and reproduction of the individuals can improve, as well as the survival tax of captive animals released into the wild (Carlstead and Shepherdson 2000). Concerning behaviour, it is documented that enrichment opportunities can increase the physical and mental activity while decreasing the aggression among individuals and the performance of abnormal behaviours (Manteca 2015). In addition, it is important to maintain behavioural diversity in captivity; providing the animals with good, stimulating, and species-specific enriched environments, especially if the final goal is the release of the animals into the wild (Mellen and MacPhee 2001; Swaisgood 2007).

Furthermore, this new tendency does not only have positive direct effects on the animals, but also positive indirect effects. This is due to humans' perception of different species are changing. Being able to see the animal in a more naturalistic enclosure performing natural behaviours makes the public more aware, interested, and empathetic with the animals kept in captivity (Mellen and MacPhee 2001).

4. Frequent welfare issues in zoos

Zoos and other centres that keep wild animals in captive conditions tackle different issues that can directly affect the individuals' welfare. Some of the frequent zoo animal welfare concerns are not only related with the actual limitations of resources, but also with health issues and the stress that animals endure daily due to different situations or factors.

4.1. Lack of space

Because facilities are usually limited in the amount of space they have, one of the most frequent welfare issues in zoo animals is providing them with adequate room. Frequently, captive animals have access to less physical space compared to the area the same species encounters in the wild.

It is true that the amount of space available for the animals is important. However, if that space is poor in stimuli, more quantity does not necessarily mean better quality of life. In fact, it seems that space quality is more important than quantity (Carlstead and Shepherdson 2000) if the enclosure design provides the animals with the opportunity to perform a wide range of behaviours. This includes behaviours important to their welfare that allow the animals to exercise some control over their environment and to not compete for resources such as space, food, water, or shade (Swaisgood 2007; Manteca 2015).

Apart from the design of the facility, a good environmental enrichment program helps to increase the complexity of the enclosures, as well as providing other benefits (see 3. The importance of zoo animal welfare). A complex environment is more valuable than an abundance of space available to the animal (Carlstead and Shepherdson 2000).

4.2. Social stress

Social stress appears as a consequence of aggressive interactions between animals. Aggressive behaviours can include physical contact, displacement, or threats. The consequences of aggressive interactions might cause stress, injuries, and the appearance of negative emotions. Situations that cause pain, frustration, fear and chronic stress in an animal may at the same time cause and/or escalate aggressive behaviour (Arnone and Dantzer 1980; Kruk et al 2004).

Lack of space and management of captive populations for breeding or conservation purposes results in the creation of human-made groups of animals. According Manteca (2015), this artificial arrangement of groups may increase social stress if:

- Animals that had no previous contact are mixed. A gradual introduction of unknown individuals (establishment of olfactory contact first, followed by visual contact and, finally, physical contact) is usually recommended to allow the animals to have a period of social habituation to the new member and/or group.
- There is competition for resources (such as space, shade, water or food) and it is not possible for all the animals to have access to resources at the same time.
- Animals are kept in groups whose size or composition is different from the groups observed in the wild.

Nevertheless, aggressive behaviour is part of the normal behavioural repertoire of all species. While the frequency of aggressive interactions is important, their intensity is also essential to determine if certain aggressive behaviours are indicative of a welfare problem.

4.3. Visitor effect

The presence of visitors nearby the enclosure or, in fewer occasions, inside the facility or in close contact with the animals is a situation that animals face daily during the zoo’s open hours. The presence of people that are strangers to the animals, the sounds and smells that the visitors produce, all can impact the welfare of the animals.

Different studies have observed the effect the presence of unfamiliar people has on zoo animals. In some occasions, no evident visitor effect has been found (Sherwen et al 2014; Hosey et al 2016; Jones et al 2016). Most of the studies related with visitor effect considered that the constant presence of unfamiliar people can produce stress and have a negative impact on welfare. Specifically, an increase of abnormal (Mallapur et al 2005; Vidal et al 2016) and aggressive behaviours (Sellinger and Ha 2005; Sekar et al 2008) have been observed as a consequence of the presence of visitors. On other occasions, an increase in visitor-avoidance behaviour was also considered negative for the welfare of animals (Smith and Kuhar 2010; Ozella et al 2015). Other studies have found a positive correlation between the number of visitors and the concentration of cortisol or its metabolites (Davis et al 2005; Rajagopal et al 2011; Pifarré et al 2012).

Some studies also suggested that the negative effect could be mitigated if some measures were taken to further enrich the environment of the animal (Carder and Semple 2008). An example of this could be a specific enclosure design with hidden spots or panels that prevent the animals from having visual contact (Blaney and Wells 2004; Sherwen et al 2015) with visitors.

4.4. Diseases and other health problems

In some species, the prevalence of diseases is higher in captive conditions than in the wild (Munson et al 2005). Even when in captivity, the prevention, diagnosis, and treatment of possible diseases is vitally important and should be routinely checked. The causes of this higher prevalence are not always known, although some problems are clearly related to inadequate diet, facilities, and a lack of physical activity (Manteca 2015).

Chronic stress can induce the performance of abnormal behaviours that may turn into injury or diseases and reduce the reproductive success of stressed animals. Stress can also weaken the immune system (Möstl and Palme 2002), contributing to the development of certain diseases.

An inadequate diet can lead to chronic hunger and weight loss. Yet, the excessive consumption of food or an energy rich diet can also lead to other health problems like obesity. This disease is a relatively frequent problem with some animals in captivity and can also be a consequence of a lack of physical exercise. This is either because there is not enough space, or because the environment does not stimulate the animal's normal behaviour and activity levels. Obesity has negative effects on animal health and can increase the risk of lameness (Kurt and Kumarasinghe 1998).

Lack of physical activity is also involved in the development of diseases like lameness in mammals or ulcerative pododermatitis in birds (Manteca 2015). Environmental enrichment can help to increase the activity of the animals, therefore reducing the prevalence of these diseases.

The frequency or incidences of injuries provide relevant information on the welfare of animals. Abnormal behaviours such as stereotypies or other detrimental behaviours can be responsible for the appearance of lesions on the body that can cause pain (Mason 1993). Injuries or even death can be the consequence of aggressive behaviour in social groups. Moreover, a bad design, or maintenance of the facilities could also harm the animals.

4.5. Medical procedures

As it has been previously stated, every modern zoo or aquarium should routinely check the animals under its care for the prevention, diagnosis, or treatment of injury or disease. However, during these procedures, animal welfare could be negatively affected because some veterinary interventions cause pain. The use of appropriate analgesic and anaesthetic protocols help to reduce pain in some situations.

Moreover, some other procedures for medical evaluations can produce stress to the animals, especially if they are not used to them or if the individuals need to be captured. The goal of medical training is to train animals to facilitate certain procedures (Melfi 2013) such as general exploration, sample collection, and drug administration. This is the reason why medical training should be, when possible, included in any animal welfare routine protocol.

Medical training is based on the principles of operant conditioning and each session should be a positive and pleasant moment for the animal. Rewards, not punishment, should always be employed. This technique can be useful not only to reduce animal stress during veterinary or other routine procedures, but to increase the safety of personnel working with animals.

5. Welfare indicators

Animal well-being can be measured and assessed scientifically using a combination of welfare indicators (Hill and Broom 2009; Manteca et al 2016) that can be divided in resource-based or animal-based indicators. What follows is an explanation of these indicators.

5.1. Resource-based indicators

Resource-based indicators are easier to apply and assess the environment surrounding the animal, but not the animal itself. Examples of this type of indicator are water provision, enclosure size and design, and size and composition of a group or environmental enrichment.

5.2. Animal-based indicators

Animal-based indicators are more important for the individual assessment of animal welfare as they include all those variables that are measured directly in individuals. These are related to changes in the animals' behaviour, overall appearance and health, and include physiological parameters.

5.2.1. Indicators related with the behaviour of the animals

The observation of changes in animal behaviour is a non-invasive method for the assessment of welfare (Hosey et al 2009). These behavioural changes include the appearance of abnormal behaviours, while also noting alterations in the frequency, duration, or intensity of normal behaviours (Manteca et al 2016).

Abnormal behaviours are indicative of poor welfare and include behaviours that are never or rarely observed in the wild, such as stereotypies and apathy. Stereotypies are described as repetitive behaviours caused by frustration or repeated attempts to adapt to the current or previous environment (Rushen and Mason 2006) and they can also appear as a consequence of a dysfunction in the central nervous system.

Stereotypies differ depending on the species. For instance, ungulates usually perform oral repetitive movements (Bergeron et al 2006), while carnivores often present locomotory stereotypies, such as pacing (Clubb and Vickery 2006).

Apathy is defined as an abnormal state of inactivity accompanied by a lack of response to environmental stimuli (Manteca 2015). Apathy can occur in animals that live in barren and/or stressful environments, especially when the individuals cannot have any kind of control over their environment. Depending on the species, however, it may be difficult to differentiate between apathy and normal activity or resting behaviour.

The presence of alterations or changes in the frequency, duration or intensity of normal behaviours is particularly useful to assess animal welfare (Manteca et al 2016). Changes in food intake and behaviours such as play, maternal, vigilance, aggressive and affiliative can be the reflection of a poor welfare state.

Aggressive behaviours appear when conflicts between individuals arise, especially if resources such as mates, food, water, shade, or space are limited. Yet not all conflicts end in overt aggression and fights can be avoided by a submissive gesture or posture from one animal towards another (Nelson 1995).

Agonistic or aggressive behaviours in animals might be useful indicators of poor welfare because they can cause damage, injuries, stress, and negative emotions such as pain, fear or frustration. Aggressive behaviours can be caused, or at least increased by negative emotions or chronic stress (Arnone and Dantzer 1980; Kruk et al 2004).

However, the use of aggressive behaviour as welfare indicator has several problems that difficult its objective assessment (Manteca et al 2016). Aggressive behaviour is part of the normal behaviour repertoire of all species and it may be not possible to eliminate its expression completely. Moreover, not only the frequency of aggressive behaviours is important, but also the intensity of the attacks. Additionally, it is not easy to evaluate the intensity of a negative interaction.

Affiliative behaviours, on the other hand, are considered pleasant behaviours for the animals that induce the release of oxytocin (Neumann 2008), which contributes to the reduction of the stress response (Amico et al 2004; Neumann et al 2000). These behaviours are positive indicators of welfare (Boissy et al 2007) because they also contribute to social cohesion and reduce tension in groups of animals.

However, some affiliative behaviours such as social grooming might increase after stressful events, or after an aggressive interaction (de Waal and van Roosmalen 1979; Webb et al 2014).

5.2.2. Indicators related with the appearance of the animals

The assessment of different indicators related with the physical appearance of individuals, such as body, hair or feather condition, or posture and facial expression, are also used for an overall welfare evaluation (Manteca et al 2016).

Both poor and excessive body condition may be indicative of a welfare problem. Weight loss or poor body condition may be indicative of a presence of disease, as well as of an inadequate diet, or chronic hunger. However, an excessive body condition or obesity is also problematic (Kurt and Kumarasinghe 1998), since it may predispose the animal to develop lameness and/or other health problems.

An anti-algid posture is the body pose that an animal adopts to reduce pain on an anatomic area. This posture, as well as others such as postures that indicate fear, may be a useful indicator of negative emotions (Manteca 2015).

Like body posture, changes in facial expression could be useful to assess pain (Langford et al 2010) and other emotions in some species.

The condition of the hair on mammals and the feathers on birds is also important for the assessment of welfare (Manteca 2015). The accumulation of dirt on the body may indicate that the individual does not have a suitable place to lie down and it can also increase the risk of some diseases. Besides, it can also be an indicator of a disease or an expression of the inability of the animal to perform adequate grooming behaviour.

5.2.3. Indicators obtained from records

It is important for zoos to have updated registers of health and other issues related with the animals under their care. Information collected can prove to be very useful for the animal. For instance, the prevalence and incidence of diseases and also life span are welfare indicators that can be obtained from well-kept records (Manteca et al 2016).

Health is a very important aspect of welfare and therefore, any disease can be considered a negative welfare indicator. Diseases that cause pain, discomfort or otherwise weaken an animal may prevent it from getting access to resources. That may make an animal more vulnerable to aggressive behaviour and should especially be taken into account. Diseases that prevent the animal from expressing certain behaviours, or that reduce the possibility of the individual to experience positive emotions should also be carefully considered. Multifactorial diseases such as lameness, diarrhoea and respiratory problems, whose prevalence or incidence increases as a result of stress or environmental conditions, are also important to note when keeping health records.

Life span is also a useful welfare indicator that helps to assess the welfare of groups of animals rather than specific individuals in a retrospective way. Captivity can lead to negative effects on the life expectancy due to different factors such as prolonged periods of stress or anxiety, high prevalence of diseases, inbreeding, impaired maternal behaviour, or aggression between the animals.

5.2.4. Physiological indicators

Physiological measures such as oxytocin concentration (Seltzer and Ziegler 2007), heterophil:lymphocyte ratio (Maxwell 1993), and acute phase proteins can also provide useful information on the welfare of animals (Bertelsen et al 2009). The physiological indicators most commonly used are the glucocorticoids concentrations, because they measure the hypothalamic-pituitary-adrenal (HPA) axis activity.

The stress response involves the activation of the HPA axis, which in turn results in an increased secretion of glucocorticoids hormones such as corticosterone and cortisol, that mobilise fatty acids and glucose from the cells with the aim of obtaining energy. These stress hormones are part of the endocrine mechanism that the organism uses for self-protection in case of stressful conditions (Lane 2006; Keeling and Jensen 2009). However, an increase of secretion of stress hormones is also related to other situations that are not detrimental for welfare and can even provide pleasure to the individuals, such as hunt, sexual, or play behaviours (Lay 2010).

Nevertheless, the absence of chronic or long-lasting stress is important to ensure welfare. Prolonged episodes of elevated glucocorticoids concentrations might weaken the immune system (making the individual more susceptible to bacterial and viral infections) and can impair reproduction (Möstl and Palme 2002).

The concentration of glucocorticoid hormones such as cortisol, corticosterone or their metabolites is used to measure the stress response and can then be used to assess an animal’s welfare (Manteca et al 2016). The concentration of glucocorticoids can be measured in various biological matrixes. The most common samples used in zoo animals are plasma, saliva, faeces, hair, or feathers.

5.2.4.1. Plasma

Plasma concentration of glucocorticoids presents a circadian rhythm and a wide variability among individuals (Mormède et al 2007; Möstl and Palme 2002). Some animals suffering from chronic stress do not present a plasma concentration higher than normal, so plasma concentration of cortisol or corticosterone might not be a valid measure of chronic stress.

The method used for obtaining a blood sample is an invasive procedure that can provoke a stress response and consequently might affect the plasma concentration of glucocorticoids, especially if the animal is handled or caught. However, this artefact can be evaded using an in-dwelling catheter, habituating the animal to handling or medical training, and by taking a blood sample before two or three minutes after the individual has been restricted as this is the time before the adrenal cortex is activated (Mormède et al 2007).

5.2.4.2. Saliva

The method used for the collection of saliva for the assessment of glucocorticoids can be less invasive and stressful than obtaining a blood sample if the animals are previously trained for the aspiration of saliva or the chewing of cotton buds (Mormède et al 2007). The determination of glucocorticoids in saliva can be used to measure acute stress.

5.2.4.3. Faeces

Faeces can be collected non-invasively. Therefore, the metabolites of cortisol can be determinate by avoiding the stress caused by other sampling methods (Mormède et al 2007). Faecal samples can be obtained from individually identified animals and have to be kept frozen at -20ºC until their analysis.

Cortisol metabolites concentrations found in faeces are a reflection of the total amount of cortisol excreted during a time lag that range between a few hours to more than a day, depending on the digestive transit time of the species (Palme et al 1999; Möstl and Palme 2002).

Since the excretion of cortisol metabolites in faeces does not take place immediately after a stressful event, the concentration of glucocorticoids in faeces might be a better estimation of the production of glucocorticoids than plasma, where cortisol concentrations change quickly (Möstl and Palme 2002). However, there can be circadian and seasonal variations and a large variability in the concentration of cortisol metabolites in faeces depending on the species (Möstl and Palme 2002).

5.2.4.4. Hair and feathers

The quantification of cortisol in hair in all mammals, except rodents, or corticosterone in feathers in birds and in the hair of some rodents, is used for the assessment of chronic stress. In these matrixes, the values are not influenced by momentary stress of the sampling.

The method for the collection of hair is painless, although the animal can suffer stress while sampling due to capture or restriction if it has not been trained for that purpose. Both hair and feathers can be stored in ambient temperature (Gow et al 2010).

It may not be possible to assess cortisol levels in hair if the individuals have little hair or if they are small. Moreover, hair and feather glucocorticoids concentrations need to be validated for each species, because there are a lot of differences between species in relation to the stress response and secretion of glucocorticoids (Bennett and Hayssen 2010).

6. Animal welfare protocols

Animal welfare includes the behaviour, emotional state and physical health of an individual (Fraser et al 1997). Due to this multidimensional concept of animal well-being, there is not a single indicator that can provide enough information about the welfare of an individual.

However, zoo animal well-being can be measured and assessed scientifically using a combination of several indicators (Hill and Broom 2009; Manteca et al 2016).

6.1. The Welfare Quality® project

In 2004 a partnership called Welfare Quality® (Welfare Quality® 2009) was formed with the aim to develop tools for the assessment of welfare of farm animals from a scientific point of view. With that objective some protocols were developed to assess welfare of cattle, poultry, and pigs. These protocols included animal and resource-based welfare indicators.

The Welfare Quality® protocols take into account four parameters (Botreau et al 2007): feeding, housing, health and behaviour, as an expression of optimal emotional states. These four parameters produce 12 criteria that permits the welfare assessment in farm animals:

Good feeding	Absence of prolonged hunger Absence of prolonged thirst
Good housing	Comfort around resting Thermal comfort Ease of movement
Good health	Absence of injuries Absence of disease Absence of pain due to management procedures
Appropriate behaviour	Expression of social behaviours Expression of other behaviours Good human-animal relationship Positive emotional state

Although these protocols were developed specifically for the evaluation of the welfare of farmed animals, they could also be used as a base to develop welfare assessment protocols to evaluate the well-being in other species.

6.2. Zoo animal welfare protocols

The development of welfare protocols specifically designed for the assessment of the well-being of species kept in captivity should include a combination of several welfare indicators (Hill and Broom 2009; Manteca et al 2016). These protocols could help to detect shortcomings in areas for improvement of facilities, management, and to identify specific welfare problems at an individual level.

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OBJECTIVES

The general objective of the present thesis is to advance in the study and assessment of animal welfare in wild ungulates in captivity through the use of case studies in dorcas gazelles (*Gazella dorcas*), fallow deer (*Dama dama*) and Spanish ibex (*Capra pyrenaica*).

The specific objectives are:

1. To study social stress in captive dorcas gazelles using behavioural and physiological welfare indicators.

2. To assess the visitor effect in captive fallow deer and Spanish ibex using behavioural and physiological welfare indicators.

3. To develop a protocol to assess welfare in captive dorcas gazelles.

4. To apply this welfare protocol to find if it is sensitive enough to detect shortcomings or areas for improvement in different groups of dorcas gazelles.

CHAPTER 1

Aggressive behaviour and hair cortisol levels in captive dorcas gazelles (*Gazella dorcas*) as animal-based welfare indicators

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Abstract

Ensuring welfare in captive wild animal populations is important not only for ethical and legal reasons, but also to maintain healthy individuals and populations. An increased level of social behaviours such as aggression can reduce welfare by causing physical damage and chronic stress to animals. Recently, cortisol in hair has been advanced as a non-invasive indicator to quantify long-lasting stress in many species. The sensitivity of social behaviour and hair cortisol concentration was evaluated in several groups of dorcas gazelles (*Gazella dorcas*). Four different groups of gazelles from three different zoos were observed and the expression of intra-specific affiliative and negative social behaviours was assessed across the different groups. Hair samples were taken from sub-groups of animals and analysed for cortisol concentrations. Significant differences between groups of dorcas gazelles were found in frequency of negative social behaviour and hair cortisol concentration. Despite the low sample size, these two parameters had a positive Spearman correlation coefficient ($r_s = +0.80$, $P = 0.20$). These results suggest that hair cortisol levels are sensitive to differences in the social structure of dorcas gazelles.

1. Introduction

Providing high standards of welfare in wild animals kept in captivity is important not only for ethical reasons (Moran 1987), but it is also a legal mandate for wild animals living in captive conditions (Council Directive 1999/22/EC 1999). Moreover, the World Association of Zoos and Aquaria (WAZA) highlights in its Conservation Strategy (2005) that ensuring optimal animal welfare is important for the establishment and maintenance of viable populations of animals in good health. To ensure good animal welfare, factors which may impair it must be overcome.

For some species, sociality is an adaptive strategy to survive and to face environmental difficulties. Sociality is dependent on two types of social interactions: agonistic or negative interactions and affiliative or positive interactions (Rault 2012). Affiliative interactions contribute to group cohesion. Moreover, they cause an increased release of oxytocin (Neumann 2008), which in turn buffers the stress response (Amico et al 2004; Neumann et al 2000). By contrast, aggressive behaviours elicit a stress response and the subsequent release of glucocorticoids. Furthermore, there is positive feedback between stressful conditions and negative social behaviours (Kruk et al 2004).

According to Nelson (1995), a given behaviour is considered aggressive when it is performed with the intention of causing damage or an unpleasant feeling upon another animal. Aggressive behaviours appear when conflicts between two or more individuals arise. They are especially prone to appear if resources such as mates, food or territories are limited. Not all conflicts end in overt aggression; in most of the cases, a fight for a resource is avoided by a submissive posture or gesture from one animal.

Aggression can impair animal welfare through physical damage, like wounds, bone fractures or even death of the individuals. However, non-injurious aggression might also be associated with reduced welfare because animals might suffer from stress (Galindo et al 2011). Chronic stress can impair an optimal welfare because it can induce performance of abnormal behaviours, it can weaken the immune system and also reduce the reproductive success of the stressed animal, among other affected body functions (Möstl and Palme 2002).

The stress response is driven by the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis along with other systems (Keeling & Jensen 2009). The HPA axis releases glucocorticoids, such as cortisol, as part of the endocrine mechanism for self-protection of the body in presence of a stressor. The quantification of cortisol or its metabolites is one physiological indicator for the assessment of stress (Manteca 2009). Several studies have advanced the evaluation of cortisol or cortisol metabolites levels in plasma, faeces, urine, saliva, and milk (Mormède et al 2007).

Hair has also been advanced as a matrix for the evaluation of accumulated cortisol (Bennett & Hayssen 2010; Tallo-Parra et al 2013), and increases of hair cortisol have been associated to stressful situations in different species (Carlitz et al 2014; Qin et al 2013; Siniscalchi et al 2013). However, the baseline cortisol levels in hair has not yet been evaluated for dorcas gazelles. We have empirical evidence that hair has a continuous growth in this species. However, it is unknown if it follows seasonal growth patterns. Due to lack of knowledge on hair growth rate in dorcas gazelles, we took an estimate growth rate (of about 10 mm/month) based on other species because this information allows the quantification of cortisol in hair as an integral measure of the production of cortisol during recent weeks (Russell et al 2012). This way, the conditions that the animal had been facing during the weeks prior to hair sampling can be assessed.

Dorcas gazelles (*Gazella dorcas*) belong to the family Bovidae and are one of the smallest species of antelopes. They are distributed across North Africa around the Sahelo-Saharan region, living in a variety of habitats that include savannah, semi-desert plains and desert (Yom-Tov et al 1995). The dorcas gazelle is a social species with a strong hierarchical structure (Lawes & Nanni, 1993). It is listed as ‘vulnerable’ in the IUCN Red List of Threatened Species (2013). Since 2002, one of its subspecies (*Gazella dorcas neglecta*) is managed within the European Endangered species Programme (EEP) of the European Association of Zoos and Aquaria (EAZA).

This study presents a descriptive analysis of social behaviour and hair cortisol levels in captive Dorcas gazelles within the EEP captive breeding program as well as an evaluation of the sensitivity of each of these two animal-based indicators to discriminate between different groups of animals.

2. Materials and methods

2.1. Study population

Four groups of dorcas gazelles housed in the three following zoological institutions were studied: Parc Zoològic de Barcelona, Zoo Aquarium de Madrid and Zoobotánico Jerez. These three centres are participants of the EEP of the EAZA for this species. The four groups (Table 1) were named as F (all female group, n = 17), FY (female with young group, n = 10), M1 (all male group, n = 3) and M2 (all male group, n = 5). Group M1 and group F were each in a different zoo and groups M2 and FY were on the same zoo but in different areas and animals from different groups did not have visual contact between them.

In the three zoos, animals were identified using the same methods, following the recommendations of the EAZA's Best Practice Guidelines for this species. Males and females wore various ear-tags on the right or left ear, respectively, of different colours and numbered differently to facilitate the individual identification.

Table 1. Composition of the dorcas gazelle groups studied (female group F; bachelor groups M1 and M2; and the group of females with young group FY).

Group	Males	Females	Animals less than 10 months of age	Totals per group	Animals sampled for cortisol analysis
F	0	17	0	17	8
FY	0	4	6	10	3 (all sub-adults)
M1	3	0	0	3	3
M2	5	0	0	5	5
Total	8	21	6	35	19

2.2. Behavioural observations

Observations of intra-specific social behaviours (affiliative and negative interactions) were performed by the same observer for a total of 180 minutes per group (nine 20-minute-long sessions each) from the outside of the enclosures between May and June 2013. Observations were done at mornings between 10 am and 1 pm and the frequencies of social behaviours were recorded using continuous focal behavioural sampling. Keepers had already done the daily husbandry activities and they did not go into the enclosures while observations were done. Before starting and after finishing each session the number of animals present in the field of vision and the number of sleeping animals were counted. The descriptions of affiliative and agonistic behaviours are based on pilot observations and on previous literature reports in dorcas gazelles and similar species (Alados, 1985; Welfare Quality®, 2009).

Affiliative behaviour was divided into three categories ('social grooming', 'social smelling' and 'horning') which are described below:

'Social 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 seconds and then starts brushing the same receiver again, this was 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 seconds 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 reversal role between actor and receiver.

'Horning': head play with 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 in order to become victorious. It is taken as a new bout if the same animals start horning after stopping for10 seconds or more or if the horning partner changes.

Agonistic or aggressive behaviour was divided into four categories ('displacement with physical contact', 'displacement without physical contact', 'chasing' and 'fighting') which are described below:

'Displacement with physical contact': interaction where the aggressor is butting, hitting, thrusting, striking or pushing the receiver with forehead, horns, horn base or any other part of the body with a forceful movement and as a result the receiver gives up its position.

'Displacement without physical contact': the aggressor threats or interacts with the receiver without making any physical contact and as a result the receiver gives up its position.

'Chasing': the aggressor makes an animal flee or give up its current position by following or running behind it, sometimes also using threats like jerky head movements. Chasing is recorded even if it does not follow 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 and both exert force against each other. A new bout starts if the same animals restart fighting after more than 10 seconds or if the fighting partner changes.

2.3. Hair sampling

Opportunistic samples of hair were obtained from a total of 19 of the animals studied when they were captured for routine health checks and/or for husbandry reasons (Table 1). Behavioural observations were performed during the same week but different day from the opportunistic hair sampling. This way, the recording of possible alterations in the normal behaviour of the gazelles due to the stress caused by capture was avoided.

About 250 mg of hair per animal was collected with a shearer or scissors from the rump. The region shaved was the same for all individuals. Skin damage was avoided and the hair was not pulled to prevent hair follicle removal as well as potential pain. The hair was placed in individual bags, identified and stored at environmental temperature.

2.4. Hair cortisol extraction and quantification

Cortisol was extracted from hair using a modified methanol-based technique (Tallo-Parra et al 2015). Briefly, hair samples were washed three times for 2.5 min with isopropanol. The hair was then minced into < 2 mm length fragments by using an electric hair clipper. Fifty milligrams of trimmed hair were placed into an Eppendorf tube and pure methanol was added. Samples were moderately shaken for 18 hours at 30ºC for steroid extraction. Following extraction, samples were centrifuged and the supernatant transferred into a new Eppendorf tube and placed in a heater at 38ºC. Once the methanol was completely evaporated, the dried extracts were reconstituted with EIA Buffer provided by the ELISA assay kit. Samples were immediately stored at -20ºC until analysis.

The quantification of cortisol in hair was performed using an enzyme-linked immunosorbent assay (Cortisol ELISA KIT; Neogen® Corporation, Ayr, UK). Intra-assay CV was 2.85%, the recovery percentage was 99.2%± 16.9% and the R^2 from the parallelism test 0.99.

2.5. Statistical analysis

Statistical analyses were performed by means of the Statistical Analysis System (SAS® 9.2. Institute Inc., Cary, NC). Negative social interactions were expressed as a proportion of the total number of social interactions at group level. The GENMOD procedure was used to investigate whether significant differences existed between groups of dorcas gazelles in terms of frequencies of social negative behaviours. A Poisson distribution was applied according to the value of the deviance (Cameron and Trivedi, 1998). Normal distribution (Kolmogorov-Smirnov test; Q-Q, scatter and box plots) of residuals was achieved after a log transformation of hair cortisol data. Differences between group of dorcas gazelles in concentrations of hair cortisol were assessed by means of a one- way ANOVA. A log transformation was applied to hair cortisol data and further analysed at individual level. The residual maximum likelihood was used as a method of estimation and the least square means of fixed effects (LSMEANS) were used when analysis of variance indicated differences (P < 0.05). Spearman's correlation coefficients between the mean frequency of social negative interactions of the focal sampling and mean hair cortisol concentrations were calculated at group level. Significance was declared at $P < 0.05$.

3. Results

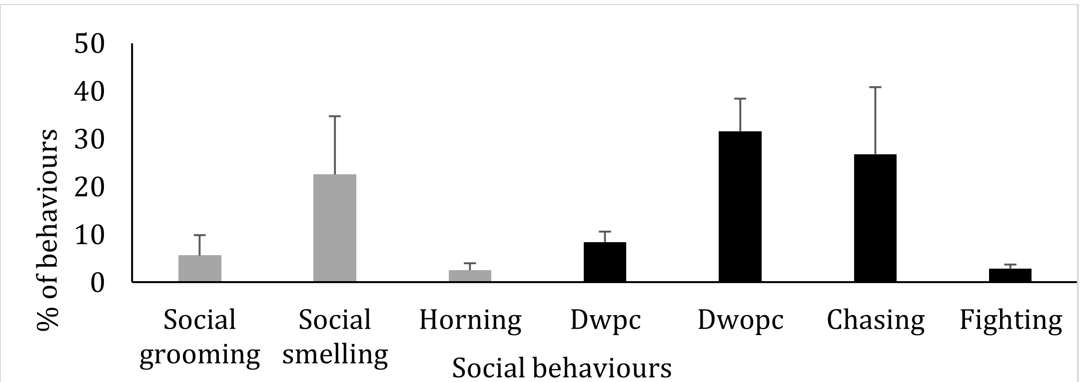
Out of the global population of gazelles studied (n = 4 groups), 29.61% of recorded behaviours were considered affiliative, while the 70.39% were aggressive or negative behaviours. Table 2 presents the total amount of social behaviours per group and per animal, as well as the frequencies of affiliative and aggressive behaviours per group and per individual.

Table 2. Frequencies of social, affiliative and aggressive behaviours per dorcas gazelle groups studied (female group F; bachelor groups M1 and M2; and the group of females with young group FY) and per individual.

Group	Number of animals	Total of social behaviour	Total of social behaviour per animal	Total of affiliative behaviours	Total of affiliative behaviours per animal	Total of aggressive behaviours	Total of aggressive behaviours per animal
F	17	77	4,5	34	2	43	2,5
FY	10	83	8,3	53	5,3	30	3
M1	3	55	18,3	0	0	55	18,3
M2	5	54	10,8	8	1,6	46	9,2

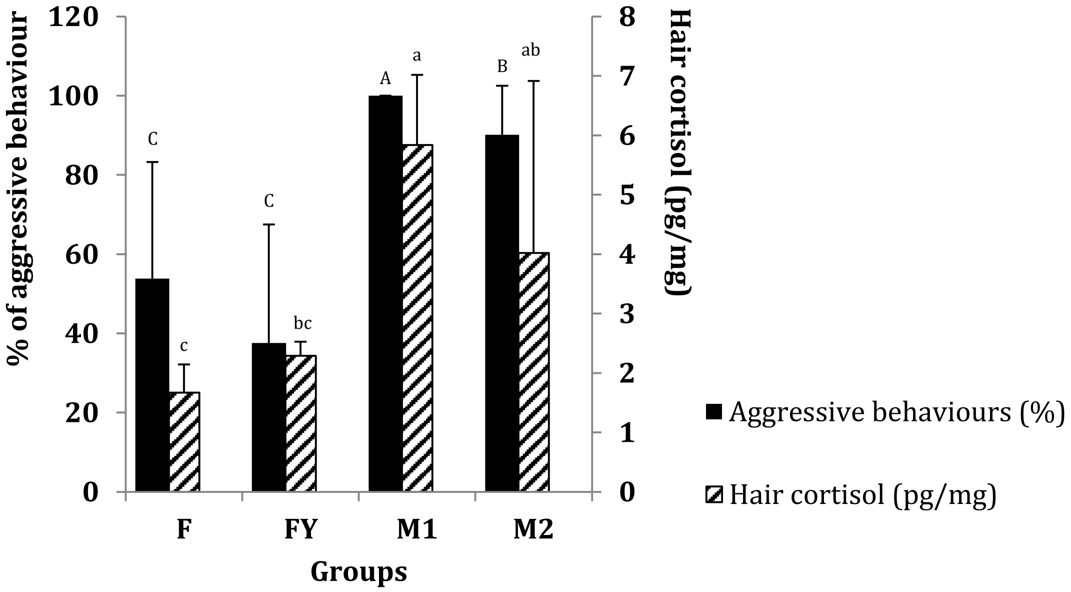
As Figure 1 shows, the occurrence of affiliative behaviours ranges from 'social smelling' (23%), to 'social grooming' (6%), and 'horning' (3%). On the other hand, the most prevalent aggressive behaviour was 'displacement without physical contact' (32%), followed by 'chasing' (27%), 'displacement with physical contact' (8%), and 'fighting' (3%).

Figure 1. Percentage of affiliative behaviours ('social grooming', 'social smelling', 'horning') (grey bars) and aggressive behaviours ('dwpc' = displacement with physical contact, 'dwopc' = displacement without physical contact, 'chasing', 'fighting') (black bars) of the study population of dorcas gazelles studied (n = 4 groups).



Significant differences (*P* = 0.03) were found among groups in the frequency of aggressive behaviour (Figure 2). Animals from the bachelor group M1 displayed the highest frequency of aggressive behaviour (100%) compared with M2 (90.17%, *P* = 0.03), F (53.80%, *P* = 0.0003) and FY (37.58%, *P* < 0.0001). M2 presented significantly higher frequencies of aggressions compared with F (*P* = 0.003) and FY (*P* = 0.002). Both female (F) and female with young (FY) groups presented similar frequencies of aggressive interactions (*P* = 0.33).

Figure 2. Percentage of aggressive behaviours out of the total of social interactions recorded for each group of dorcas gazelles studied (female group F (n=17); bachelor groups M1(n=3) and M2 (n=5); and the group of females with young group FY (n=10)) and the average results of cortisol concentration in hair (pg/mg) of the animals sampled for each group (F, n=8; M1, n=3; M2, n=5; FY, n=3). Different capital letters indicate significant differences between groups with regard to behaviour (*P* < 0.05). Different lowercase letters indicate significant differences between groups with regard to cortisol (*P* < 0.05).



The average concentration of hair cortisol of the 19 animals sampled was 3.45±1.88 pg of cortisol/mg hair. Cortisol level in hair was significantly different (*P* = 0.0005) between groups of dorcas gazelles (Figure 2). M1 presented significantly greater cortisol levels (5.84±1.18 pg/mg) than F (1.67±0.48 pg/mg, *P* = 0.0005) and FY (2.29±0.23 pg/mg, *P* = 0.03). Cortisol levels for M2 (4.02±2.9 pg/mg) were significantly greater than F (*P* = 0.01). Cortisol levels were not significantly different between bachelor groups M1 and M2 (*P* = 0.23) and between F and FY (*P* = 0.51). The Spearman correlation coefficient between the frequency of aggressive behaviours and the amount of cortisol was found to be r_s = +0.80 (*P* = 0.2).

4. Discussion

Dorcas gazelles are social animals which form groups in the wild. The challenge in zoos is that surplus males are frequently kept together, mainly due to a lack of space and also for avoiding keeping animals of the same species individually without contact to conspecifics, which is considered a welfare problem in most cases.

López and Abáigar (2013) stated that adult males of dorcas gazelles in captivity usually perform high levels of aggressive behaviours. In the present study, the two bachelor groups studied performed significantly more aggressive behaviours compared with the female and offspring groups. Therefore, group composition seems to significantly modify the balance between affiliative and aggressive social behaviours. Males of any vertebrate species are generally more aggressive than females, and androgenic steroid hormones have been linked to aggressive behaviour (Nelson 1995).

Dorcas gazelles are known to have a complex and habitat-related social organization in the wild. The different social structures are largely a consequence of the availability and distribution of food resources: dorcas gazelle group size increases with increased forage quality (Grettenberger 1987; Lawes & Nanni 1993). Four different situations have been seen in the wild: harem-like structure (social units with one male accompanied by one to five females), satellite groups of immature males, female herds unaccompanied by males and male pairs. Therefore, keeping adult males in captivity together would not be inherently artificial for this species, since sometimes males are seen living together in the wild. Nevertheless, particular attention should be given to levels of aggressive behaviour in all-male groups.

Differences were observed between groups regarding cortisol concentration in hair. More concretely, males presented higher levels of hair cortisol than the female group. This difference between groups could be explained due to a gender effect as a consequence of the activity of the sexual hormones. In fact, there is evidence that the gonads modulate the HPA axis, so there are sexual differences in the secretion of glucocorticoids (Van Lier et al 2014). It is still unknown which are the basal levels of glucocorticoids in males and females of dorcas gazelles; however, one study assessed hair cortisol concentration in 21 gazelles (Tallo-Parra et al 2014). They did not find that sex had a significant effect over hair cortisol concentration, although females shown higher levels of this hormone compared with males. In some species, females can have higher basal levels of corticosteroids compared with males (in rat, Ogilvie & Rivier 1997; in sheep, Van Lier et al 2003). According to Ogilvie and Rivier (1997), testicular androgens suppress the adrenal secretion of glucocorticoids and circulating levels of estradiol enhance the secretion of adrenocorticotropin (also called ACTH) that stimulates the secretion of glucocorticoids.

The difference between male and female groups that we observed regarding cortisol concentration could also be associated with the level of aggressive behaviours that these groups presented. This comes reflected by the relatively high Spearman correlation coefficient (r_S = +0.80) found between the frequency of aggressions and the levels of cortisol in hair. Kruk et al (2004) suggested that there is a quick, mutual, positive feedback between the activation of the HPA axis and the presence of aggressive behaviours. Therefore, the high presence of agonistic behaviours that we observed in the bachelor groups could be causing a stress response in the animals, activating their HPA axis and therefore increasing the release of cortisol.

Other aspects should also be considered when interpreting those results. One aspect to take into account is the location of the enclosures inside the zoo. Group F was housed near big cats, whose sound and scent could cause stress in prey animals like gazelles (Morgan and Tromborg, 2007). However, it presented the lowest cortisol concentration in hair. In the other zoos, dorcas gazelles shared their enclosures with other species: white rhinoceros (*Ceratotherium simum*), common ostriches (*Struthio camelus*) and Rothschild's giraffes (*Giraffa camelopardalis rothschildi*). The presence of other species in the same enclosure might also be a potential stressor, although no inter-specific agonistic interactions were observed in any of the groups studied.

Differences in visitor numbers among zoos could also have caused differences in hair cortisol levels. According Hosey (2008), however, it is not clear which influence visitors might have on captive animals, as human audience could be stressful to some species (especially in primates), enriching for others, or even no affect the animals at all.

5. Conclusion

Evaluation of welfare in wild animals kept in captivity is very challenging and finding feasible and valid indicators of poor welfare is necessary. The frequency of negative social behaviour and the hair cortisol concentration were sensitive to detect differences between groups of dorcas gazelles. Both animal-based indicators should be taken into account in welfare assessment systems for dorcas gazelles.

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CHAPTER 2

Visitor effect on vigilance behaviours and faecal cortisol metabolites concentrations in captive fallow deer (*Dama dama*) and Spanish ibex (*Capra pyrenaica*)

Abstract

Modern zoos are focusing their efforts in improving the welfare of the animals under their care. However, animal welfare status can potentially be affected by different issues such as the presence of visitors. The aim of this study was to use vigilance behaviours and faecal cortisol metabolites concentrations as animal-based welfare indicators to assess the effect that zoo visitors had in two species of wild ungulates housed in free-range exhibits: the fallow deer (*Dama dama*, n=13) and the Spanish ibex (*Capra pyrenaica*, n=8). The amount of visitor presence significantly affected the occurrence of vigilant behaviour in fallow deer (0.6±0.84 events/animal/day in low visitor presence, and 3.3±2.26 events/animal/day in high visitor presence, $P < 0.0001$) and Spanish ibex (1.0±1.01 events/animal/day in low visitor presence, and 3.2±2.14 events/animal/day in high visitor presence, $P < 0.0001$). In Spanish ibex, visitor presence significantly affected the expression of displacement caused by the visitors behaviour (0 events/animal/day in low visitor presence, and 0.5±0.71 events/animal/day in high visitor presence, $P < 0.0001$). Faecal cortisol level was not significantly affected by a high presence of visitors in Spanish ibex (5.2±3.49 ng/100 mg dry faeces in low visitor presence, and 6.4±4.00 ng/100 mg dry faeces in high visitor presence, $P = 0.16$), nor in fallow deer (1.1±0.40 ng/100 mg dry faeces in low visitor presence, and 1.0±0.36 ng/100 mg dry faeces in high visitor presence, $P = 0.22$). It was concluded that visitor presence affected fallow deer and Spanish ibex vigilance behaviours, but did not have a negative impact on their physiological response in terms of cortisol metabolites.

1. Introduction

Animal welfare is a multidimensional concept that includes the health, emotional state and also behaviour expression of an individual (Fraser 1997). The leading zoos and other centres that keep wild animals in captivity are focusing their efforts in improving the welfare of the animals under their care, in addition to their conservation, entertainment, education and research roles (Reade and Waran 1996). However, animal welfare status can potentially be affected due to different aspects such as space limitations, health problems, or inability to express certain important behaviours for that species (Manteca 2015). Another factor that could impair animal welfare is the presence of zoo visitors, and several studies have tried to assess the visitor effect on captive animals.

Most of the studies suggested that visitor effect could have a negative impact on welfare. Researchers observed an increase in the expression of visitor-avoidance (Smith and Kuhar 2010; Ozella et al 2015), aggressive (Sellinger and Ha 2005; Sekar et al 2008) and abnormal (Mallapur et al 2005; Vidal et al 2016) behaviours, as well as a positive correlation between the number of visitors and the concentration of glucocorticoids or glucocorticoid metabolites (Davis et al 2005; Rajagopal et al 2011; Pifarré et al 2012). On other occasions, though, it was suggested that visitors did not have an evident visitor effect, at least in the animals and situations studied (Sherwen et al 2014; Sherwen et al 2015a; Hosey et al 2016; Jones et al 2016).

The main concern when studying the visitor effect is to determine if the presence of unfamiliar people produces a stressful situation for the individuals, compromising their welfare. Chronic or long-lasting stress can weaken the immune system, restrict the reproduction success and/or facilitate the presence of abnormal behaviours (Möstl and Palme 2002).

A stressor activates the stress response, which is driven, along with other systems, by the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. The HPA axis releases glucocorticoids such as cortisol as part of the endocrine mechanism for self-protection of the body in the presence of a stressor. The quantification of cortisol or its metabolites can be determined in different matrixes such as plasma, faeces, urine and saliva (Mormède et al 2007), and it is used as a physiological indicator for the assessment of stress. Some studies have used the concentration of cortisol or its metabolites, as well as changes on the expression of certain behaviours, as indicators to assess the effect of visitor presence in animal welfare (de Azevedo et al 2012; Clark et al 2012; Pifarré et al 2012; Sherwen et al 2015a; Sherwen et al 2015b).

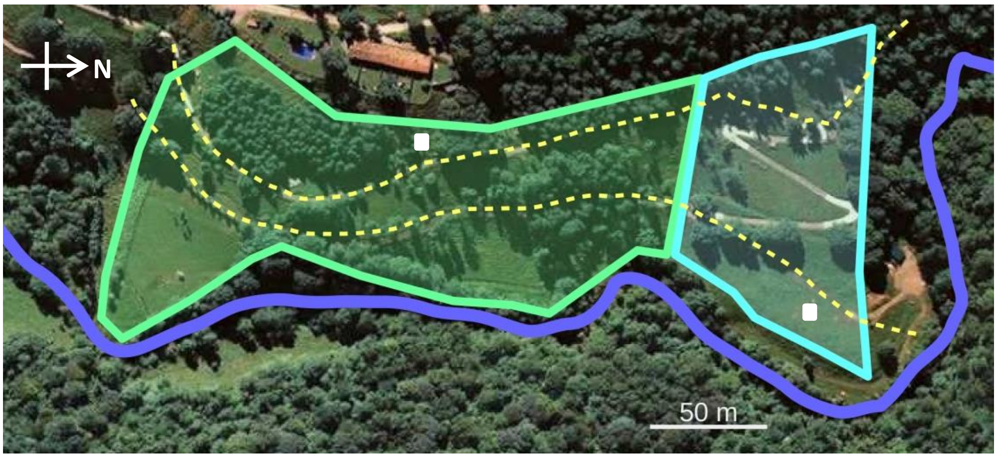
The aim of this study was to use animal-based welfare indicators to assess the effect that zoo visitors had in two species of wild ungulates housed in free-range exhibits. As welfare indicators, we used vigilance behaviours and the concentration of cortisol metabolites in faeces. The species included in the study were the fallow deer (*Dama dama*) and the Spanish ibex (*Capra pyrenaica*).

2. Materials and methods

2.1. Description of the enclosures

The study was carried out in Molló Parc, a zoological institution situated in the Pyrenees that holds autochthonous species. The enclosures of the animals assessed during this study (fallow deer and Spanish ibex) were adjoining spaces and were both free-range exhibits. The area of the fallow deer's enclosure was about 14200 m^2 and the enclosure for the Spanish ibex was about 5800 m^2 (Figure 1).

Figure 1. Representation of the enclosures of the fallow deer (left) and the Spanish ibex (right) studied. The dotted lines represent the visitor pathways and squares represent the feeding and water troughs. At the highest point the terrain reached 1125 m, and the lowest point, close to the river, was 1080 m. Image adapted from the Insitut Cartogràfic i Geològic de Catalunya (Ortofoto ICGC 1:2500; http://www.icgc.cat; license https://creativecommons.org/licenses/by/4.0/deed.ca).



The visitor route plan suggested by the zoo was circular and the visitors passed at least twice through each enclosure of these two species, usually first through a lower pathway and then through an upper pathway (Figure 1). The pathways for people were well marked, but it was easy for a person to leave the defined path and get closer to the animals. Since visitors walked through the exhibits, there were doors to separate one enclosure from the other. These doors closed themselves once the visitors passed through, but some fallow deer had learned to open the doors by pushing if the last person entering had not locked the door. It was forbidden for the visitors to feed the animals or to leave the defined pathway.

2.2. Populations studied

One group of Spanish ibex (n=8) and one group of fallow deer (n=13) were studied (Table 1). Animals did not have individual tags or any other kind of artificial external identification mark. However, it was possible to tell the individuals apart after dedicated observation of their individual physical differences in colour and pattern of fur, horn shape or other external characteristics.

Table 1. Composition of the groups of Spanish ibex (n=8) and fallow deer (n=13) studied and number of faeces collected per species and per treatment.

Species	Number of males	Number of females	Number of faeces collected per treatment	
			Low visitor presence	High visitor presence
Spanish ibex (*Capra pyrenaica*)	4	2	41	56
Fallow deer (*Dama dama*)	4	5	67	81

2.3. Behavioural observations and faecal sampling

The same procedures were done on both species: behavioural observations and faeces sample collection. Two treatments were established: low visitor presence (ranging from 0 to 25, median = 6 visitors per day) and high visitor presence (ranging from 117 to 1125, median =196 visitors per day). Observations were done by the same observer in February-May 2015. Each species was observed for a total of 16 days: 8 days with high visitor presence (weekends and holidays) and 8 days with low visitor presence (weekdays)). Observations were done in two time slots per day, one in the morning (starting at 1130h) and one in the afternoon (starting at 1500h). The observation method used was scan sampling of all the animals every 5 minutes during 18 points (1.5 hours per session).

In order to study the visitor effect, we decided to observe the expression of vigilance behaviours, and the ones recorded were 'vigilant behaviour' and 'displacement caused by the visitor'. 'Vigilant behaviour' was defined as 'the animal lifts its head away from the ground and pays attention to its surroundings, indicated by the head held high, either with or without a scan of the environment' (Hunter and Skinner 1998). 'Displacement caused by the visitor' was defined as 'the animal gives up its position as a consequence of the approach or the presence of humans'.

We decided to include a physiological welfare indicator, and we collected faeces of the individuals in order to quantify the concentration of cortisol metabolites. Faeces were non-invasively collected in the afternoon after the behavioural observations, from as many individuals as possible. A minimum of 6g of faeces per sample were collected and put in individual bags properly identified and were kept refrigerated once collected and frozen at -20ºC until the evaluation of cortisol metabolites. Although the aim was to get samples from all the animals in all determined time slots, it was not possible due to difficulties during the sample collection. A total of 97 faecal samples from Spanish ibex and of 148 from fallow deer were collected (Table 1).

2.4. Faecal cortisol metabolites extraction and quantification

Cortisol metabolites were extracted from faeces using the same technique in both species. First, the samples were dried in a desiccator at 60ºC during a week, until the moisture was completely evaporated. Secondly, each sample was chopped using a Mixer Mill 200, Retsch® at 25 Hz for 3 minutes. Thirdly, the powder of each sample was precise weight to obtain 300 mg. After that, the 300 mg of sample powder were deposited in a Falcon tube and then 2.5 ml of distilled water and 3 ml of pure methanol were added. Then each Falcon tube was shaken using a vortex for 30 minutes. Later, each sample was centrifuged at 3000 rpm for 15 minutes at 25ºC. To finish, 20 µL of the supernatant was put in an Eppendorf tube and this was frozen at -20ºC until analysis.

The quantification of cortisol metabolites in faeces was performed using an enzyme-linked immunosorbent assay (DetectX® Cortisol Enzyme Immunoassay, Arbor Assays Inc., Ann Arbor, MI, USA). The intra-assay coefficient of variation was 16.19% for Spanish ibex, and 7.87% for fallow deer. The inter-assay coefficient was 26.21% for Spanish ibex, and 18.65% for fallow deer. The average recovery percentage from spike-and-recovery test was 81.30±25.62% for Spanish ibex, and 83.04±33.43% for fallow deer. The sensitivity was 1.760ng of cortisol/100mg dry faeces for Spanish ibex, and 0.594ng of cortisol/100mg dry faeces for fallow deer. The curves with standard and faecal samples showed parallel displacement for Spanish ibex (standard curve y = -0.239x + 0.455, R^2 = 0.81; pooled faecal samples y = -0.240x + 0.148, R^2 = 0.80), and fallow deer (standard curve y = -0.187x + 0.514, R^2 = 0.84; pooled faecal samples y = -0.202 + 0.128, R^2 = 0.92).

2.5. Statistical analysis

For all data, the individual animal is the experimental unity. Faecal cortisol metabolites levels were analysed using the proc mixed procedure of SAS (SAS® 9.4. Institute Inc., Cary, NC) after log transformation. The occurrence of vigilance behaviours expressed as number of events per animal and per day was analysed using the glimmix procedure. A negative binomial distribution was used according to the value of the deviance (Cameron and Trivedi 1998). For each species, the models studied the effect of the treatment (low vs. high visitor presence), the sex and the interaction treatment x sex. The day of observation was included in the models as a random variable. In addition, the between-individual variability for faecal cortisol metabolites levels was estimated after considering the variable "individual" as a fixed effect. The residual maximum likelihood was used as a method of estimation and the least square means of fixed effects (LSMEANS) were used when analysis of variance indicated differences ($P < 0.05$). Spearman's correlation coefficients between the faecal cortisol level and each vigilance behaviour were calculated. Significance was declared at $P < 0.05$, and tendencies are discussed at $P < 0.10$.

3. Results

The results obtained from the behavioural observations and the quantification of the faecal cortisol metabolites are represented in Table 2 (Spanish ibex) and in Table 3 (fallow deer).

Table 2. Mean occurrence (mean number of events per day and per individual) and SD of vigilant behaviour and displacement caused by the visitors, and faecal cortisol metabolites level measured at individual level, depending on the treatment and the sex of the animals, in the group of Spanish ibex studied (n=8). Different lowercase letters indicate significant differences (*P* < 0.05).

	Variables	Treatment		Sex	
		Low visitor presence	High visitor presence	Females	Males
Spanish ibex	Vigilant behaviour (events/animal/day)	1.0±1.01[a]	3.2±2.14[b]	1.9±1.82	2.3±2.13
	Displacement caused by the visitors (events/animal/day)	0[a]	0.5±0.71[b]	0.2±0.44	0.3±0.65
	Faecal cortisol metabolites (ng/100 mg dry faeces)	5.2±3.49	6.4±4.00	6.5±4.51	5.2±2.79

Table 3. Mean occurrence (mean number of events per day and per individual) and SD of vigilant behaviour and displacement caused by the visitors, and faecal cortisol metabolites level measured at individual level, depending on the treatment and the sex of the animals, in the group of fallow deer studied (n=13). Different lowercase letters indicate significant differences (*P* < 0.05).

	Variables	Treatment		Sex	
		Low visitor presence	High visitor presence	Females	Males
Fallow deer	Vigilant behaviour (events/animal/day)	0.6±0.84[a]	3.3±2.26[b]	2.5±2.37[x]	1.6±1.94[y]
	Displacement caused by the visitors (events/animal/day)	0	0.2±0.46	0.1±0.22	0.1±0.39
	Faecal cortisol metabolites (ng/100 mg dry faeces)	1.1±0.40	1.0±0.36	1.2±0.42[x]	1.0±0.36[y]

No significant correlation was found between the faecal cortisol metabolites concentrations and the occurrence of vigilant behaviour and displacement caused by visitors in Spanish ibex, nor in fallow deer.

No significant interactions were detected between treatment and sex for any of the variables studied in both species.

A high individual variability was reflected by high standard deviations in the faecal cortisol metabolite concentrations in both species. However, there was no individual effect on faecal cortisol metabolites concentration in Spanish ibex (P = 0.84) nor fallow deer (P = 0.15).

4. Discussion

Both Spanish ibex and fallow deer displayed more vigilance behaviours when there was higher visitor presence. An increase in vigilance behaviours has also been described in species such as sika deer (*Cervus nippon*, Shen-Jin et al 2010), western lowland gorilla (*Gorilla gorilla* grilla, Carder and Semple 2008; Clark et al 2012), Kangaroo Island kangaroo and red kangaroo (*Macropus fuliginosus fuliginosus* and *Macropus rufus*, Sherwen et al 2015a), and greater rheas (*Rhea americana*, de Azevedo et al 2012). Different reasons could explain the appearance of vigilance behaviours towards humans. In ungulates, the described vigilance or alertness behaviours could be interpreted as antipredator responses due to fear, but also as a form of curiosity towards visitors (Hosey 2013).

This ambiguity in interpreting the motivation behind the expression of vigilance behaviours when visitors are present, makes it difficult to draw a conclusion on the visitor effect on the welfare of the ungulates observed based only on these behavioural measures.

We included not only behavioural indicators, but also a physiological indicator. As it has been previously stated, a stressor activates the stress response, which is driven by the activation of the HPA axis. The HPA axis releases glucocorticoids such as cortisol as part of the endocrine mechanism for self-protection of the body in presence of a stressor. Faecal cortisol metabolites come from the bloodstream, are metabolised in the liver, and are excreted by the bile duct (Palme et al 1996).

Therefore, cortisol metabolites found in faeces are a reflection of the total amount of cortisol excreted during the past few hours. This range of hours depends on the digestive transit time of the species (Möstl and Palme 2002). Fallow deer and Spanish ibex are ruminants, so the results of faecal cortisol metabolites represent the amount of cortisol excreted during the past 10-12 hours (Palme et al 1999).

We collected the faecal samples in the afternoon, after the observation session, in order to analyse the concentration of cortisol metabolites excreted during each observation day. However, no differences in the concentration of faecal cortisol metabolites were found between the days with high visitor presence and the days with low visitor presence. These results suggest that visitor presence did not have a negative effect on the stress response of the animals measured by means of faecal cortisol.

Female fallow deer had slightly significant higher average concentration of faecal cortisol metabolites, as well as a significance higher expression of vigilant behaviour independently of the treatment, compared with males. This difference between sexes could be explained due to a gender effect as a consequence of the activity of the sexual hormones. There is evidence that the gonads modulate the HPA axis and that there are sexual differences in the secretion of glucocorticoids (Van Lier et al 2014). In some species, females have higher basal levels of corticosteroids compared with males (in rat, Ogilvie & Rivier 1997; in sheep, Van Lier et al 2003). It is still unknown which are the basal levels of glucocorticoids in males and females of fallow deer, but the higher average of faecal cortisol levels in females could be due to a gender effect.

The enclosures of the fallow deer and the Spanish ibex were adjacent spaces. The first one had an area of about 14200 m^2 and second one of about 5800 m^2, respectively. They were free-range exhibits and the visitors walk through both enclosures using two marked pathways. It could be possible that these large exhibits give the animals more freedom of movement, allowing them to choose which distance they want to keep from visitors. It has been suggested necessary to give the individuals the opportunity to choose and have some control over their environment (Ozella et al 2015; Sherwen et al 2015a; Bonnie et al 2016). This could potentially reduce the negative impact from visitor presence.

The increase in vigilance behaviours could be due to a natural instinct to check for movements in the surrounding area, and may not unleash the stress response unless a real threat is perceived. Therefore, the possibility needs to be considered that animals have been habituated to the presence of visitors, who could be potential stressor cues otherwise. Different studies about the visitor effect in greater rheas (de Azevedo et al 2012), African penguins (*Spheniscus demersus*, Ozella et al 2015), Kangaroo Island kangaroos and red kangaroos (Sherwen et al 2015a), and crowned lemurs (*Eulemur coronatus*, Jones et al 2016) have also hypothesised that animals have been habituated to the visitor presence.

It was forbidden for the visitors to deviate from the marked pathways, but on some occasions people were observed leaving the pathways and getting closer to the animals. The displacement behaviours were observed in these situations or if animals were standing or lying in the visitor pathway and people approached. The steeper areas of the enclosures provided quieter retreat areas that visitors could not climb on, but animals could not avoid being seen. Therefore, the current retreat areas did not provide enough protection for the animals to avoid the visitors' view, and retreat areas or hidden spots should be provided. Visual barriers and good enclosure designs can help to avoid the direct view of the visitors, and have been considered important in other species such as the western lowland gorilla (Kuhar 2008) and the black-capped capuchin (*Cebus apella*, Sherwen et al 2015b).

5. Conclusion

When visitor presence was high, both Spanish ibex and fallow deer express more vigilance behaviours. However, the quantification of faecal cortisol metabolites did not show any significant negative effect of the presence of visitors on the animal's stress response.

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CHAPTER 3

Development and application of a protocol to assess welfare in captive dorcas gazelles (*Gazella dorcas*)

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Abstract

There is a lack of protocols especially developed for the assessment of welfare in captive animals, even when providing good standards of welfare to wild animals kept in captivity is important. The aim of this study was the development and the application of a protocol for the assessment of welfare in captive dorcas gazelles (*Gazella dorcas*). The protocol was developed taking into account the available literature of the biology of this species in the wild, the Husbandry Guidelines especially developed for the captive breeding and management of this species, and the protocol for the assessment of welfare in cattle from the Welfare Quality® project. The protocol was specifically developed for dorcas gazelles and included four principles, 10 criteria, and 23 animal and environmental-based indicators. To test its utility, this protocol was applied to five different groups of gazelles (three bachelor groups, one group of adult females and one group of females with young) from three different zoos. Its application made possible to detect some areas for improvement in all groups assessed.

1. Introduction

Despite the ethical (Moran 1987) and legal (Council Directive 1999/22/EC 1999) importance of welfare in wild animals kept in captivity and that ensuring optimal animal welfare is essential for the establishment and maintenance of viable populations of animals in good health (WAZA 2005), there is a lack of standardized and validated tools to assess welfare in captive animals.

Welfare assessment protocols can provide such tools by using a combination of several welfare indicators that provide information gathered by simple survey, enclosure inspection, and remote observation of animals. These protocols can be a simple, economic way to assess the welfare of captive animals, as well as to gauge the effect on animal well-being when an improvement is made.

Dorcas gazelles are part of the family Bovidae and are one of the smallest species of gazelles. These animals are naturally distributed around the Sahel-Saharan region (North Africa), living in a variety of habitats that include savannah, semi-desert plains, and desert (Yom-Tov et al. 1995). They are ruminants; therefore, their diet is strictly herbivore and they eat succulent plants, hard desert herbs, and fruits and leaves from different species of acacias (Alados 1982). They are gregarious animals with a strong hierarchical structure (Lawes and Nanni 1993).

Dorcas gazelles are considered 'vulnerable' in the IUCN Red List of Threatened Species (IUCN SSC Antelope Specialist Group 2008). The European Association of Zoos and Aquaria (EAZA 2011) established in 2002 the European Endangered species Program (EEP) for the subspecies Saharawi dorcas gazelle (*Gazella dorcas neglecta*).

The aim of this project was the development and application of a protocol for the assessment of welfare of captive dorcas gazelles. The project was executed in two phases: first, the development of the welfare protocol; and second, the application of the protocol in facilities holding dorcas gazelles.

2. Development of the welfare protocol

2.1. Materials and methods

The welfare protocol was developed using a combination of the study of dorcas gazelles' biology and behaviour in natural conditions, the Husbandry Guidelines for the captive breeding and management especially developed for this species (López and Abáigar 2013), and the welfare protocols for cattle of the Welfare Quality® (2009) project, since dorcas gazelles are also bovids.

In order to obtain general information about dorcas gazelle's biology and behaviour, we used the Web of Science™ search engine using the keywords "dorcas gazelle*". A total of 48 papers were reviewed and we found useful information to add to the protocol in three of the papers.

The Husbandry Guidelines for the captive breeding and management developed for dorcas gazelles provided information on current best practices and recommendations for the management of this species in captivity.

The Welfare Quality® project protocols take into account four principles: good feeding, good housing, good health, and appropriate behaviour as an expression of optimal emotional states. These four principles lead to 12 criteria that allow the development of the welfare assessment indicators: absence of prolonged hunger and thirst; comfort around resting, thermal comfort, and ease of movement; absence of injuries, diseases, and pain induced by management procedures; and expression of social and other behaviours, good human-animal relationship, and positive emotional state (Botreau et al. 2007).

2.2. Results

The protocol developed for the welfare assessment of captive dorcas gazelles includes four principles, 10 criteria and 23 indicators (Table 1). A description of the 10 criteria, together with their proposed indicators, follows.

Table 1. Principles, criteria and indicators of the protocol to assess welfare in captive dorcas gazelles. There are 10 animal-based indicators (indicated by '*') and 13 resource or management-based indicators.

Principles	Criteria	Indicators
Good Feeding	1. Absence of prolonged hunger	1.1. Body condition*
	2. Absence of prolonged thirst	2.1. Number of water points 2.2. Availability of water 2.3. Cleanliness of the water points
Good Housing	3. Thermal comfort	3.1. Availability of shade 3.2. Availability of shelter
	4. Ease of movement	4.1. Enclosure size (area) 4.2. Square meters available per animal
Good Health	5. Absence of injuries	5.1. Lameness* 5.2. Integument alterations*
	6. Absence of disease	6.1. Nasal discharge* 6.2. Ocular discharge* 6.3. Hampered respiration* 6.4. Diarrhoea*
Appropriate Behaviour	7. Expression of social behaviours	7.1. Affiliative behaviour* 7.2. Intra-specific aggression*
	8. Group size	8.1. Number of gazelles 8.2. Composition of the group 8.3. Number of animals of other species
	9. Expression of other behaviours	9.1. Stereotypies* 9.2. Environmental enrichment program
	10. Good human-animal relationship	10.1. Medical training program 10.2. Capture, immobilization and handling

2.2.1. Absence of prolonged hunger

Even though there are descriptions on other considerations related with feeding and diet requirements, no parameter related with this indicator is described in the Husbandry Guidelines for this species. However, body condition is included as an animal-based indicator in several protocols to assess welfare in animals. Poor body condition might be a consequence of inadequate nutrition, poor health, or chronic hunger; and it also can have negative effects on health, behaviour and reproduction.

Excessive body condition may increase the risk of lameness and other conditions and it may be a consequence of lack of physical exercise (Kurt and Kumarasinghe 1998).

Both poor and excessive body condition are indicative of a welfare problem. However, there is no body condition scale developed for dorcas gazelles currently. Therefore, gazelles' body condition (indicator 1.1.) is to be scored following the guidelines for the assessment of this indicator in another ruminant, the deer (Audigé et al. 1998). The animals are visually assessed from behind and from the side in the loin. Gazelles are scored as 'Poor body condition': pelvis, ribs and spine prominent; concave rump area; 'Normal body condition': pelvis, ribs and spine not readily distinguished or appear rounded rather than sharp; rump area is flat; or 'Excessive body condition': pelvis concealed by fat cover; rump very convex; spine not visible.

2.2.2. Absence of prolonged thirst

Ad libitum access to good quality water is considered a welfare requirement and welfare assessment protocols for farm animals include provision of water as a resource-based indicator. Gazelles should have easy access to drinking water area and troughs should be cleaned daily. The Husbandry Guidelines consider important that gazelles have *ad libitum* access to clean water changed daily.

The number of water points has to be checked (indicator 2.1.), as well as the availability of water (2.2.). The cleanliness of the water points (2.3.) with regard to the presence of old or fresh dirt on the inner side of the bowl or trough as well as staining of the water are also checked. Water points are considered clean when there is no evidence of crusts of dirt (e.g. faeces, mould), and/or decayed food residue, although some amount of fresh food is acceptable.

2.2.3. Thermal comfort

Even though dorcas gazelles are the most widespread of the gazelle species in the wild (Alados 1982), in zoos they are often kept in climates that are very different from those of their natural habitat. However, it seems that gazelles can adapt to a diversity of climates. Nevertheless, wet and muddy conditions are likely to compromise welfare, as they may increase the risk of feet conditions. Very high temperatures might cause heat stress and sunburn if animals do not have access to shade. Currently, there is no precise information on the range of temperatures that is adequate for dorcas gazelles.

The Husbandry Guidelines describe that gazelles should have protection from bad climatic conditions, as well as from a prolonged exposure to the sun. They state that all enclosures should provide a shelter or stable with roof, enough vegetation or objects to provide shade, and, if possible, for indoor facilities to have controlled temperature.

Two indicators were developed to assess the thermal comfort: availability of shade (indicator 3.1.) and shelter (3.2.). For the assessment of these indicators, it has to be recorded whether all animals in the enclosure can have access at the same time to a non-damp or non-muddy area, shade, and shelter from bad climatic conditions.

2.2.4. Ease of movement

Dorcas gazelles are found in a variety of habitats in the wild and can move fairly long distances depending on which habitat they live (Lawes and Nanni 1993). Animals kept in small enclosures are more likely to develop physiological and behavioural changes indicative of poor welfare than animals kept in larger enclosures. However, although the amount of space available for the animal is important, the quality and complexity of the space (e.g. whether there is any sort of environmental enrichment) is likely to be even more important (Carlstead and Shepherdson 2000). Recommendations on the minimum space per animal are very diverse and the rationale for such recommendations is not clear. According to the Husbandry Guidelines each gazelle should have a minimum of 46.5 m^2 and 11.61 m^2 for each additional animal. According to the American Association of Zoos and Aquariums (Smith et al 1997), each gazelle should have a minimum of 18.6 m^2.

The enclosure size (area) (indicator 4.1.) and the square meters available per animal (4.2.) are two indicators developed to assess the ease of movement of dorcas gazelles in captivity. For the assessment of enclosure size, the area of the enclosure has to be calculated. For the determination of the square meters available per animal the area of the enclosure is divided by the total number of animals found in that enclosure. Also, it has to be recorded if there are adequate resting places for all the animals at the same time.

2.2.5. Absence of injuries

Two indicators were developed to assess the absence of injuries: lameness and integument alterations.

Hoof problems are common in Artiodactylds (Boever 1986) and these and other feet problems, traumas due to aggressive behaviours, consolidated bone fractures or other conditions can lead to lameness. In farm animals, lameness is considered a major welfare problem as it is indicative of pain and may interfere with normal behaviour.

Animals are observed in motion because lameness (indicator 5.1.) is an abnormality of gait that is more evident when the legs are in motion. Animals have to be scored as 'not lame' (when the animal walks without any apparent abnormality) or 'lame' (when the animal walks with an apparent abnormality or without resting one or more legs on the floor).

Integument alterations such as hairless patches and lesions or swellings may be a consequence of disease, rough handling, intra-specific aggression, or inappropriate physical environment. In farm animals, presence of injuries on the integument is commonly used as indicator of poor welfare. The Husbandry Guidelines recommend paying attention to any object, handling procedure or animal (especially when intra-specific aggressive behaviours have been observed and in the case of enclosures shared with other species) that could cause lesions to gazelles.

Only skin alterations (indicator 5.2.) of a minimum diameter of 2 cm at the largest extent have to be counted. A hairless patch includes an area with hair loss, with the skin not damaged, an extensive thinning of the coat due to parasites and hyperkeratosis. A lesion/swelling includes damaged skin either in form of a scab or a wound, dermatitis due to ectoparasites and ear lesions due to torn off ear tags. Without touching the animals, three body regions on one side of the assessed animal have to be examined: body, hind leg and front leg. These body regions are scanned from the rear to the front, excluding the bottom side of the abdomen and the inner side of the legs, but including the inner side of the opposite hind leg. Random side selection (right or left) before the examination has to be ensured, in order to prevent biased results.

Animals are scored as follows: 'no integument alterations'; 'mild integument alterations': at least one hairless patch, but no lesion/swelling; and 'severe integument alterations': at least one lesion/swelling or large hairless patch.

2.2.6. Absence of disease

According to the Husbandry Guidelines, the most frequent diseases or afflictions in captive dorcas gazelles are traumatisms, behavioural disorders, gastrointestinal and respiratory affections, and birth problems. Traumatisms are due to fights, accidents at capture or management, or by accidental trampling by larger species. These can be assessed through the inspection of integument and the presence of lameness.

The Welfare Quality® protocols for ruminants include some indicators that can be used as a tool to assess (through remote observation) the gastrointestinal and respiratory affections described in the Husbandry Guidelines.

Nasal discharge (indicator 6.1.) is a 'clearly visible flow/discharge from the nostrils that can be transparent to yellow/green and often is of thick consistency'. Ocular discharge (6.2.) is a 'clearly visible flow/discharge (wet or dry) from the eye, at least 3 cm long'. Hampered respiration rate (6.3.) is a 'deep and overtly difficult or laboured breathing; expiration is visibly supported by the muscles of the trunk, often accompanied by a pronounced sound'. Diarrhoea (6.4.) is a 'loose watery manure below the tail head on both sides'. Animals are scored with regard of each described indicator as 'no evidence' or 'evidence' of the specific indicator.

2.2.7. Expression of social behaviours

Affiliative behaviours are considered self-rewarding and they may have a buffering effect on stress. However, in domestic cattle, social grooming may increase after stressful events. The Husbandry Guidelines do not include information regarding affiliative behaviours among adult animals.

Intra-specific aggression may lead to injuries and social stress and has been included in several protocols to assess welfare in farm animals. According to the Husbandry Guidelines, adult males of dorcas gazelles usually present high levels of aggression while in captive conditions. Also it is usual that a breeding male attacks the other males when he is moved from a reproductive group to a bachelor group. Some degree of intra-specific aggression may be normal or even unavoidable. Therefore, only "excessive" aggression should be indicative of poor welfare, and currently there is no definition of "excessive" intra-specific aggression in dorcas gazelles. Presence of overt aggression and frequent threats between animals should be considered indicators of poor welfare.

Two indicators were developed to assess the expression of social behaviours: affiliative behaviours (indicator 7.1.) and intra-specific aggression (7.2.). An ethogram with the social behaviours is included in Table 2. Overall observation time is 180 minutes per group, in nine 20-minute long sessions. The frequency of social behaviours has to be recorded using continuous focal behaviour sampling, since social behaviours may be subtle and of short duration.

2.2.8. Group size

Three indicators were developed to assess the group size, including number of gazelles in the group, composition of the group and number of animals of other species.

Dorcas gazelles have a complex and habitat-related social organisation. The different social structures are largely a consequence of the availability and distribution of food resources: dorcas gazelle group size increases with increased forage quality (Grettenberger 1987; Lawes and Nanni 1993). Four situations have been observed in the wild: harem-like structure (social units with one male accompanied by one-five females), satellite groups of immature males, female herds unaccompanied by males, and male pairs. In zoos, animals are usually kept in four groups: females and young with only one reproductive male; females and young without adult males; bachelor groups of males; and isolated males (they usually were the reproductive males of a harem).

Table 2. Description of the social behaviours that are included in the welfare protocol for dorcas gazelles.

	Behaviour	Definition of the behaviour
Affiliative behaviour	Social 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 seconds 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 seconds 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 reversal role between actor and receiver.
	Horning	Head play with 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 in order to become victorious. It is taken as a new bout if the same animals start horning after 10 seconds or more or if the horning partner changes.
Aggressive behaviour	Displacement with physical contact	Interaction where the actor is butting, hitting, thrusting, striking, pushing or penetrating the receiver with forehead, horns, horn base or any other part of the body with a forceful movement and as a result the receiver gives up its position.
	Displacement without physical contact	The actor threats or interacts with the receiver without making any physical contact and as a result the receiver gives up its position.
	Chasing	The actor makes an animal flee or give up its current position by following fast or running behind it, sometimes also using threats like jerky head movements. Chasing is recorded even if it not follows 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 and both exert force against each other. A new bout starts if the same animals restart fighting after more than 10 seconds or if the fighting partner changes.

Social contact is necessary for good welfare in group-living animals and social isolation has been associated with stereotypies, chronic stress, and incompetence on the performance of reproductive and social behaviours (Price and Stoinski 2007). In domestic social species, being kept in groups is considered a requisite for good welfare.

Group size is not the only factor to consider, as group composition and the compatibility between individual animals are also important. The Husbandry Guidelines propose approximate numbers of how big captive groups should be; however, the rationale for such recommendations is not clear: one adult male and 3-7 adult females and their young in the case of reproductive groups, and between 3-7 adult males in the case of bachelor groups. They do not give a number in the case of the group with adult females and young. Only aggressive adult males should be kept isolated if a treatment with long-action tranquilizers does not work. Females should not be kept isolated because they might suffer high stress levels.

For the assessment of the number of gazelles in the group (indicator 8.1.) and composition of the group (8.2.), it has to be recorded how many animals form the group, their age and sex. Also it needs to be recorded if any individual (regardless of age, sex, or if it is castrated or not) is kept alone without any contact from other conspecifics.

The current tendency in zoos is to make larger and more naturalistic enclosures (Reade and Waran 1996), and mixing species that share the same space is done as an enrichment source sometimes. However, this has to be carefully done. In the case that dorcas gazelles share their enclosure with individuals of species bigger than them, the Husbandry Guidelines recommend the use of selective gates that only gazelles can go through. Gazelles could use those gates to evade or avoid individuals from another species.

For the assessment of the number of animals of other species (indicator 8.3.) is recorded which species and how many animals of each species share the enclosure with dorcas gazelles. Any information related with the social inter-specific interactions or the use of space could provide valuable information for welfare assessment.

2.2.9. Expression of other behaviours

Stereotypies were defined as behaviours that are repetitive, invariable, and without any apparent function. More recently, Rushen and Mason (2006) described them as repetitive behaviours resulting from illness or repeated attempts to adapt to a difficult environment. In general, stereotypies are considered to be indicators of a lack of good welfare. This is due to both the circumstances that favour their development, such as restrictive environments that prevent the expression of normal species-specific behaviours, and the fact that some stereotypies have negative consequences to the animal, causing injury or loss of body condition (Mason 1993).

The Husbandry Guidelines do not contain any information regarding stereotypies in dorcas gazelles. However, although stereotypies are not widely reported in this species, ungulates are particularly at risk of developing oral stereotypies in captivity (Hosey et al. 2009). In fact, repetitive, seemingly functionless oral and oro-nasal activities (e.g. object-licking, dirt-eating, tongue-rolling, etc.) are prevalent in captive ungulates (Bergeron et al. 2006).

The relationship between stereotypies and individual welfare is complex, as stereotypies may persist even after the environment in which the animal is kept has been considerably improved, as well as not always poor welfare status results in developing stereotypies.

All the animals are to be assessed for the presence or absence of stereotypic behaviours (indicator 9.1.) during the observations of the expression of social behaviours. Animals are scored as 'no presence of stereotypic behaviour' or 'presence of stereotypic behaviour'. If any stereotype is recorded, the behaviour has to be described.

Environmental enrichment has positive effects on welfare in captivity. Dorcas gazelles in the wild use different kinds of trees with different purposes (Attum and Mahmoud 2012). Larger acacia trees are used for territorial purposes because gazelles use middens (or dung piles) for activities related to territory maintenance, advertisement, and olfactory communication. Larger trees also provide more shade, another food source (such as seed pods) and cover from predators than smaller trees. The loss of large trees in the wild may indirectly affect social behaviour of dorcas gazelles because animals are losing conspicuous landmarks that could be used for midden sites. Dorcas gazelles in the wild spend much of their total time foraging and browsing, and shorter trees are a source of browsing material.

The Husbandry Guidelines consider essential that gazelles have the opportunity to perform natural behaviours for the species, as well as grazing, browsing, marking the territory, keeping a healthy physical condition and also running away or hiding. With that aim, they recommend the use of structural components, such as rocks, vegetation, irregular ground, and also to change the feeding routine or to provide sensorial stimulation with new sounds or smells.

For measuring the environmental enrichment program (indicator 9.2.) it has to be recorded if the animals are given opportunities to browse and if there are trees/poles/sticks/post/other vertical objects of different form and size in the enclosure and if they are accessible to the animals. If the centre has an enrichment program established for dorcas gazelles, it has to be described which enrichments they use and how often is the enrichment material changed.

2.2.10. Good human-animal relationships

If properly done, medical training is likely to reduce the stress caused by veterinary procedures. Training based on positive reinforcement could have positive effects on welfare and be considered as a form of enrichment. Poor training techniques (e.g. training based on punishment or carried out by inexperienced staff) have negative effects on welfare and are strongly not recommended. If medical training is not possible, handling systems that minimize stress during veterinary interventions are recommended.

For measuring the medical training (indicator 10.1.) it has to be recorded if the centre is currently using a medical program for this species and if it is based on positive or negative reinforcement techniques, and if carried out by experienced staff.

The Husbandry Guidelines do not mention the implementation of a medical training program, although it recommends that the capture, immobilisation and handling of the animals have to be intended to cause as little stress as possible.

The methods used to capture, immobilise and handle the animals (indicator 10.2.) have to be recorded.

3. Application of the welfare protocol

3.1. Materials and methods

The welfare assessment protocol was applied between May and June 2013 to five groups of dorcas gazelles held in three centres participating in the EEP of the EAZA for this species: Parc Zoològic de Barcelona, Zoo Aquarium de Madrid and Zoobotánico Jerez. The five groups assessed are named as F (for 'female group', n=17), FY (for 'female and young group', n=10) M1 (for 'male group 1', n=7), M2 (n=3) and M3 (n=5).

3.2. Results

The results obtained after the application of the protocol are as follows:

3.2.1. Absence of prolonged hunger

The application of the protocol for body condition (indicator 1.1.) showed that all the animals assessed had a 'normal body condition'.

3.2.2. Absence of prolonged thirst

The assessment of the water points (indicator 2.1.) for group M1 was not possible. Groups F and FY had three water points, and groups M2 and M3 had two.

The water point quality (both presence of water (indicator 2.2.) and cleanliness of the troughs (2.3.) should be improved in all centres assessed; especially in group FY, which of three water points only two had water and in both of them water was dirty. Only two groups had at least one clean water trough (groups M2 and M3), all the others were dirty or partly dirty.

3.2.3. Thermal comfort

Animals of groups F, FY and M1 had shade (indicator 3.1.) and shelter from bad conditions (3.2.) available to all the animals at the same time. Gazelles of group M2 shared the enclosure with two white rhinoceros (*Ceratotherium simum*). During the hottest hours of the day there was only one source of shade available, provided by an acacia next to the enclosure. The shade was exclusively used by the rhinoceros, and therefore the gazelles were continuously exposed to the sun. They also did not have shelter from bad conditions. Group M3 also did not have shelter from bad conditions, although the location where this zoo is located has a low frequency of precipitation and rain and storms are not very common. So, in this case, a shelter from bad weather might not be so important.

3.2.4. Ease of movement

The area (indicator 4.1.) and the square meters available per animal (4.2.) were different in all groups (Table 3). Group M2 had one of the largest enclosures, and the enclosure with more space per animal. However, it was also the enclosure with less chance of shelter from bad conditions and from the sun, and with less trees or vertical objects that gazelles use with territorial purposes and social communication. All groups fulfilled the minimum space per animal requirement according to the Husbandry Guidelines.

Table 3. Enclosure size (area) and space per animal available in each group of dorcas gazelle assessed.

Group	Number of animals	Enclosure size (area) (m^2)	Space per animal (m^2/animal)
F	17	728	43
FY	10	1143	88
M1	7	1117	93
M2	3	1143	229
M3	5	1287	184

3.2.5. Absence of injuries

One animal from group M2, one from group M3 and two from group FY were scored as ‘lame’. All the lameness had been previously documented in the veterinarian records of each institution and no new lameness (indicator 5.1.) was detected during the application of the protocol.

The assessment of integument alterations (indicator 5.2.) showed that none of the animals presented lesions, swellings or large hairless patch in their integument. However, due to the difficulty to observe the animals at closer distance, it was not possible to look for small hairless patches in every animal.

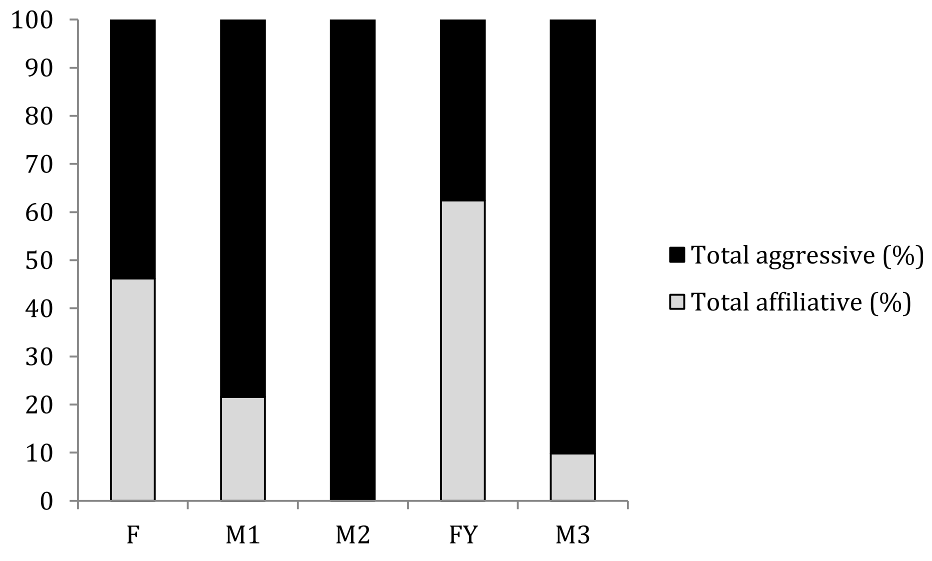
3.2.6. Absence of disease

None of the animals observed had nasal or ocular discharge, hampered respiration or diarrhoea (indicators 6.1., 6.2., 6.3., and 6.4., respectively) during the application of the protocol.

3.2.7. Expression of social behaviours

The application of the protocol for the indicators affiliative behaviour (7.1.) and intra-specific aggression (7.2.) showed that male groups (M1, M2 and M3) displayed more aggressive behaviours than the female (F) and female with young groups (FY) (Figure 1).

Figure 1. Percentage of aggressive and affiliative behaviours out of the total number of social interactions recorded for each group of dorcas gazelles studied (n = 42). The five groups assessed are named F (for 'female group', n=17), FY (for 'female and young group', n=10) M1 (for 'male group 1', n=7), M2 (n=3) and M3 (n=5).



3.2.8. Group size

When assessing the number of gazelles in the group (indicator 8.1.) and the composition of the group (8.2.), it was observed that none of the zoos kept gazelles individually and that all animals were part of a social group (F, n=17 (all females); FY, n=10 (four females and six sub-adult or young with less than 10 months of age); M1, n=7 (all males); M2, n=3 (all males); and M3, n=5 (all males)). Also, no mature males were sharing the same space with a group of adult females.

When assessing the number of animals of other species (indicator 8.3.), it was observed that, except for group F, all groups shared their enclosures with other species. However, although group M1 shared its space with five scimitar-horned oryx (*Oryx dammah*), group FY with three common ostriches (*Struthio camelus*) and group M3 with two Rothschild's giraffes (*Giraffa camelopardalis rothschildi*), no inter-specific agonistic interactions were observed. As previously stated, the gazelles of group M2 shared space with two white rhinoceros that occupied the only shade available in the enclosure during the hottest hours of the day, and therefore the gazelles were subjected to more hours of sun exposure. This problem could be solved by planting more trees in the enclosure or in the perimeter, or by installing a structure to provide shade. All the enclosures assessed where gazelles shared the space with other species had gates to allow them to hide.

3.2.9. Expression of other behaviours

None of animals observed presented any kind of stereotype (indicator 9.1.).

No formal enrichment program (indicator 9.2.) was implemented at any zoo. None of the animals were provided with browsing material regularly, although in all centres browsing material for the animals were provided at some point. However, this was sporadic and not scheduled. A good enrichment program is strongly recommended in all the zoos, with special opportunities to browse regularly. Group M2 had only three vertical objects but all of them were of the same size and material. More trees, posts, or other vertical objects could be added into the enclosure to give the animals more opportunities to communicate. At the same time, more shades would be provided, solving two shortcomings with only one action.

3.2.10. Good human-animal relationships

None of the groups of dorcas gazelles assessed was part of a medical training program (indicator 10.1.).

The capture, immobilization, and handling of the animals (indicator 10.2.) varied from one centre to another. In groups FY, M1 and M2 the capture of the animals was performed in dark indoor stables where usually the animals lied down, making the capture easier. In groups F and M3 a net was used to capture the animals. The posterior immobilization and handling of the animals was generally the same in all groups: the eyes of the gazelles were covered to decrease stress and their legs firmly held to avoid self-inflicted harm due to escape attempts.

4. General discussion

This welfare protocol developed for dorcas gazelles is the first documented work towards developing a standardised welfare assessment tool for this species. This is not the first welfare protocol for wild animals in captivity that has been developed using the Welfare Quality® protocols as a reference. An on-farm welfare assessment protocol for foxes (*Vulpes* spp) and mink (*Neovison vison*) (Mononen et al. 2012) was developed adapting the Welfare Quality® framework to a novel species. Clegg et al. (2015) also presented their work about the development of a welfare assessment index for captive bottlenose dolphins (*Tursiops truncatus*).

Animal welfare includes the emotional and the physical health, and also the behaviour of the animals. There is no single indicator capable of providing enough information to thoroughly assess animal well-being. Animal welfare can only be appropriately assessed when using a combination of several indicators (Botreau et al. 2007).

However, even if many indicators are needed, it is important to develop protocols of easy application. The ultimate goal of a protocol to assess welfare in wild animals kept in captivity is to have it be regularly used as a management tool in the centres where these animals are held. A successful protocol should be practical and easy to apply. The largest group of dorcas gazelles assessed in this study had 17 individuals, and it was possible to evaluate all the indicators in less than six hours per group.

The application of the protocol in five participant groups of the EEP of the EAZA for this species and the posterior analysis of the results made possible to detect some areas for improvement in all the centres assessed. Therefore, we think that this protocol can be a useful tool for those centres that keep dorcas gazelles in captivity. However, validation of the protocol has not been completed and consequently this protocol is presented as an initial phase to assess welfare of captive dorcas gazelles.

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GENERAL DISCUSSION

Through Chapters 1, 2, and 3 of this thesis on captive wild ungulates, different aspects related with two welfare issues such as social stress and visitor effect have been studied, as well as a protocol to assess welfare has been developed and applied to different groups of animals.

1. Frequent welfare issues in zoos: social stress and visitor effect

Zoos and other institutions that hold wild animals in captivity face different issues that can have a direct impact on the individuals' welfare. Lack of space, social stress, visitor effect, diseases and other health problems, and medical procedures (Manteca 2015) are some of the main challenges facing zoos when they want to guarantee an optimal welfare status for the animals under their care.

We used three species of ungulates to focus our attention on two main zoo welfare issues: social stress and visitor effect. For this purpose both physiological and behavioural indicators were used. We studied how social stress (Chapter 1) affected dorcas gazelles (*Gazella dorcas*) and if visitors' presence had an effect (Chapter 2) on the welfare of fallow deer (*Dama dama*) and Spanish ibex (*Capra pyrenaica*).

The management of captive populations for breeding and conservation purposes have resulted in the creation of human-made groups of animals. In particular, the management of the population of surplus males in limited spaces represents a challenge for zoos. They try to minimise the stress due to negative social interactions without reaching the point to keep animals of the same species individually without contact to conspecifics, which usually is considered a welfare problem.

In our study about social stress in dorcas gazelles (Chapter 1), we observed that the two bachelor groups studied displayed significantly more aggressive behaviours when compared with the reproductive groups. Aggressions can negatively affect welfare through physical damage such as wounds, bone fractures or even death of the individual. However, non-injurious aggression may be associated with reduced welfare because individuals might suffer from stress (Galindo et al 2011).

Zoo visitors are another factor that may be a stress source for captive animals, especially if the animals do not have any kind of control over their environment, enrichment opportunities, or if the enclosure does not have an adequate design that allows the animal to hide from the visitors' view if it chooses to (Blaney and Wells 2004; Choo et al 2011). The noise generated by the zoo public can also have a negative impact on animal welfare (Quadros et al 2014).

Our study related with the visitor effect in free-range exhibits of fallow deer and Spanish ibex (Chapter 2) could not conclude that visitors had a negative effect on the welfare of these animals. The amount of vigilance behaviours performed by the animals in days with more visitors was significantly higher than the days with fewer visitors. However, after the assessment of cortisol metabolite concentrations in faeces, we did not find any differences at hormonal levels in any species between the days of high and low visitor presence.

A similar study (Sherwen et al 2015) with Kangaroo Island kangaroos (*Macropus fuliginosus fuliginosus*) and red kangaroos (*Macropus rufus*) in free-range exhibits determined that there was no evidence of adverse effects in the welfare of these animals due to visitors. They found an increase in visitor-directed vigilance behaviour when visitor numbers were higher, but there was no effect of visitor number on the distance kangaroos positioned themselves from the visitor pathway or on the faecal glucocorticoid metabolites concentration in either species.

Other studies have assessed the visitor effect in different species of zoo animals. On some occasions, it has been suggested that visitors do not have a negative impact on the welfare of animals or at least that there is not an evident visitor effect in some species or situations (Sherwen et al 2014; Hosey et al 2016; Jones et al 2016). Most of the studies considered that the visitor effect could have negative impact on the individuals' welfare, increasing the expression of abnormal (Mallapur et al 2005; Vidal et al 2016), aggressive (Sellinger and Ha 2005; Sekar et al 2008), and visitor-avoidance (Smith and Kuhar 2010; Ozella et al 2015) behaviours.

The possibility that the visitor effect as a potential welfare issue has been overestimated and that previous studies that concluded that visitors did not have a negative impact on welfare have not been published should be considered.

We should also take into account the possibility that other environmental factors could have a more pronounced negative effect on welfare in some animals in captivity compared with the effect that visitors might produce on them, especially if the animals have been habituated to their presence.

In wild killer whales (*Orcinus orca*) it has been observed that prey availability has a greater physiological impact than the tourist vessel presence (Ayres et al 2012). In particular, faecal glucocorticoid metabolites concentrations were lower during the peak season in vessel traffic, which also coincided with the peak in prey availability.

Similar results have been observed in wild red deer (*Cervus elaphus*), whose higher faecal glucocorticoid metabolites concentrations seem to be more related with nutritional stress than with the presence of hikers or park visitors (López-Bejar M, unpublished data).

The protocol for the welfare assessment of captive dorcas gazelles developed during this thesis (Chapter 3) included several indicators whose goals were to detect deficiencies of the enclosures or the management procedures that could be challenging the individuals' well-being.

With the application of the protocol, it was possible to detect areas for improvement in all groups assessed. This welfare protocol could be a valuable tool for all centres that held dorcas gazelles in captive conditions, as it might help to detect and overcome welfare problems.

2. Zoo animal welfare indicators

We chose animal-based welfare indicators in order to study welfare issues related with social stress and visitor effect.

2.1. Using physiological indicators

The determination of glucocorticoids hormones concentration (such as cortisol or its metabolites) is a tool to measure the adrenal activity, which is related with the stress response (Mormède et al 2007). The concentration of cortisol or its metabolites can be determined in various biological matrixes such as plasma, milk, urine, saliva, faeces and hair.

In this thesis, we worked exclusively with two different matrixes: hair in dorcas gazelles (Chapter 1), and faeces in fallow deer and Spanish ibex (Chapter 2). Some considerations must be taken into account when working with these physiological indicators.

2.1.1. Cortisol concentration in hair

We decided to determine cortisol concentrations in hair because of its capacity to represent an integrative value of long-term adrenal activity. The 'multicompartment model' described by Henderson (1993) is the most accepted theory of substance accumulation in hair. According to this model, cortisol is incorporated into hair mainly via passive diffusion from the bloodstream. However, cortisol can also be found in hair due to sweat, sebum and other external sources. The glucocorticoid concentration values of this matrix are not influenced by momentary stress of the sampling, and hair can be stored in ambient temperature (Gow et al 2010).

Hair collection is easy and painless and we took opportunistic samples of hair with a razor when the animals were captured for routine health checks or for husbandry reasons. However, if the animal is not trained for sample collection and physical manipulation, its capture and restrain during sampling can be stressful and unsafe both for the animal and for the people involved in the sampling procedure.

Animals can be trained with the purpose of obtaining hair samples in a stress-free and even voluntary procedure. Moreover, training based on positive reinforcement may be considered as a form of enrichment and have positive effects on welfare.

2.1.2. Cortisol metabolites concentration in faeces

The method for the collection of faeces samples is easy, painless, and can be non-invasive. The enclosures where we carried out the study in fallow deer and Spanish ibex were free-range enclosures where visitors walk through them using a defined pathway. Because of this, sample collection after defecation was easy and non-invasive and it was possible to individually identify each sample once the behavioural observations were finished.

Faecal samples cannot be stored in ambient temperature and they need to be kept frozen at -20ºC until analysis. This was not a problem in our study because it was done during winter-early spring in an area with average low temperatures, and samples were refrigerated just after collection and frozen at -20ºC shortly after.

Faecal cortisol metabolites come from the bloodstream before being metabolised in the liver and excreted by the bile duct (Palme et al 1996). Therefore, cortisol metabolites are not present in faeces immediately after the stressful event, but they are a reflection of the total amount of cortisol excreted between the past few hours to more than 24 hours, depending on the digestive transit time of the species (Palme et al 1999; Möstl and Palme 2002). Fallow deer and Spanish ibex are ruminants, so the results of cortisol metabolites obtained represented the amount of cortisol excreted during the past 10-12 hours (Palme et al 1999).

A variability in faecal cortisol metabolites concentrations depending on factors such as the species, the circadian rhythms and the seasons, has been described (Möstl and Palme 2002). Due to this reported variability, we decided to treat each species independently and we sampled at the same time every day during a short period of the year.

2.1.3. Cortisol and cortisol metabolites concentration as welfare indicators

A stressor can unleash the stress response, which includes the activation of the hypothalamic-pituitary-adrenal axis (Lane 2006; Keeling and Jensen 2009). As a result, an increase in the secretion of glucocorticoid hormones, also known as stress hormones, with the aim to obtain energy from the cells, will take place. The concentration of these hormones (such as cortisol and corticosterone) or its metabolites is usually determined and used as a welfare indicator. However, interpretation of the results has to be carefully done as glucocorticoids excretion takes place not only in welfare detrimental situations, but also in other circumstances that may provide pleasure to the animals, such as sexual, play or hunt behaviours (Lay 2010).

Other variables need to be taken into account, because the concentration of cortisol or its metabolites can be affected by sex, age or the animal's rank within a social group (Lane 2006). Variations in cortisol concentrations due to diurnal and seasonal rhythms, humidity, temperature, and other environmental factors have also been described (Mormède et al 2007), as well as stage of breeding and body condition (Cockrem 2013).

Animal welfare includes the behaviour, emotional state and physical health of an individual (Fraser et al 1997). Due to this multidimensional concept of animal welfare, there is not one single indicator that can assess welfare by itself, but a combination of different indicators is needed. Hence, the determination of the concentration of cortisol or its metabolites as a welfare indicator is not an exception, and it should always be accompanied by the assessment of other indicators. In our case, we used behavioural indicators such as social (Chapter 1) and vigilance (Chapter 2) behaviours.

Our results in the study related with visitor effect (Chapter 2) showed an increase in the behavioural variables (vigilant and displacement caused by the visitor behaviours) when the number of visitors was high. However, the faecal cortisol metabolites concentration did not show significant differences depending on the number of visitors, in both species assessed. Conversely, in dorcas gazelles (Chapter 1) we found that the more intra-group aggressive behaviours they displayed, the higher hair cortisol concentration they had. So, it was possible to relate the higher hair cortisol concentration values with higher display of intra-group aggressive behaviours, which are considered detrimental for welfare.

The determination of cortisol concentration in zoos can be a useful tool to assess stress in captive animals, as well as the observation of certain behaviours, if always they are done accompanied by other welfare indicators that will help to properly assess welfare.

2.2. Combination of physiological and behavioural indicators

The observation of the behaviour of the animals is a useful way to study their welfare. As behavioural indicators, the intra-group aggressive and affiliative behaviours in dorcas gazelles were observed (Chapter 1), and the vigilant behaviour and the displacement of the animals due to the visitors were assessed in Spanish ibex and fallow deer (Chapter 2). However, it is important to use other indicators to fully understand and make conclusions from the observations. In our case, we could have concluded that visitors had a negative effect on fallow deer and Spanish ibex only by studying their vigilance behaviours. They expressed vigilant behaviour and displacement caused by the visitor in higher proportion the days when more visitors came at the zoo.

The increase in vigilance behaviours could be due to a natural instinct to check for movements in the surrounding area, but it could be possible that these behaviours do not provoke a stress response unless a real threat is detected. The results of the analysis of cortisol metabolites concentration in faeces showed that there were not statistical differences between the days with low and high visitor presence. If we had only studied the behaviour of the animals without taking the cortisol into account, we might have decided on other conclusions.

In the case of the study of social behaviour and cortisol concentration in hair in dorcas gazelles we found that both aggressive behaviours and concentration of cortisol in hair increased. So both indicators helped us to reach the conclusion that in some of the groups studied there was social stress that could be leading to aggressive behaviours that, probably at the same time, resulted in social stress.

Hair cortisol concentration and proportion of aggressive behaviours were sensitive to detect differences between groups of dorcas gazelles. We concluded that these two animal-based welfare indicators were sensitive to detect differences in the social structure of dorcas gazelles.

2.3. Potential uses of glucocorticoid determination and behaviour to assess zoo animal welfare

The combination of behavioural observations and the determination of cortisol or cortisol metabolites concentrations have been used to evaluate the welfare in several wild species housed in captive conditions. Different welfare aspects have been approached as well as attention having been focused on a variety of behaviours and matrixes to assess cortisol or its metabolites.

For instance, abnormal behaviours such as stereotypies have been related with an increase in cortisol metabolites concentration in faeces in giant pandas (*Ailuropoda melanoleuca*, Liu et al 2006). Moreover, self-directed hair plucking has been associated with an increase in cortisol concentration in urine in female bonobos (*Pan paniscus*, Brand et al 2016).

The effect that transportation has in animal welfare has been studied in Asian elephants (*Elephas maximus*, Laws et al 2007) and in tigers of different subspecies (*Panthera tigris*, Dembiec 2004). In both studies behavioural and physiological welfare indicators were used, and they concluded that transportation had an effect on animal welfare. In the study with tigers, they also demonstrated that previous experience to a stressful event such as being transported might help to reach some level of habituation that may reduce the effects of transport stress.

Research has been done to study social stress in Asian elephants (*Elephas maximus*, Dathe et al 1992) and in western lowland gorillas (*Gorilla gorilla gorilla*, Peel et al 2005) using behavioural indicators and cortisol in saliva and cortisol metabolites in faeces, respectively.

Visitor effect has been studied in repeated occasions using behavioural and physiological indicators and different outcomes have been found. In a study with captive Mexican wolves (*Canis lupus baileyi*, Pifarré et al 2012), the concentration of cortisol metabolites in faeces as well as some behaviours (related with posture, eating and locomotion) were assessed during days with different zoo visitor attendance. Results showed that higher visitor presence was related with changes in behaviour of the wolves and with higher levels of faecal metabolite cortisol.

Behaviours such as visitor-directed vigilance increased as visitor number increased in Kangaroo Island kangaroos (*Macropus fuliginosus fuliginosus*) and red kangaroos (*Macropus rufus*) held in free-range exhibits (Sherwen et al 2015). However, no effect of visitor numbers was observed on avoidance behaviour or in faecal glucocorticoid metabolite concentration. Researchers have concluded that there was no evidence of adverse visitor effects on animal welfare in their study.

Cortisol concentration in faeces has also been used to demonstrate the use of behavioural diversity as a behavioural indicator of animal welfare in cheetahs (*Acinonyx jubatu,* Miller et al 2016).

3. Integration of several indicators to create a welfare protocol

The inclusion of several welfare indicators is essential when putting together a welfare protocol, which is necessary in order to have a comprehensive assessment of welfare and to have a tool that helps to evaluate animal well-being.

The development and application of a protocol to assess welfare in captive dorcas gazelles (Chapter 3) developed during this thesis is the first documented work towards creating a standardised tool to evaluate the welfare of these animals in captive conditions.

Several indicators were considered useful to detect areas for improvement in all groups of gazelles visited. The welfare indicators included aspects related with good feeding of the animals, good housing, good health, and appropriate behaviour. A total of 10 animal-based indicators and 13 resource or management-based indicators were identified. Most of the indicators that we used were extracted from the welfare protocols of the Welfare Quality® (2009) project, which previously had tested their indicators for validity, reliability and feasibility. However, the indicators that we selected for the assessment of welfare in dorcas gazelles still need to be tested for validity, reliability and feasibility in a posterior stage in order to be specifically applied to this captive wild species.

3.1. Validity, reliability and feasibility of the indicators

In order to create a welfare protocol, it is important to consider the validity, the reliability and the feasibility of the indicators that will be used to measure different aspects related with the animal well-being (De Rosa et al 2003).

One concern when assessing animal welfare is the degree to which we are actually measuring what we are supposed to be measuring (Knierim and Winckler 2009). A measure will not be valid when it can explain many different and non-correlated properties (Garner 2005). Regarding resource and management-based measures, it is frequently argued that their validity is potentially low because of their indirect essence and complex interaction with other environmental conditions, as well as the individual itself, leading to unforeseeable outcomes. Animal-based measures reflect directly how the individual is coping, but they can present reliability problems (Knierim and Winckler 2009).

Reliability or repeatability of the indicators is essential in a welfare assessment protocol (De Rosa et al 2003; Knierim and Winckler 2009). The inter-observer reliability evaluates if different assessors with a certain and equal degree of training reach very similar or the same results when using an indicator. The intra-observer reliability assesses if the same observer assigns similar or the same results to the same object. Finally, the test-retest reliability assesses if results are mostly the same in repeated test in the same individuals (Meagher 2009). When assessing the reliability of an animal-based measure, a large agreement between different observers and within the same observer should be achieved.

An indicator needs to be feasible, meaning that it has to be easy to carry out and not too time consuming in order to be included in a welfare protocol. In our protocol for the assessment of welfare in dorcas gazelles we included several indicators, but none were physiological indicators. The assessment of the hypothalamic-pituitary-adrenal axis activity can be useful to study chronic stress and welfare, but the collection of samples for the posterior glucocorticoid levels determination may be too invasive or require too much time. The economic cost of the analysis needs also to be taken into account when developing a protocol that is intended to be a useful tool to any centre that keeps wild animals in captivity.

3.2. Protocols developed to assess welfare in wild animals held in captive conditions

We have knowledge of only two protocols specifically developed for the assessment of welfare of wild animals held in captive conditions: the WelFur project (Mononen et al 2012) and the C-Well® (Clegg et al 2015).

The WelFur project developed on-farm welfare assessment protocols with feasible and accurate indicators for three wild animals bred in captivity with productive goals: blue fox (*Vulpes lagopus*), silver fox (*Vulpes vulpes*), and mink (*Neovison vison*). The development of a welfare assessment protocol for captive bottlenose dolphins (*Tursiops truncatus*), named C-Well® or the Cetacean Welfare Assessment, found validated, reliable and feasible indicators for the welfare assessment in this species.

Both welfare assessment protocols were based on those developed for domestic farm animals by the Welfare Quality® project. In the WelFur project they identified 15 fox and 9 mink animal-based welfare indicators, and 11 fox and 13 mink resource or management-based indicators. On the other hand, the C-Well® suggested 21 animal-based and 15 resource or management-based indicators.

The WelFur project considers that a complete welfare assessment of a farm would require three visits during different times: 1) between pelting time and mating, 2) between the offspring leaving the nest and until they are weaned, 3) adult breeding and juvenile animals. The average time of each visit is not specified and they accept that the narrow time windows for the assessment visits make the implementation of the protocol for these wild species in farmed conditions challenging in practice. On the other hand, the C-Well® assessment affirms that it can be fully applied in ten dolphins in two days.

The ideal aim of a protocol to assess welfare in wild animals held in captive conditions should be to have it regularly used as a management tool in the centres where these animals are kept. Therefore, a successful protocol should be practical, economic and easy to apply. With our protocol for the welfare assessment of captive dorcas gazelles, it was possible to evaluate all the indicators chosen (10 animal-based indicators and 13 resource or management-based indicators), in all five groups of gazelles (the largest one having 17 individuals), and in less than six hours per group. We assessed all animals at an individual level.

4. Welfare assessment of individual animals: individual differences

The knowledge obtained from the studies developed in Chapters 1, 2 and 3 of the present thesis and the information currently available in the literature make us feel the need to question the possibility that it is important to study and guarantee animal welfare at the individual level.

4.1. Glucocorticoid concentrations

Glucocorticoid concentrations vary not only between species, but also between individuals (Palme et al 1999; Cockrem 2013). A stressor might evoke little or no response in some animals, but a relatively large response in others. These variations can be the result of genetic differences between animals, as well as experiences during pre-natal, post-natal and adult life.

Moreover, glucocorticoid responses have been associated with individual variation in personality, having relatively low or high stress responses associated with behavioural differences between individuals facing similar situations (Koolhaas et al 1999). Bolder individuals tend to have lower glucocorticoid responses than shyer animals. Individual differences in responsiveness are related to differences in the temperament of the individuals and to environmental stimuli. Glucocorticoid responses to stressors are critical components of individuals' responses to environmental stimuli (Cockrem 2013).

A common issue when dealing with zoo animal welfare assessment is the frequent dependency on opportunistic sampling, which can hinder the implementation of certain experimental designs. In our case, the ideal study would have included several measures of hair cortisol concentration of the same individual of dorcas gazelles (Chapter 1) in order to consider individual differences at this level. However, due to the opportunistic hair sampling when the animals were captured for husbandry reasons or for routine health checks, it was not possible to collect repeated samples that could have been helpful to study individual differences in hair cortisol concentration.

In the study of the visitor effect in fallow deer and Spanish ibex (Chapter 2), we took several faecal samples of the individuals that were later analysed for the detection of cortisol metabolites. The results revealed a high individual variability shown by the high standard deviations in the faecal cortisol metabolite concentrations, but there was not an individual effect detected.

4.2. Behavioural differences due to temperament or personality

Behavioural differences between individuals of the same species are not only attributed to the animal's age or sex. Temperament or animal personality is defined as an individual's consistent reaction to different environmental variables that differ from the behaviour of the other individuals of the same species, and that are not related to the age or sex of the animal (Manteca 2015).

The understanding of an individual's personality enables us to predict how the animal will perceive and react to factors in the environment, which can be helpful in order to assess its welfare under a variety of conditions (Hosey et al 2009).

It is possible to objectively and repeatedly measure how individuals differ in their response to the environment using two different methodologies (Manteca 2015). The first one measures the frequency, duration or intensity of different behaviours that are supposed to reflect the individual's temperament. The second methodology consists of the keepers or humans close to the individual to score the animal for personality traits previously defined (Whitham and Wielebnowski 2009).

A study assessed personality in snow leopards (*Uncia uncia*, Gartner and Powell 2012) using a survey completed by keepers and by observing the reactions of the individuals to novel objects. They compared both methods and determined that both keeper assessments and novel object tests identified individual differences in snow leopards.

The study of temperament in wild animals held in captivity has diverse practical applications. For instance, the assessment of animals' personality has been used to evaluate the compatibility of breeding pairs, their reproduction success, and in order to create stable social groups.

A study assessed different personality traits in captive black rhinoceros (*Diceros bicornis*, Carlstead et al 1999) and concluded that the most compatible rhinoceros pairs (and the ones with greater breeding success) were those with submissive males and assertive females. They also concluded that these animals bred best in larger enclosures with few concrete walls.

Individual behaviour variation was assessed in 44 adult captive-born cheetahs (*Acinonyx jubatus*, Wielebnowski 1999) using observer and keeper ratings. Non-breeder individuals of both sexes scored higher on the personality trait related with fearfulness than their breeding counterparts.

In a study with giant pandas (*Ailuropoda melanoleuca*, Powell et al 2008) it was observed that timid or shy females had less developed socio-sexual performances than bolder, more confident females. Bolder females were more likely to show interest in males and were less likely to be aggressive to them. Females that were aggressive to males took longer to approach a novel stimulus and showed less interest in it.

Temperament assessment may also predict the survival success of animals reintroduced into the wild, depending on their personality traits such as their boldness or timidity (Réale and Festa-Bianchet 2003; Bremner-Harrison et al 2004).

In conclusion, evidence suggests that personality traits in wild animals in captive conditions need to be taken into account when considering their welfare, breeding in captivity, and reintroduction into the wild.

4.3. Individual welfare assessment

The importance of individual differences in wild animal management, conservation, and welfare is gradually being acknowledged. Therefore, more emphasis is focused on the significance of assessing welfare at an individual level, because each animal has its subjective impression about its own welfare (Tetley and O'hara 2012).

In zoos, individual welfare should be considered when environmental enrichment programs are applied, because the response of each animal to the enrichment will depend on its personality (Manteca 2015). In a non-fearful animal, a new stimulus can stimulate exploratory behaviour and have a positive effect on its welfare. However, the same stimulus in a very fearful individual can have a negative effect on its well-being.

Personality evaluations can also be used to assess the reproductive success of the animals and to improve captive breeding recommendations by identifying compatible breeding pairs. Moreover, social stress resulting from housing incompatible animals together could be avoided, as well as potential aggressive interactions reduced, if the temperaments of the group members are known (Tetley and O'hara 2012).

There is some evidence that suggests that the susceptibility to certain diseases are also determined by individual differences such as temperament (Manteca 2015). Actually, the same stressor or a similar disease challenge may lead to different responses in each animal due to individual differences, and so the physical health and the emotional state of an individual cannot be completely understood or assessed without considering that individual as an independent being (Mills 2010).

To summarize, the assessment of welfare at an individual level is important when taking care of wild animals in captivity. Zoos need to develop tools and protocols in order to properly assess the welfare of all the animals under their care, as it is their responsibility to ensure an optimal well-being of each individual in captive conditions.

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CONCLUSIONS

After the research conducted in our experimental conditions, it was possible to reach the following conclusions:

1. Group composition has an effect in the balance between affiliative and aggressive social behaviours in dorcas gazelles.
2. Hair cortisol levels reflect differences in the social structure of dorcas gazelles.
3. Both the frequency of negative social behaviours and the hair cortisol concentration are sensitive welfare indicators to detect differences in animal well-being between groups of dorcas gazelles.
4. Conflicting results between indicators related with the expression of vigilance behaviours and the faecal cortisol metabolites concentrations in fallow deer and Spanish ibex suggest that a multidimensional approach is necessary in order to properly assess welfare.
5. The visitor presence increased the expression of vigilance behaviours, but did not have a negative effect on the faecal cortisol metabolites concentration in Spanish ibex and fallow deer.
6. A protocol that included 10 animal-based indicators and 13 resource or management-based indicators was found to be comprehensive enough to assess all relevant welfare aspects in captive dorcas gazelles.
7. Our results suggest that the farm animal welfare assessment protocols could be a useful framework to develop tools to assess the welfare of captive wild animals.
8. The application of the protocol allowed the detection of areas for improvement in all groups of dorcas gazelles assessed.
9. The protocol presented in this thesis can be a useful tool for the centres that keep dorcas gazelles and want to routinely check the welfare of the animals under their care.