

Evidence-based practice

Breeding success of dholes *Cuon alpinus* at Nehru Zoological Park, Hyderabad: lessons learned

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Abstract

Captive breeding programs play an important role in the conservation of endangered species however; many species struggle to breed in captivity. Therefore, understanding the factors that influence breeding success is crucial in order to develop species-specific strategies to reduce the chances of breeding failure. The dhole *Cuon alpinus* is facing increasing threats in the wild including habitat loss, prey depletion, disease transmission from domestic dogs and human-wildlife conflict thus a species recovery program has been initiated in several Indian zoos through captive breeding programs. Dholes are known to show lower reproductive success in captivity due to limited knowledge about their reproductive biology, nutritional requirement and poor husbandry practices. However, by providing appropriate environmental conditions and dietary supplements significantly enhance the breeding performance of dholes in captivity. The Nehru Zoological Park received two pairs of dholes from Pilikula Biological Park. Unfortunately, the first litter did not survive due to perceived housing conditions. The puppies died after one month of birth due to bone disorder likely attributed by the smooth flooring and lack of sunlight in night houses. In the subsequent breeding cycle, changes were made to the housing conditions for the dholes. A day kraal with natural substrate (sandy soil), abundant sunlight and an artificial cavernous den was used for whelping. Additionally, we improved the quantity of diet and supplements for females during the both pre-partum and post-partum periods to improve the reproductive health. These changes resulted in no pup mortality. Our study highlights the significant knowledge gap regarding species habitat requirements in captivity, particularly with respect to breeding. Our observations provide valuable insights: keeping pups on a smooth surface without sunlight access is not ideal as it affects on their growth and survival. Further future captive breeding programs for dholes and other similar species can use these observations as a reference.

Background

The Asiatic wild dog, also known as the dhole *Cuon alpinus*, is a medium-sized, pack-living predator that resembles wolves and domestic dogs (Wildlife Institute of India 2018). This species has a wide distribution and can be found in South and Central Asia, as well as in small numbers in the Russian Federation (Kamler et al. 2015). Dholes live in a wide array of habitats, however prefer medium to dense forest areas (Karanth and Sunquist 2000). Currently there are thought to be less than 2,500 mature individuals in the wild due to several intrinsic and extrinsic factors such as habitat loss and fragmentation, prey depletion, disease transmission from domestic dogs, and inter-specific competition (Kamler et al. 2015). Thus, the IUCN has listed the dhole as 'Endangered' (Kamler et al. 2015).

Owing to various disturbances, the remnant dhole population is restricted to few habitat patches in India (Modi et al. 2021). This dispersed population suffers from poor genetic diversity and inbreeding; hence concerted efforts from multiple departments are required to conserve this species from extinction (Modi et al. 2021). Zoos serve an important role in maintaining species genetic diversity through scientifically managed conservation breeding programs (Paulraj et al. 1992; Pandey and Murthy 2008) and also creating stock populations for further captive breeding and reintroduction programs.

Many studies on dhole have concentrated on understanding its distribution and occupancy (Jenks et al. 2015; Ngoprasert and Gale 2019), behaviour (Johnsingh 1982; Karanth and Sunquist 2000; Ghaskadbi et al. 2016), and population factors (Selvan et al. 2014). Due to its elusive nature, limited

information is available on its reproductive biology and denning behaviour (Johnsingh 1982; Fox 1984). Dens and associated habitat features are important factors in the distribution and abundance of Canids like dholes. The selection of suitable den sites increases the chance of dholes breeding success as well as rearing and survival of pups.

The dholes in South-western India are known to use four types of dens: a) simple earth dens with one entrance, b) complex earth dens with multiple entrances, c) simple cavernous dens excavated under or between rocks, and d) a complex cavernous dens; which consist of several dens in the same area with many interconnected entrances (Johnsingh 1982; Fox 1984). However, the comparative study of these den sites and their impact on the breeding performance of dholes and other canines is not well understood (Jackson et al. 2014; Nurvianto et al. 2015; Comley et al. 2023). However, some research has focused on den site selection of African wild dog *Lycaon pictus*, indicating that species tend to prefer den sites close to food and water while avoiding areas with high human and predator disturbance (Jackson et al. 2014; Comley et al. 2023). Thus, knowledge of den ecology is critical for understanding the species requirements and resource use, which contribute greatly to the conservation of dholes in the wild and captivity.

Dholes are seasonal breeders. Breeding occurs between October-April (India) and January-May (Java). The gestation period lasts 60-63 days. Litter size is 4-12 pups (Hunter and Barrett 2011). The structure and flooring of animal-holding facilities significantly affect the health and breeding activity of animals in captivity (Haspesslagh et al. 2013; Kilbridge et al. 2015). Inadequate flooring can lead to foot problems, joint swelling, reduced traction, and injuries due to transitional movements (Elmore et al. 2015; Kilbridge et al. 2009). Currently, various flooring types such as concrete, slatted surfaces, rubber, wire mesh, PVC carpet, and bio-floor are used in captive environments, each with its own advantages and disadvantages (Elmore et al. 2015; Kilbridge et al. 2015; Leinwand et al. 2021). Therefore, a careful assessment of flooring is essential for animal welfare. Despite this, research on the effects of different flooring types on the welfare of wild animals in captivity remains limited (Meller et al. 2007; Leinwand et al. 2021). Few studies have highlighted that housing animals on natural surfaces, such as sand or soil, reduces the risk of foot problems and musculoskeletal disorders (Meller et al. 2007 and 2016; Kilbridge et al. 2009). Additionally, husbandry practices are continually evolving, and the introduction of advanced self-sterilizing bio-floors has been suggested to improve the health, behaviour, and activity budgets of animals in outdoor facilities (Beisner and Isbell 2008; Leinwand et al. 2021).

Metabolic bone disease (MBD) is a common issue in captive animals is often resulting from inadequate dietary supplements such as calcium, vitamin A and D deficiencies (Jayathangaraj 2007; Dittmer and Thompson 2011; Kumar et al. 2018). MDS refers to a range of issues typically marked by poor bone mineralization, skeletal deformities, tetany, and weakness, all of which can result in fractures. MBD encompasses various bone disorders, including rickets, osteomalacia, hyperparathyroidism, osteoporosis, fibrous osteodystrophy, and nutritional bone disorders (Jayathangaraj 2007). In addition to dietary supplements, metabolic bone disease (MBD) in captive animals is likely linked to a lack of adequate daylight access and inappropriate housing conditions; however, these factors are often overlooked in captivity (Ross et al. 2013; Kumar et al. 2018; Woods et al. 2023). Thus, this paper attempts to document and share knowledge of effective management interventions adopted to decrease puppy mortality and improved dhole breeding success at Nehru Zoological Park and further these best practices can be implemented in other zoos for similar species that failed to reproduce.

Action

Study area

This study was conducted in Nehru Zoological Park (NZP) located in Hyderabad, the capital city of Telangana state, India (17°21'04.9"N, 78°26'57.6"E). It is one of India's largest zoos, with an area of 380 acres. The zoo houses nearly 200 species of animals, including mammals, birds, reptiles, and amphibians. The zoo is well-known for its effective captive breeding conservation and reintroduction programs of Indian mouse deer *Moschiola indica* (Sandeep et al. 2019; Kumar and Umopathy 2023). In 2021, Nehru Zoological Park received two pairs of dholes (2.2.0) from Pilikula Biological Park in Mangalore as part of an animal exchange programme. The individuals were housed in the zoo's quarantine centre before being released into the exhibit area. Later, it was decided to integrate the four individuals into two breeding pairs.

Housing

The dhole enclosure in the zoo is an open, irregular shaped dry moat (Figure 1; Table 1 for information of major enclosure features of dholes in the zoo). The enclosure has four holding rooms (Figure 2). One of the night cells was equipped with an artificial den with a wooden framework (1X1 m, Figure 3). Each breeding pair (1:1) occupied one night cell each. Animals were exhibited in pairs (1:1) in the exhibit area on alternate days to avoid the disturbance and prolonged periods of stay in the night cells.

Table 1. The main enclosure features of the dhole exhibit at the Nehru Zoological Park, Hyderabad.

| Enclosure features | More information | Reference |
|--------------------|---|------------------|
| Display enclosure | It is open, irregular shaped dry moat with numerous trees, bushy vegetation and drinking water facilities resembling natural environment of the species. | Figure 1 and 2 |
| Night houses | The enclosure has four off display night cells or retiring cells (7.5 ft. length X 8.5 ft. width X 5.9 ft. Height) with smooth floor and a metal sliding door. The night cells lack provision of adequate sunlight. | Figure 3 |
| Day Kraal | Two day kraals (15 ft. length X 16 ft. width X 7 ft. height) were built in the immediately adjacent the display enclosure to house surplus and sick animals. The day kraal's wall is a sturdy concrete structure with a height of 1.5 metres; on top of it a chain link cage was constructed. The day kraal provides ample amount of sunlight to the animals and is connected to the night cells through sliding doors allowing animal to move freely. The surface area of the day kraal is filled with medium to fine soil about 30 cm height. | Figure 4 a and c |



Figure 1. The naturalistic display enclosure for Dholes at Nehru Zoological Park, Hyderabad.

Diet and health management

The diet of dholes in captivity consists of beef and chicken. Each individual is fed approximately 1.5 kg of dressed beef and 0.5 kg of fresh chicken with bones once a day. Additionally, calcium powder and OstoVet (which contains calcium, phosphorus, vitamin D3, vitamin B12, and carbohydrates) are mixed into their feed and provided daily. Numerous studies have documented the significance of dietary supplements like calcium, phosphorus, vitamin D for the growth and reproductive success of canines and other species (Jayathangaraj 2007; Ravi et al. 2015; Brown and

Thompson 2019; Smith et al. 2020; Jones et al. 2021; Martinez and King 2022). A study by Jones et al. (2021) found that African wild dogs had larger litter sizes and lower perinatal mortality rates when provided with calcium and phosphorus supplements. Therefore, it is essential to supply dietary supplements to captive animals to meet their physiological needs that can influencing both breeding outcomes and overall health (Marker et al. 2021; Martinez and King 2022). Besides that, routine vaccination and de-worming were conducted in the dholes to maintain their health (Table 2).

Table 2. Diet and health management of dholes at Nehru Zoological Park, Hyderabad.

| Category | Item | Quantity/individual/day | Details |
|-------------|------------------------|-------------------------|--|
| Diet | Beef | 1.5 kg | Increased to 2 kg for pregnant females |
| | Chicken | 0.5 kg | Increased to 1 kg for pregnant females |
| | Liver | 50 gram | Increased to 100 gram for pregnant females |
| Supplements | Calcium carbonate | 10 gram | Increased by 20% for pregnant females |
| | OstoVet | 5 ml | Increased by 20% for pregnant females |
| | Vimrel | - | 2-3 ml weekly twice for pregnant females |
| Vaccination | CANIGEN 8 DHPPiI (CDV) | - | Annually for Canine Distemper |
| | Anti-Rabies Vaccine | - | Annually |
| De-worming | Fenbendazole | - | Every three months |

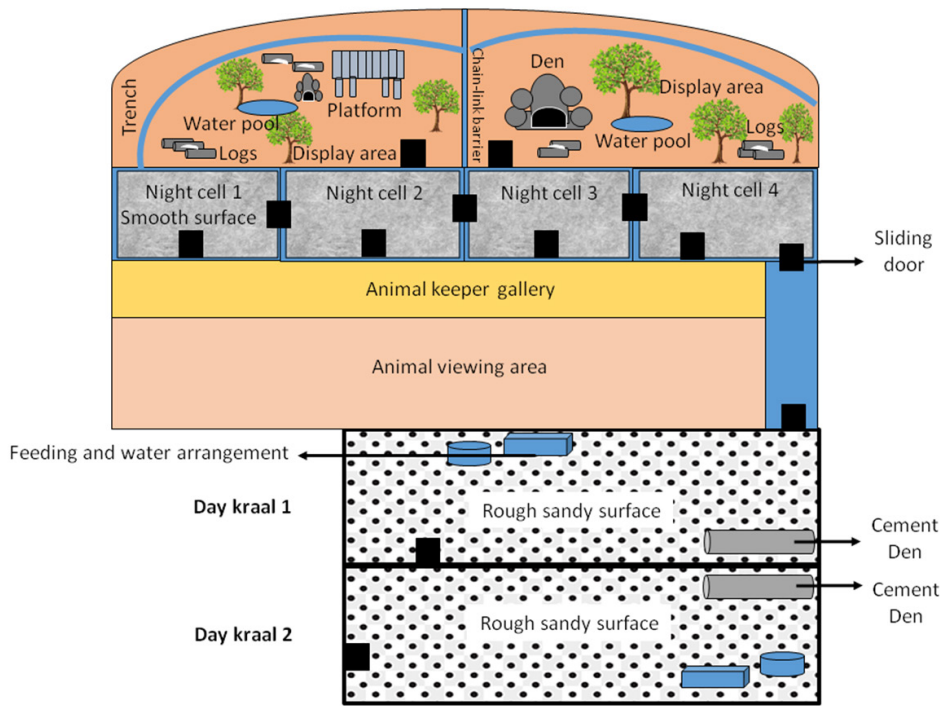


Figure 2. A schematic representation of the dhole enclosure at Nehru Zoological Park Hyderabad, featuring night cells and day kraals.

Consequences

First breeding attempt (2022)

In September 2022, one of the breeding pairs engaged in its first mounting behaviour in the display area. Mounting frequencies were found to be greater in the morning (3-4 times), with a typical canine copulation tie lasting 5-10 minutes. The pregnancy was detected one month after copulation by a clearly expanded abdomen and enlarged mammary glands. Consequently, the diet was increased to 1.5–2 kg of beef and 0.5–1 kg of chicken (Table 2). Additionally, the dietary supplement OstoVet was administered twice daily. When the female was about to give birth, access was provided to a night cell with a den for whelping and nursing the puppies (Figure 3). After 61 days, the female gave birth to five puppies. At the time of birth, the puppies appeared normal and remained in the night cell for 30 days. Later, we found that the puppies had difficulties in walking on the smooth floor in the night cells without sunlight and eventually died with a bone disorder

(Figure 3). This tragic experience highlighted the importance of understanding species-specific requirements in captive environments. Consequently, the design of enclosures must consider whether animals need concrete hard or smooth flooring. Smooth concrete flooring can restrict movement and provide inadequate grip, leading to bone deformities such as rickets. For example, African elephants *Loxodonta africana* and Asian elephants *Elephas maximus* housed on hard concrete surfaces have been observed to develop arthritis and foot-related issues compared to those kept on natural substrates (Miller et al. 2016). Similarly, Wyss et al. (2013) found that flamingos with access to ponds containing natural substrate were less likely to develop foot lesions compared to those housed in ponds with concrete flooring. Additionally, von Houwald and Flach (1998) noted that greater one-horned rhinoceroses *Rhinoceros unicornis* exhibited a higher prevalence of foot disease when housed on concrete flooring and lacking access to ponds and wallowing areas. Thus, our study emphasizes the necessity for rough surfaces in night

Table 3. Mating instances, gestation period and litter size of dholes in Nehru Zoological Park, Hyderabad.

| Year | First mating | Last mating | Length of estrus (days) | Whelp date | Gestation days | Litter size |
|---------|--------------|-------------|-------------------------|-------------|----------------|-------------|
| 2022 | 02 Sep 2022 | 15 Sep 2022 | 14 | 15 Nov 2022 | 61 | 5 |
| 2023 | 05 Sep 2023 | 20 Sep 2023 | 16 | 22 Nov 2023 | 63 | 5 |
| 2024 | 07 Nov 2023 | 01 Dec 2023 | 25 | 03 Feb 2024 | 64 | 2 |
| Mean±SD | | | 18.33±5.85 | | 62.66±1.52 | 4.00±1.73 |



Figure 3. The images showcase the off display facilities of the night cells/retiring cells, which have smooth flooring and wooden framework specifically designed for dhole parturition and rearing of puppies at the time of first litter.

houses to ensure adequate grip and alleviate discomfort while walking. Furthermore, a small section of the night house could be constructed with natural substrate, or advanced flooring systems such as bio-floor could be implemented.

Second breeding attempt (2023)

The second breeding activity was observed in the same breeding pair during September 2023; this continued breeding of dholes showed that they were not likely under extreme stress and were living in suitable environmental conditions. This time, the gestation period lasted 63 days (Table 3). When the female was identified as pregnant, we gave her dietary supplements such as vitamins and minerals (Ostovet, Vimrel) and increased the amount of feed quantity (1.5–2 kg beef and 0.5–1 kg of chicken). Since the first litter failed to survive we opted not to house the animal in the night cells. Instead, we drove the animal into the day kraal one week before to the delivery date, to acclimatise to the new environmental conditions (Figure 4a-c). Prior to that, the day kraal was properly cleaned and disinfected. The day kraal provides a natural sandy substrate, ample open space, and outdoor access to natural daylight features that were lacking in the first breeding cycle (Figure 4 and 5). This time instead of a wooden box, we installed a cement pipe (2 m long and 0.6 m diameter) by giving access from one end for whelping and nursing puppies that resembles more naturalistic den structure in the day kraal. Inside the cement pipe (hereafter den), dry hay grass was laid to provide smooth bedding for the puppies and mother (Figure 4b and 5). Dholes are highly timid; any disturbance can have a significant impact on their reproductive success. Therefore, to reduce unnecessary stress, the view of animal keepers was obscured by covering the chain link mesh with Jute Gunny Cloth and Green Agro Shade Net (Figure 4a). Further, the day kraal was also provisioned with drinking water ad libitum.

On 22 November 2023, the female gave birth to five puppies in the den. In the first week, the female spent the majority of her time in the den feeding the puppies. At the end of the fourth week, puppies emerged from the den site and began exploring the enclosure by playing with their dam and other members of the pack. During this time, puppies were observed having regurgitated food provided by their mother. Thus, in the second month, the mother was offered with 2 kg of boneless domestic chicken, and

the puppies were occasionally spotted eating on their own. The feed quantity was gradually increased from 2 kg to 4 kg of chicken (with bone). The dietary supplements (OstoVet and Vimrel) were continuously given for better milk production. When the puppies reached roughly 90 days of age, on trail basis, they were released in a display cage on Monday, which was a holiday period for the zoo (visitors are not permitted) with no visitor disturbance. For the first few minutes, the puppies remained with their mother before gradually moving around the area. The enclosure wall was built in such a way that it resembles the natural rock boulders of dhole habitat, and puppies were regularly seen climbing and sleeping on these structures (Figure 2). To improve physical activity and cognitive skills, enrichment activities such as wooden logs, water puddles, and earthen pots were included in the enclosure.

On 3 February 2024, the second breeding pair also produced two puppies in the day kraal. No mortality was recorded during the subsequent reproductive cycle when they were housed in a day kraal for whelping. This indicates that understanding the specific need of animal for any functional characteristics is very essential in captivity.

Implications

The successful breeding of dholes at the Nehru Zoological Park was influenced by several factors, including the presence of cavernous dens, a natural sandy substrate and relative open space in the day kraal and access to natural daylight (Figure 5). No single underlying factor dominates breeding activity; therefore, it is necessary to consider multiple interrelated factors that affect the breeding and well-being of these animals. From our experience with the successful breeding and rearing of dholes in the zoo, we emphasize that simply providing a wooden box for whelping will not solve the problem of reproductive failure as it did not mimic the animal's natural den features. The availability of a covered area, specifically the concrete cement den with a single opening, is a potential factor contributing to the greater survival rate of puppies in the day kraal, though the precise causal mechanism remains unclear. It is well understood that dholes typically use cavernous dens between rocks which offer greater security from predators. Thus, the artificial den structure might have encouraged both breeding pairs to whelp successfully.



Figure 4. Day kraal A) outfitted with Jute Gunny Cloth and Green Shade Net to obscure keeper disturbance B) A cavernous den filled with dry hay grass C) day kraal with sufficient sunlight and soil substrate, where puppies are playing with their mother

Furthermore, cavernous dens provide better protection from predators and extreme weather conditions compared to earthen dens, which require more energy to excavate (Comley et al. 2023). This indicates that our efforts to align with their natural den site selection have led to improved reproductive success. Additionally, the captive conservation breeding program for dholes at Indira Gandhi Zoological Park in Vishakapatnam has shown increased breeding success with the use of cavernous dens (unpublished data). Therefore, this study recommends providing suitable hides or cavernous dens to fulfil the ecological requirements of dholes and enhance their breeding performance. Future enclosure designs for canids should consider dens as an essential component of enclosure features.

MDS typically results from nutritional deficiencies; however, smooth flooring can also contribute to musculoskeletal disorders during the early developmental stages of pups. This issue has received little attention in captive environments due to a lack of awareness. In our study, moving dholes to a day kraal with a natural soil substrate and open space increased the physical activity of the puppies, leading to no observed abnormalities. Similar findings were reported in pigs and elephants when they were provided outdoor access with natural substrates (KilBride et al. 2009, Miller et al. 2016). Therefore, we recommend that each animal enclosure includes a separate day kraal with natural elements suited to the species, as this is ideal for the breeding and early developmental stages of puppies.

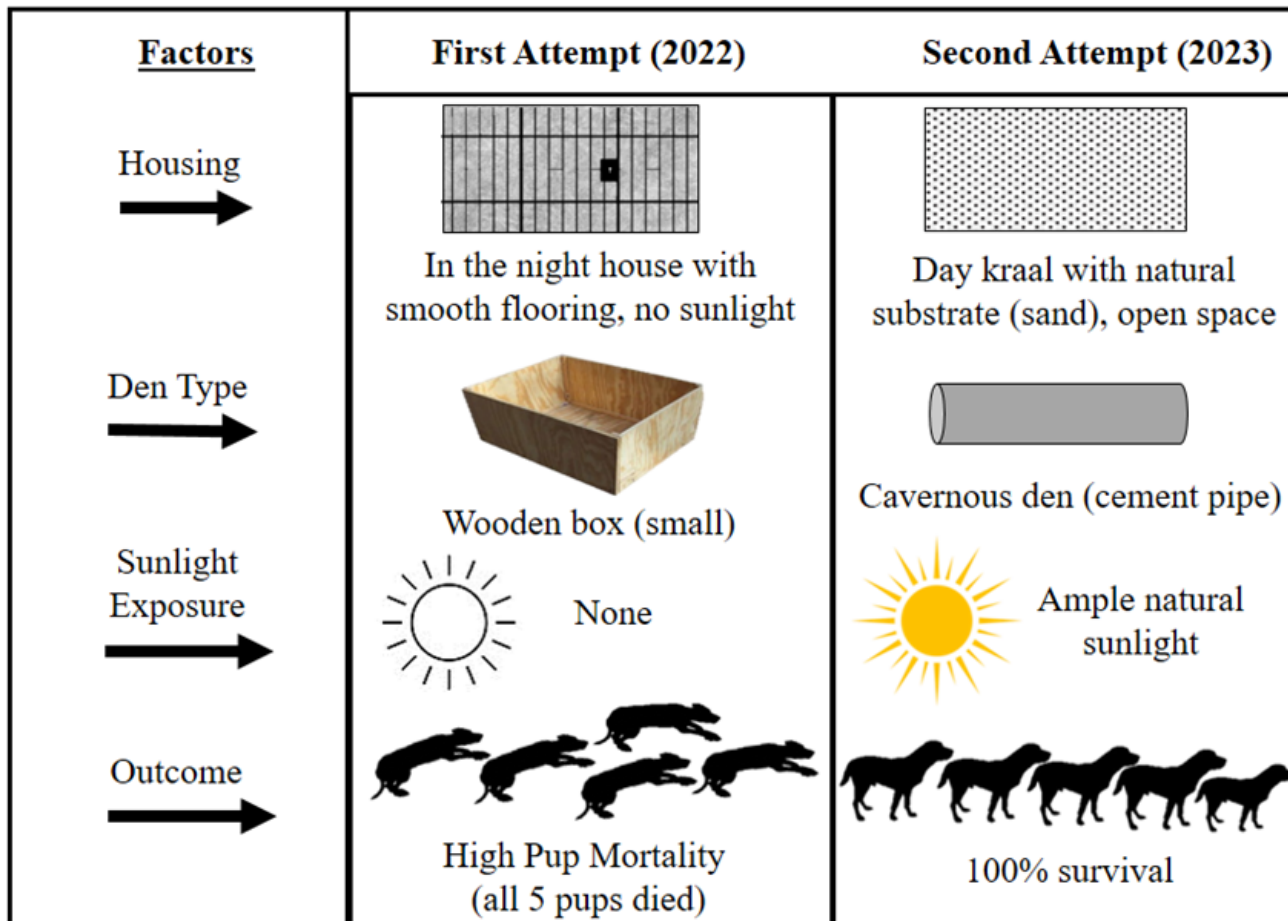


Figure 5. Graphical illustration of the factors that influence the breeding performance of Dholes.

Among the various features of enclosures, access to natural daylight is a key element for animal welfare. Numerous studies have shown that the vitamin D deficiency in animals may also increase due to reduced sunlight exposure, resulting in musculoskeletal disorders (Hurst et al. 2020; Woods et al. 2023). A critical aspect of enclosure complexity is the availability of natural daylight, which supports animal growth and development while regulating their circadian rhythms and behaviours (Kavanau and Ramos 1975; Edwards 2003; Farrell et al. 2015). Adequate UV light has been shown to influence the foraging and breeding success of birds and mammals (Ross et al. 2013; Mohapatra et al. 2019; Woods et al. 2023). Despite its importance, this factor has not received sufficient attention in captive settings (Ross et al. 2013; Woods et al. 2023).

In the present study, during the second breeding cycle, individuals were housed in a day kraal with free access to natural daylight throughout a three-month period. The animals were observed actively utilizing the day kraal during early mornings and late evenings. This ample sunlight and temperature regimes might have contributed to vitamin D synthesis, musculoskeletal development, and overall well-being of the species in the day kraal. Furthermore, empirical evidence clearly indicates that exposure to natural daylight improves breeding rates, growth, and foraging activity in animals. The present findings align with

empirical data suggesting that outdoor access could serve as a potential future refinement for captive animals, allowing them to exhibit natural behaviours and reduce stress. We recommend that dholes should have access to exterior spaces. This is essential because their activity levels are significantly influenced by daylight conditions, even for nocturnal species. The outdoor access for daylight exposure in captivity is an emerging field, and future research is essential to understand the extent to which sunlight plays an important role in animal productivity.

Conclusion

This study suggests that adjustments to key environmental factors can contribute to improvements in breeding success for dholes. The features implemented in this study, such as large open day kraals with natural sandy substrates, outdoor access, and cavernous dens, appear to be important for dhole breeding and are elements that may warrant greater consideration in captive settings. This study underscores the importance of carefully considering various environmental components to support successful dhole breeding. While the findings are specific to dholes, the principle of considering habitat complexity may be relevant to the breeding of other canids. Often, captive animal facilities are designed based on human perceptions, and as such may overlook the specific environmental needs and preferences

of the animals themselves. Therefore, recognising the complexity of a species' habitat, encompassing both abiotic factors like structures, size, and microclimate, and substrates, and biotic (plant diversity) is important for the overall welfare of captive animals, including their reproductive success. This study provides a valuable case history highlighting the potential benefits of environmental changes that may influence breeding outcomes and underscores the need for continued investigation into the nuanced environmental requirements of species in managed care.

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