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Vitamin A deficiency and its treatment in captive Sunda pangolins

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Abstract

The high incidence of disease in captive pangolins is a major obstacle in pangolin-conservation breeding programs. Therefore, elucidating pangolins' susceptibility to disease is the key to conservation progress. At the Pangolin Research Base for Artificial Rescue and Conservation Breeding of South China Normal University (PRB-SCNU), vitamin A deficiency was diagnosed in 14 captive Sunda pangolins. Typical eye signs included lacrimal eyes, keratopathy and a blank, milky orb. The afflicted pangolins were treated with vitamins A and D for 15–30 days; all individuals recovered. We report the detection and treatment of vitamin A deficiency in captive Sunda pangolins at the PRB-SCNU. Our results could provide guidance for the future prevention and treatment of vitamin A deficiency and associated diseases in pangolin species, both to reduce the incidence of these diseases in captive pangolins and to aid conservation efforts.

KEYWORDS

captive pangolins, conservation breeding, Pholidota, vitamin deficiency

1 | INTRODUCTION

The Sunda pangolin (*Manis javanica*) is one of the eight extant pangolin species; it is widely distributed in south-eastern Asia (Challender et al., 2019), but found only in Menghai and Menglian Counties of Yunnan Province in China (Wu et al., 2005). Due to overharvesting and habitat destruction, the wild Sunda pangolin population has declined sharply, leading to its classification as Critically Endangered in the International Union for Conservation of Nature Red List of Threatened Species (Challender et al., 2019). In China, confiscated smuggled Sunda pangolins are confined in artificial environments for conservation research for the protection of the species and to counter potential negative impacts on local ecology caused by random releases (Zhang et al., 2017; Zhang, Yu, Wang, Xu, & Wu, 2019).

Pangolins are difficult to maintain in captivity because they are prone to various diseases under the conditions of an artificial environment. The most common causes of mortality in captive Chinese pangolins and Sunda pangolins are pneumonia and gastrointestinal diseases (Chin et al., 2012; Yang et al., 2001; Zhang et al., 2017); appetite loss, skin disease, trauma, liver disease, heart disease, kidney disease, parasites, bleeding from the mouth and nose and limb-movement disorders are also health concerns in these two species (Chin et al., 2012; Hassan et al., 2013; Zhang et al., 2015; Mohapatra et al., 2016; Zhang, Zou, et al., 2019). Captive Indian pangolins also have a high reported incidence of pneumonia, gastroenteritis, hepatitis and kidney disease (Mohapatra & Panda, 2014). The prevalence of diseases is related to their captive diets, which are often nutritionally incomplete and incompatible with their digestive morphology and physiology (Zhang et al., 2017; Zhang, Xu, et al., 2019). Some studies have found that adding chitin and soil to their diets in moderation can improve the health of captive pangolins (Cabana & Tay, 2020). Pangolin feeding and treatment research at

the Pangolin Research Base for Artificial Rescue and Conservation Breeding of South China Normal University (PRB-SCNU) have also indicated that some diseases common in captive pangolins may be related to vitamin B deficiency (Zhang, Zou, et al., 2019).

In this paper, we report the signs and treatment of vitamin A deficiency in Sunda pangolins maintained at the PRB-SCNU. Our results could provide guidance for developing formulated feed and disease-prevention strategies for pangolin species and could play an important role in reducing the incidence of vitamin A deficiency in captive pangolins.

2 | MATERIALS AND METHODS

2.1 | Subjects

We focused on Sunda pangolins with a vitamin A deficiency at the PRB-SCNU.

2.2 | Housing and animal husbandry

The pangolins were housed separately in enclosures with an area of 15 m^2 and were fed once per day. The artificial food comprised of ants (20%), mealworm powder (50%), silkworm pupa powder (20%), yeast powder (4.5%), sand (5%) and multivitamin supplements (0.5%). Housing and husbandry details for these pangolins have been described previously (Zhang et al., 2017).

2.3 | Physical examination

During captivity, physical observations were performed weekly. Some pangolins were weighed weekly to monitor changes in body mass. Their appetite was monitored, and their eyes were checked to ensure they were black, bright and absent from abnormal secretions.

2.4 | Determination of vitamin A levels in feed

High-performance liquid chromatography (National Standards of the People's Republic of China GB/T 17817-2010) was used to measure the levels of vitamin A in the pangolin feed.

3 | RESULTS

3.1 | Vitamin A deficiency signs in captive Sunda pangolins at PRB-SCNU

A total of 14 captive Sunda pangolins were housed for over 3 months and diagnosed with vitamin A deficiency at the PRB-SCNU. Typical eye signs included lacrimal eyes; eyelid bonding; corneal lesions; and hazy, cloudy and lackluster eyeballs. In severe cases, the eyeballs were

FIGURE 1 Representative photograph of the eye of a Sunda pangolin with vitamin A deficiency

FIGURE 2 Representative photograph of the eye of a healthy Sunda pangolin

blank, milky and protruding, with weak light reflection (Figure 1), providing a sharp contrast to healthy pangolin eyes, which appear dark and reflective (Figure 2). More than half of the individuals affected by vitamin A deficiency recorded reduced appetite, slow or no growth, or weight loss. Figure 3 shows the changes in food intake and body weight of one individual (ID DY03) during and after vitamin A deficiency. The symptoms of the different individuals are shown in Table 1.

3.2 | Treatment

During the study period, pangolins with vitamin A deficiency were fed the equivalent of one soft capsule of vitamin A (3,000 IU) and vitamin D (300 IU) per day (Xiamen Sinopharm Xingsha Pharmaceutical Co., Ltd.). We extracted the vitamins from the capsules by pricking a tiny hole in each capsule using a syringe needle, and squeezing the liquid containing the vitamin out through the hole. If the individual pangolin was able to take the initiative to eat, then the extracted liquid was added to its food immediately before feeding time every

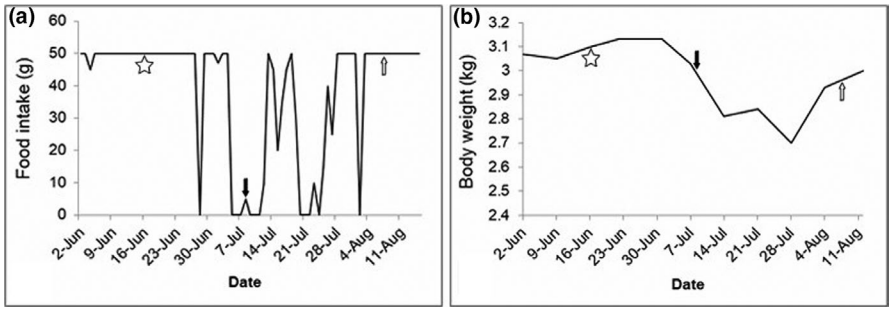

FIGURE 3 Food intake (a) and body weight (b) changes in a Sunda pangolin individual (ID DY03) during and after vitamin A deficiency. The star indicates when the eye abnormality was found, the black solid arrow indicates when the pangolin's diet was first supplemented with vitamins A and D, and the hollow arrow indicates when diet supplementation ended

TABLE 1 Symptoms of vitamin A deficiency in Sunda pangolin individuals

		Symptom						
No.	ID	Lacrimal eyes	Eyelid bonding	Lackluster eyeballs	Milky eyeballs	Protruding eyeballs	Reduced appetite	Weight loss
1	MJ12	√	√	√			√	
2	MJ58	√		√	√	√	√	√
3	FSX05			√	√	√	√	
4	FSX07	√		√		√		
5	FSX011	√	√	√		√	√	√
6	QFY01		√	√		√		
7	QFY04	√	√	√	√		√	
8	QFY13			√	√	√		
9	QFY19	√		√		√		
10	QFY25	√	√				√	√
11	QFY38	√	√	√	√	√	√	√
12	QFY42			√	√		√	
13	DY01	√		√		√		
14	DY03	√		√	√	√	√	√

Note: √ = the presence of this symptom.

day. If the individual had a poor appetite and little or no food intake, then we mixed the extracted liquid into 20 ml liquid food and used a syringe to force feed the diseased pangolin. In addition to daily diet supplementation with vitamins A and D, chloramphenicol eye drops (Guangzhou Baiyun Mountain Pharmaceutical Co., Ltd.) were administered to diseased pangolins in the morning and evening to eliminate ocular inflammation. Generally, individuals with mild signs recovered after 15 days of continuous treatment, whereas individuals with severe signs recovered completely within 30 days.

Pangolins were considered cured when the white, fog-like membrane disappeared from the surface of the eye and the cloudy, dull eyeball returned to a limpid, dark and reflective appearance. Individuals that had exhibited poor appetite or that refused to eat gradually regained their appetite and began to gain weight following treatment.

3.3 | Detection and improvement in vitamin A content in feed

The vitamin A content of the pangolin feed used by PRB-SCNU was measured. The results showed <500 IU/kg vitamin A in the feed, which is below the sensitivity level of the instrument and likely does not meet the pangolin's daily requirement. Thus, vitamin A acetate powder (0.01% of 325,000 IU/g, Zhejiang Gress Biotechnology Co., Ltd.) was incorporated into their diet, after which they did not show the signs of deficiency.

4 | DISCUSSION

Captive pangolins suffering from vitamin A deficiency at the PRB-SCNU exhibited lacrimal eyes, conjunctival lesions, a blurred white spot on the eye surface and decreased photosensitive ability. These signs are similar to those of vitamin A deficiency in humans (Rubino et al., 2015), cows (Chen, 2018), rabbits (Sun, 2017) and pigs (Liu, 2018). All affected pangolins recovered following treatment with dietary supplementation of vitamins A and D. Together with its derivatives, vitamin A regulates multiple processes including reproduction, embryogenesis, vision, growth, cell differentiation and proliferation, maintenance of epithelial cell integrity and immune function (Dadon & Reifen, 2017). Vitamin D mainly regulates calcium and phosphorus metabolism and is related to bone development in

animals (Wu, 1999). We consider that the effects of vitamin A deficiency on these processes caused the various signs observed in captive pangolins at the PRB-SCNU.

The diseased pangolins frequently exhibited other signs associated with vitamin A deficiency in rabbits (Sun, 2017), cattle (Chen, 2018) and pigs (Liu, 2018), including poor appetite or refusal to eat, growth stagnation, weight loss, increased nasal cavity watery secretions and respiratory abnormalities. Animals with long-term vitamin A deficiency can exhibit epithelial keratinization in respiratory, digestive, reproductive and urinary organs, leading to reduced resistance to bacteria and increasing susceptibility to pneumonia, diarrhoea, and kidney and urethral stones, as well as abortion, stillbirth, abnormal fetus and other signs in pregnant individuals (Chen, 2018; Goodwin & Jennings, 1958; Sun, 2017; Wu, 1999). The incidence of pneumonia and gastroenteritis is higher in captive pangolins maintained in artificial environments (Chin et al., 2012; Zhang et al., 2017). At the PRB-SCNU, increased vaginal pus discharge was observed in several female pangolins (F. Zhang unpubl. data, Figure 4), and seven stillbirths were recorded including two malformed fetuses (Zhang et al., 2013, F. Zhang unpubl. data). Necropsy conducted on two pangolins were found to have bladder calculi (F. Zhang unpubl. data). These signs were similar to those of vitamin A deficiency in other species. However, because no strict control experiments were conducted in this study, further research is required to verify an association between these signs and vitamin A deficiency.

Vitamin A intake in captive pangolins may not meet their requirements in captivity. Pangolins feed mainly on ants and termites; each adult preys on 250–400 g ants or termites per day (Liu & Xu, 1981; Shi & Wang, 1985). The vitamin A contents of the pangolin prey species *Polyrhachis dives* is 2.87–8.74 IU/g (Liu, 2004; Shi et al., 1995). Therefore, we hypothesized that an adult pangolin consumes at least 700–3,500 IU of vitamin A per day in the wild. However, we found that the vitamin A content of the artificial pangolin food used at the PRB-SCNU was <0.50 IU/g, and feeding was generally controlled to 30–60 g per day (Zhang et al., 2017). Thus, captive pangolins ingested <30 IU vitamin A per day at PRB-SCNU, which is significantly lower than their daily intake in the wild. This finding indicates that captive pangolins at the PRB-SCNU are typically fed insufficient vitamin A, inevitably leading to vitamin A deficiency. After their diets were enriched with 950–1,900 IU vitamin A, no signs of disease were exhibited suggesting that vitamin A deficiency was causing the symptoms. In addition, fat in feed is easily oxidized, leading to rancidity; fat rancidity severely degrades vitamin A in feed. Because the fat content of pangolin feed is high, long-term storage of pangolin feed must be avoided, and vitamins should be added before feeding to prevent oxidative rancidity of fats and vitamin loss in pangolin feed.

FIGURE 4 A pangolin with excessive pus flowing (arrow) from the vagina

The nutrient requirements of pangolins are largely unknown, leading to a variety of diseases in captivity. This is the first report of vitamin A as a successful treatment for eye disease in captive Sunda pangolins, which has not been reported among any of the eight extant pangolin species. Taking our results together with those of the existing reports on vitamin B deficiency and its treatment in captive Sunda pangolins, it is necessary to pay more attention to the vitamin requirements of pangolins, which may significantly reduce the prevalence of certain disease in captive pangolins.

5 | CONCLUSION

We recorded eye signs of vitamin A deficiency in captive pangolins, including lacrimal eyes, eyelid bonding, keratopathy, and cloudy and dull eyes. These signs were cured by dietary supplementation with vitamins A and D. We conclude that vitamin A deficiency is at least partly responsible for the high incidence of eye disease, loss of appetite, pneumonia and gastroenteritis observed in captive pangolins. We also determined that vitamin A intake at the PRB-SCNU is insufficient to meet their dietary needs, increasing the risk of developing associated diseases.

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AUTHOR CONTRIBUTION

Fuhua Zhang: Conceptualization; Data curation; Funding acquisition; Writing-original draft; Writing-review & editing. **Yue Min**: Data curation; Formal analysis; Investigation; Writing-original draft. **Yishuang Yu**: Data curation; Formal analysis; Writing-original draft. **Na Xu**: Data curation; Formal analysis; Investigation. **Wenhua Wang**: Data curation; Formal analysis; Writing-original draft. **Shibao Wu**: Conceptualization; Funding acquisition; Investigation; Supervision; Writing-review & editing.

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