Reconsidering the use of soy and alfalfa in southern white rhinoceros diets

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Abstract

The captive southern white rhinoceros (SWR) population is not currently self-sustaining due to the reproductive failure of captive-born females. Our research into this phenomenon points to chemicals produced by plants common to captive diets, such as soy and alfalfa, as possible causes. In other species these chemicals, called phytoestrogens, are well known to cause fertility issues due to their ability to disrupt normal hormone function. Here we present a brief overview of how phytoestrogens impair fertility and propose a mechanism for how they do so in SWR. In addition, we summarize our past findings that suggest developmental exposure to phytoestrogens is a probable cause of the low fertility captive-born female SWR exhibit. Moreover, we present recent evidence from our own institution that suggests changing to a low-phytoestrogen diet may promote the reproductive success of previously infertile captive-born female SWR. As a result, we strongly suggest dietary phytoestrogen levels should be reduced in order to increase fertility of SWR in managed settings.

Résumé

La population de rhinocéros blancs du sud (RBS) en captivité n'est pas actuellement autonome en raison de l'échec de reproduction des femelles en captivité. Notre recherche sur ce phénomène souligne les produits chimiques produits par les plantes communes aux régimes captifs, tels que le soja et la luzerne, en tant que causes possibles. Chez d'autres espèces, ces produits chimiques, appelés phytoestrogènes, sont bien connus comme la cause des problèmes de fertilité en raison de leur capacité à perturber la fonction hormonale normale. Nous présentons ici un bref aperçu de la façon dont les phytoestrogènes nuisent à la fertilité et nous proposons un mécanisme pour la façon dont ils le font chez les RBS. En outre, nous résumons nos résultats passés qui suggèrent que l'exposition aux phytoestrogènes est une cause probable de la faible fécondité que manifestent les RBS femelles nées en captivité. De plus, nous présentons des preuves récentes de notre propre institution qui suggèrent qu'un régime alimentaire à faible teneur en phytoestrogènes pourrait favoriser la réussite de reproduction des RBS femelles nées en captivité qui étaient auparavant infertiles. Par conséquent, nous suggérons fortement la réduction des niveaux de phytoestrogènes alimentaires afin d'augmenter la fécondité des RBS dans les environnements gérés.

Introduction

Over the past several decades, it has become evident to the conservation community that female southern white rhinoceros (SWR) born in captivity do not reproduce as well as their wild-born counterparts (Emslie and Brooks 1999; Schwartzenberger 1999; Swaisgood et al. 2006). As a result, the captive SWR population is not currently self-sustaining. With poaching of wild SWR reaching record levels, and showing little sign of slowing, maintaining a healthy ex situ assurance population of SWR is critical. Although a number of studies have investigated potential causes of the low fertility of captive-born female SWR, identification of the specific cause(s) remains elusive. What is clear, however, is that low fertility of captive-born female SWR is the result of some effect associated with development in a captive setting (Swaisgood et al. 2006).

One issue that is certainly a contributing factor to low SWR fertility is the widespread prevalence of reproductive pathologies within captive females. Irregular hormone cycles, tumors and cysts of the reproductive tract and endometrial hyperplasia (i.e. the thickening of the inner wall of the uterus) have all been documented in females from institutions around the world (Hermes et al. 2006). These pathologies share two things in common. First, each may significantly compromise an animal's fertility. Second, their onset can be the result of production of high levels of the hormone estrogen, or the exposure to estrogenic chemicals present in the environment. It has been proposed that because female SWR exhibit low fertility in captivity, they continually produce high levels of estrogen needed for follicle growth and egg maturation, without the interruption of long gestations and inter-birth intervals in which estrogen production is low. This high level of exposure is suggested to lead to the early onset of reproductive pathologies that further compromise fertility (Hermes et al. 2006). While this paradigm is documented in other species (Hermes et al. 2004), it does not provide a clear explanation for the difficulties captive-born SWR experience in achieving pregnancies in the first place. Therefore, we explored an alternative explanation for this phenomenon, focusing on the potential for exposure to estrogenic environmental chemicals to compromise initial fertility. Specifically, our research points convincingly to a group of chemicals called phytoestrogens, which are commonly found in captive diets, as important players in the low fertility of female SWR in captivity (Tubbs et al. 2012; Tubbs et al. 2014; Tubbs et al. 2016).

Phytoestrogens and their mechanisms of action

Phytoestrogens are chemicals naturally produced by plants that are structurally similar to the estrogens produced by vertebrates (Fig. 1). Because of this similarity, phytoestrogens consumed in the diet and absorbed into the bloodstream can interact with an animal's estrogen receptors (the proteins within cells that regulate the biological actions of estrogen). This ability to interact with estrogen receptors gives phytoestrogens the potential to disrupt developmental and reproductive processes regulated by estrogens. There are a number of different groups of phytoestrogens, but the most well-known are the isoflavones (e.g. daidzein and genistein) and the coumestans (e.g. coumestrol), which are found in high concentrations in legumes (Fig. 1). We chose to investigate the role phytoestrogens may play in reducing fertility in SWR because legumes, particularly soy and alfalfa, are usually major ingredients of commercially produced pellets fed to SWR in managed settings. Furthermore, consumption of high phytoestrogen diets in many other species has been shown to decrease fertility, as well as increase the development of reproductive pathologies identical to those described in SWR (Adams 1995; Unfer et al. 2004; Jefferson et al. 2005; see Jefferson et al. 2012 for a review).

The effects that phytoestrogens have on a particular organism depend on a number of factors including the level of exposure (dose), the duration of exposure (acute vs. chronic) and the developmental stage of exposure (age). Differences in the latter can result in profoundly different outcomes in terms of severity and duration. When exposure to phytoestrogens occurs as an adult, the effects can range from weak to severe, but are usually transient, and normal biological function is restored when exposure is stopped. In contrast, exposure to phytoestrogens during critical developmental stages, such as fetal development or nursing via the maternal diet, or during puberty though direct consumption, can alter development in such a way that the effects persist, even if exposure ceases. In terms of

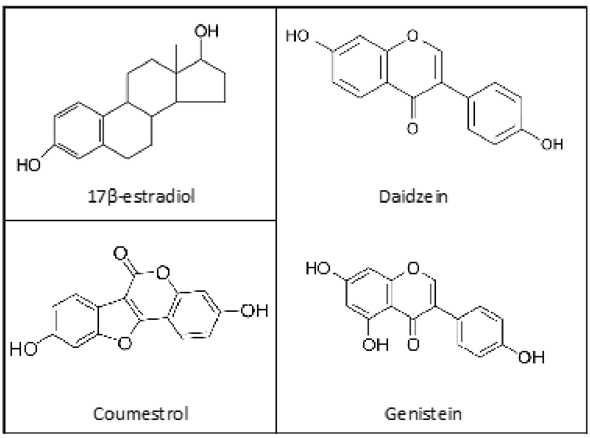

Figure 1. Chemical structures of estrogens and phytoestrogens. Due to the similarities in structure between phytoestrogens and estrogens, phytoestrogens can interact with estrogen receptors; the proteins that regulate the estrogen's actions, potentially causing reproductive harm. The structures shown represent 17ß-estradiol, the primary estrogen produced by vertebrates, and the major phytoestrogens coumestrol, daidzein and genistein.

reproduction, the consequences of developmental exposure to phytoestrogens can include subfertility or complete infertility that endure throughout an individual's lifetime.

Both adult and developmental effects resulting from eating high phytoestrogen diets have been documented in numerous vertebrate species. However, the dramatic decrease in fertility of captive-born compared to wild-born female SWR led us to propose that developmental exposure to phytoestrogens is a significant contributing factor to this phenomenon. Over the past few years, we have developed multiple lines of evidence in support of this hypothesis. First, we found that, compared to the estrogen receptors of greater one-horned rhinoceros (GOHR, a species that receives similar phytoestrogen-rich diets, but reproduce well in captive settings), SWR estrogen receptors are particularly adept at interacting with phytoestrogens (Tubbs et al. 2012; Tubbs et al. 2014). This relationship between receptor sensitivity and differences in fertility may explain how phytoestrogens could be problematic for SWR, but not GOHR (Tubbs et al. 2012; Patisaul 2012). More recently, we investigated the relationship between developmental phytoestrogen exposure and reduced fertility by comparing estrogenicity of diets and fertility at nine North American institutions with SWR breeding programs (Tubbs et al. 2016). In that study, we showed that the estrogenicity (i.e. phytoestrogen content) of a diet was directly related to the proportion of the diet accounted for by pelleted feed. We found the least estrogenic diets were from institutions that fed mostly fresh grasses, Bermuda or Timothy hay, and fewer than ~3–4 kg of pellets per animal per day (Tubbs et al. 2016). When we explored the relationship between institutional diets and fertility, we found no relationship between diet estrogenicity and fertility of wild-born female SWR. However, we clearly showed that captive-born females born at low phytoestrogen-feeding institutions had higher fertility rates than females born at moderate to high phytoestrogen-feeding institutions. In other words, our findings suggest that the more estrogenic a pregnant female's diet is, the less likely her female offspring are to reproduce during their lifetime. Consequently, those interested in breeding SWR should modify their diets by reducing or eliminating high-phytoestrogen ingredients such as soy and alfalfa, including soy and alfalfa based pellets (Tubbs et al., 2016).

Making a case for a diet change

The SWR herd at the San Diego Zoo Safari Park consists of 3.8 individuals living in a 60-acre (24.3 ha), mixed species enclosure. Within the herd there is one juvenile female, a proven captive-born 9 year-old female, a 17-year-old female who has delivered two stillborn calves and five nulliparous captive-born females ranging in ages from 10 to 32 years. Based on our research, changes were made to the herd's management in 2014 to reduce the level of phytoestrogens consumed by SWR. First, exclusion feeders were installed throughout the enclosure to reduce the SWR's consumption of pellets intended for other species sharing the enclosure. Second, a grass-based, low phytoestrogen pellet was developed and is now fed at 10% of the total diet (+90% Bermuda grass) by mass. Prior to the diet change, pellets with moderate to high phytoestrogen levels comprised approximately 50% of the SWR diet. The overall result of this new feeding regime was a significant reduction in the estrogenicity of the diet (Fig. 2).

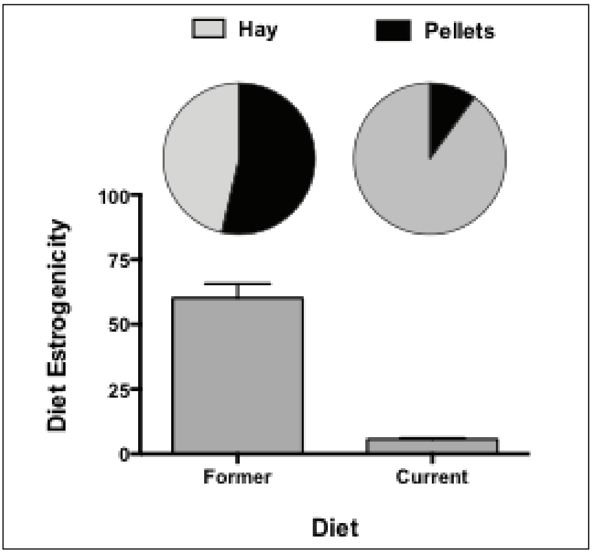

Figure 2. Differences in estrogenicity of former and current southern white rhino diets at San Diego Zoo Safari Park. The former diet consisted of approximately a 50%:50% mix of pellets with moderate to high phytoestrogen levels and Bermuda or Sudan hay. The current diet consists of 10% low phytoestrogen pellet and 90% Bermuda hay. The result of the change was a significant decrease in overall diet estrogenicity (i.e. phytoestrogen content).

Following our diet modification, routine monitoring of the reproductive status of our female SWR suggested that the diet change was having a positive impact. Approximately 10 months after the installment of exclusion feeders, levels of progesterone began rising in a 14-year-old nulliparous female SWR. Prolonged elevation in the levels of this hormone is indicative of pregnancy, as progesterone is required to maintain gestation. For this particular female SWR, we had seen no evidence of pregnancy from 2007 to 2015, despite the fact that she bred regularly during that time with proven males (Fig. 3A). Her ability to conceive and maintain her first pregnancy was unexpected, but quite encouraging as 14 years of age is well above the average age of first birth for captive-born females in North America (C. Tubbs, unpubl. obs.). Unfortunately, though, her progesterone levels dropped and the calf was stillborn approximately 2 months prematurely. Nevertheless, she was able to conceive a second time and, at the time of this writing, is maintaining a second pregnancy. We detected yet another pregnancy in a second female SWR, nearly 14 months after reducing our SWR's pellet consumption (Fig 3B). Although this female had been pregnant twice before, both of her previous calves were stillborn, and she had not maintained a pregnancy in the 10 years prior to the diet change despite also breeding regularly with proven males. In April of 2016, however, this female successfully gave birth to a healthy male calf.

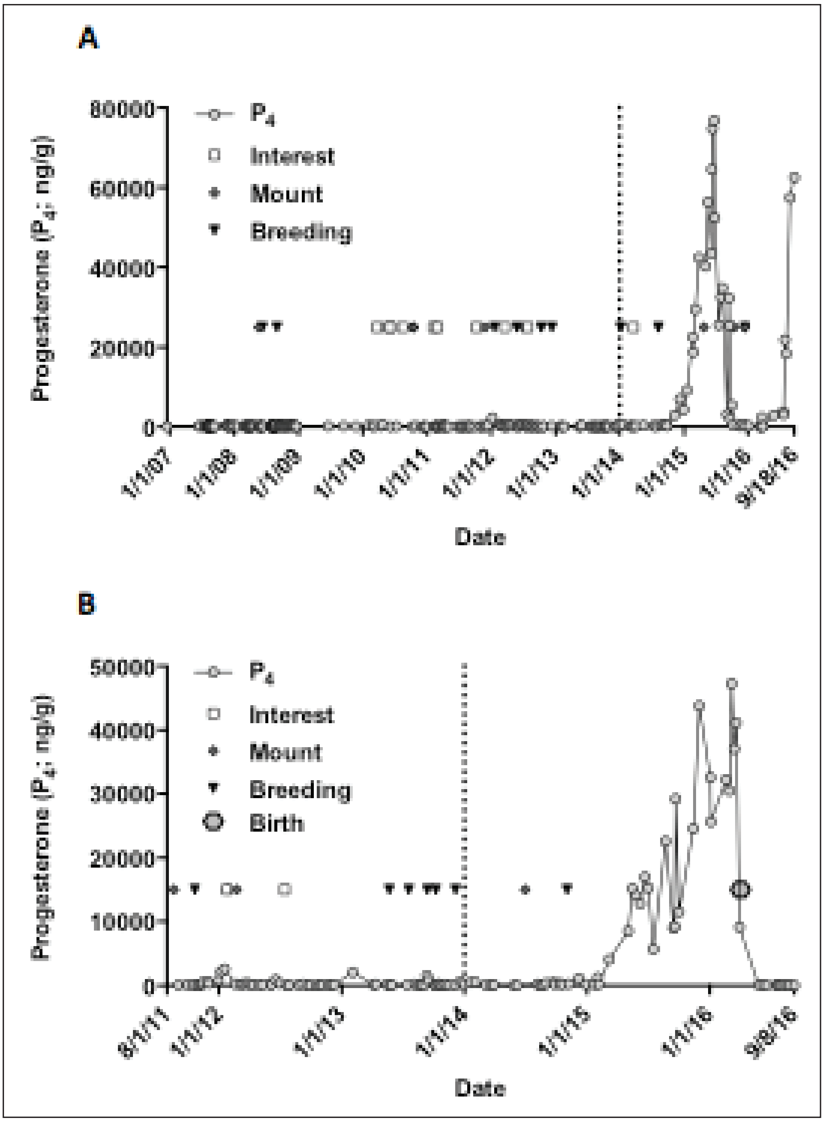

Figure 3. Progesterone profiles of two female southern white rhinoceros (SWR) before and after a reduction in dietary phytoestrogen consumption. In January 2014, SWR access to pellets in a mixed species exhibit was reduced (indicated by the vertical dotted line). After the diet change, we detected elevated progesterone levels (P4) indicative of pregnancy in two females that had never successfully reproduced, despite observed breeding with, mounting and eliciting interest from proven males. (A) One female has conceived twice since the diet change, delivering one calf premature and stillborn, and currently pregnant. (B) A second female conceived and successfully carried the calf to term.

Looking towards the future

Our initial hypothesis, based on the severity and permanence of developmental exposure to phytoestrogens in other species, painted a bleak picture for the future of SWR by suggesting that captive-born females may never reproduce. Therefore, we presumed that switching to a low phytoestrogen diet would be most beneficial in enhancing the fertility of future captive-born females and not necessarily captive-born females currently in the herd. Although this is likely to be the case for many captive-born females, the three pregnancies achieved following our diet change shed light on an added benefit of switching diets for all captive SWR immediately. While our data indeed suggest that SWR gestated at institutions feeding high phytoestrogen diets experience developmental effects, our recent pregnancies indicate that these effects alone may not be sufficient to preclude reproduction. However, if individuals exposed during gestation continue to be exposed to high phytoestrogen levels as adults, they may experience effects that, when coupled with pre-existing developmental effects, are severe enough to reduce fertility. Thus, changing diets may reduce exposure to the degree that some previously infertile SWR can successfully reproduce and contribute to the much-needed sustainability of the captive SWR population.

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