

# SUPPLEMENT

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acids) and peanut groups. In summary the results show the most positive effects on cognition and reduced cortisol concentrations in the peanut group. This seems to be in contrast to former studies, where nutrients high in n-3 fatty acids had positive behavioural and physiological effects, but not n-6 fatty acid rich diets.

#### PR1

### Faecal progestagen patterns in wild African white rhinoceros (*Ceratotherium simum*)

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The white rhinoceros (Ceratotherium simum) was rescued from extinction in southern Africa at the end of the 19th century but is still currently listed as 'near threatened' by the IUCN, primarily due to poaching. Captive breeding is a realistic option for long-term survival of the species but, to date, success has been poor. Knowledge of the reproductive status of wild animals can provide valuable information for the development of appropriate management plans. The present study aimed to establish a non-invasive protocol for monitoring faecal progestagen patterns in wild female southern white rhinoceroses. Six adult females at Lapalala Wilderness Reserve, South Africa, were located at least once every week. Three of these six animals gave birth during the study period. Faecal samples were collected at weekly intervals for 12 months (Oct 2008-Nov 2009). Samples were collected fresh and stored frozen at -20°C until analysis. They were lyophilized and approximately 0.05 g of each powdered faecal sample was extracted with 80% ethanol water (3 ml). Faecal extracts were assayed using an enzyme immunoassay for  $5\alpha$ -pregnan- $3\beta$ -ol-20-one (polyclonal antibody against  $5\alpha$ -pregnan- $3\beta$ -ol-20-one-3-HS-BSA and  $5\alpha$ -pregnan- $3\beta$ -ol-20-one-3-HS-peroxidase label). Mean faecal pregnane concentrations in each of the three pregnant females were 35 to 64-fold higher during pregnancy (55-145 days before parturition) than postpartum. The remaining three animals had mean faecal pregnane concentrations comparable to postpartum values. Our results show that noninvasive faecal progestagen measurements can provide information on the reproductive status of wild female white rhinoceroses, and be used for the detection of pregnancy in wild individuals. Collectively, the data clearly underlines the value of non-invasive hormone measurements as a tool to provide information on the reproductive patterns of free-ranging white rhinoceroses, thereby opening new opportunities to optimize breeding efforts of white rhinoceros populations in especially small and medium sized game reserves.

#### PR<sub>2</sub>

## Reproductive steroid monitoring in white rhinoceroses kept in European zoos

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White rhinoceroses within the EEP are not selfsustaining, as only 20% breed successfully. A comprehensive analysis of this worrying situation was summarized in the 2009 and 2011 in the European White Rhinoceros Studbook. Over the past two decades, faecal steroid hormone analysis in female white rhinoceroses, sometimes accomplished by rectal ultrasonographic examinations, has been used to investigate reproduction and reproductive problems. The 'normal' oestrous cycle is about 35 days in length, however cycles of 70 days in length, as well as missing ovarian activity ('flatliners'), or persistent luteal activity are common observations. Conceptions were observed following oestrous cycles of 35 days. Recently several young rhino cows were imported from South Africa into the EEP. Reaching puberty between 4-6 years of age; oestrous cycles in these young cows usually are 35