Effect of Testosterone on Antler Growth in Male Sambar Deer (*Rusaunicolor unicolor*) in Horton Plains National Park, Sri Lanka.

D. S. Weerasekera[1]*, N. L. Rathnasekara[2], D.K.K. Nanayakkara[3], H.M.S.S. Herath[3], S.J. Perera[4], K.B. Ranawana[5]

[1] Postgraduate Institute of Science, University of Peradeniya, SriLanka.
[2]Epsilon Crest Research, 6/6.67 Ward place, Colombo 07, Sri Lanka
[3]Nuclear Medicine Unit, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka.
[4]Department of Natural Recourses, SabaragamuwaUniversity, Sri Lanka.
[5] Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.

*Author for Correspondence (danushw2@gmail.com)

SUMMARY STATEMENT

Testosterone concentration in fecal pellets of antler phases of male sambar deer in Horton plains National park, Sri Lanka evaluated by Radioimmunoassay kit. The results obtained in this study were agreement with identical research work carried out in other deer species with temperate ancestry.

ABSTRACT

This study establishes the relationship between testosterone concentration with the different antler phases in male sambar deer (*Rusa unicolor*) inhabiting the Horton plains National Park, Sri Lanka (HPNP). Antler growth of sambar was categorized into seven phases; Cast (C), Growing single spike (GS), Growing into a Y as first tine appears (GIY), Growing Velvet begins to harden as third appears(GVT), Growth completed - velvet shedding begins (VS), Hard antler (HA), Casting (CT) based on phenotypic observations. The fecal samples were collected from 10 male sambar deer in each different phases of the antler growth cycle. Fecal testosterone level was estimated using radioimmunoassay (RIA). The results disclose that the dry feces of sambar deer in hard antler phase have a highest mean testosterone concentration of 18.52± 01.87 ng g^{-1}. In the GVT phase the mean testosterone concentration was 09.50±2.01ng g^{-1}, while in the VS phase the mean testosterone concentration increased to 14 ± 01.89 ng g^{-1}. The study also reveals a declination of the testosterone concentration (06.80± 01.20 ng g-1) in CS phase. The C phase had a mean testosterone concentration of 05.52± 0.84 ng g^{-1}.The fecal testosterone levels of the male sambar deer in HPNP changes according to the phase of the antler cycle.

Key words: Sambar deer, Antler cycle, Testosterone

INTRODUCTION

Sambar (*Rusa unicolor*)is the most widely spread deer in Asian continent (Chang et al., 2018; Chellappandian et al., 2014; Forsyth et al., 2014; Li, Ba, & Yang, 2016; Lin, Pei, Wang, & Liu, 2014; Oria et al., 2015; Radhakrishnan et al., 2012; Somma, Moura, Lange, Medeiros, & Montiani-Ferreira, 2016),that range from Sri Lanka(Rajapaksha, Thilakaratne, Chandrasiri, & Niroshan, 2002), India(Ajith Kumar et al., 2018; Pothana, Devi, & Goel, 2017), Southern Nepal(Thapa & Kelly, 2017), Burma(Gupta et al., 2018),throughout Southern China (Lin et al., 2014) and in South-Eastern Asia to the Pacific Coast and the islands of Borneo and Hainan (Ajith Kumar et al., 2018; Bhattacharjee & Franzmann, 1986; Brodie et al., 2015; Brodie, Helmy, Brockelman, & Maron, 2009; Hoogstraal & Wassef, 1982; Ismail & Jiwan, 2015; Jerrett, Slee, & Robertson, 1990). For the family Cervidae, most of the knowledge on reproductive biology has been originated from studies of temperate species that exhibit a distinct seasonal cycle of reproductive behavior and antler growth in males (Bhattacharjee & Franzmann, 1986; Chan, Tsai, Chen, Tung, & Chang, 2009; Lin et al., 2014; Muir et al., 1997; Vongpralub et al., 2015).Studies on Reproductive Biology and the Antler Cycle conducted in tropical regions are rare.

Antlers are growing from the skull only in male deer, with a solid calcified core and are unique in that. They go through an annual cycle of rapid growth in preparation for the rutting season and are casted after the season (Munk, Garrison, Clemons, & Keel, 2015). They are casted and regrown from a blastema annually into three branched structures of cartilage and bone that are used for sparing and attraction in adult sambar deer (Dahlan & Dawend, 2013; Lin et al., 2014).

Reproductive steroid levels contribute to reproduction of animals (Washburn et al., 2004). Hormonal levels can be estimated in animals by using plasma or fecal matter (Chen et al., 2018; Quispe, Yohannes, & Gahr, 2018). Estimating the hormone levels using fecal steroid assays provide a non-invasive method and have the capacity to develop long-term means of determining adrenocortical, testicular hormones in many vertebrate taxa(Spratt, Spratt, Bauman, & Chandler, 2018). Today the animal behavioral changes are possible to relate with changes in physiological processes (Dahlan & Dawend, 2013).

MATERIALS AND METHODS

Study area and the antler cycle

The study was carried out at the Horton Plains National Park (HPNP), Sri Lanka for a period of twelve months (01 January -31 December 2018). The HPNP was chosen for this study because of the large number of sambar deer inhabiting the park grasslands. There were over 800 sambar deer in the HPNP, during the commencement of the study (Personal communication). Based on our preliminary observations prior to the commencement of data collection, sambar males were grouped into seven 07categories based on their antler growth phase namely;(a) Cast, (b) Growing 1 – single pike, (c) Growing 2 - Antlers fork into a Y as first tine appears, (d) Growing 3 - Velvet begins to harden as third tine appears, (e) Growth completed - velvet shedding begins, (f) Hard antler and (g) Casting(Meng et al., 2015; Pereira, Duarte, & Negrao, 2005).

Antler growth categories were identified by physical appearance of the antlers, especially their sharpness and length (Suttie, Fennessy, Lapwood, & Corson, 1995), while the old males with irregular antler casting were not taken to the experiment. Additional characters that were considered for this study were the size of the body and antlers (Hu et al., 2017; Lemaitre et al., 2018).

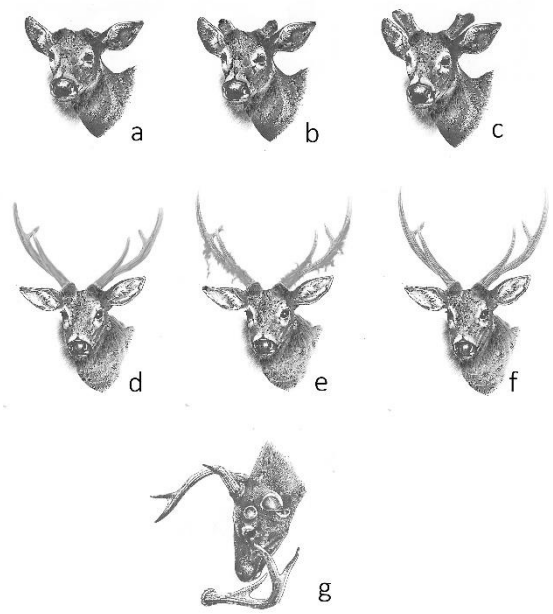


Figure 1. Antler phases: (a) Cast, (b) Growing 1 – Single Spike, (c) Growing 2 - Antlers Fork into a Y as First Tine Appears, (d) Growing 3 - Velvet Begins to Harden as Third Tine Appears, (e) Growth Completed - Velvet Shedding Begins, (f) Hard Antler and (g) Casting.

Fecal Sample collection

Fecal pellets were collected on the second week of every month within half an hour of voidance. Collected fecal pellets were labeled with antler phase and kept in polyethylene covers at -20 ^{o}C until extraction for Radioimmunoassay (RIA) analysis.

Fecal steroid extraction and enzyme immunoassay

In a standard oven, frozen fecal specimens were dried. Each sample was carefully powdered and blended. A subsample weighing 0.2 g was mixed in a test tube with 5 ml of 90% ethanol and briefly vortexed. In a water bath (90 oC) the tubes were boiled for 20 minutes, adding ethyl alcohol to prevent drying. The extract was pre-boiled with 90% ethanol and centrifuged for 20 minutes at 1500 rpm.

The extraction was poured into another storage vial. A further 90% ethanol was added to the remaining fecal powder and vortexed for 30 seconds and centrifuged for 15 minutes at 1500 rpm, combined and dried down, the first and second extracts were reconstituted in one ml of methanol and vortexed for a short time. Until the assessment of RIA, the methanol samples were placed at -20 oC.For this purpose, the 125I Testosterone RIA kit (Blottneret al.,1996) was used. The concentrations of testosterone were associated with the stage of antler.

RESULTS

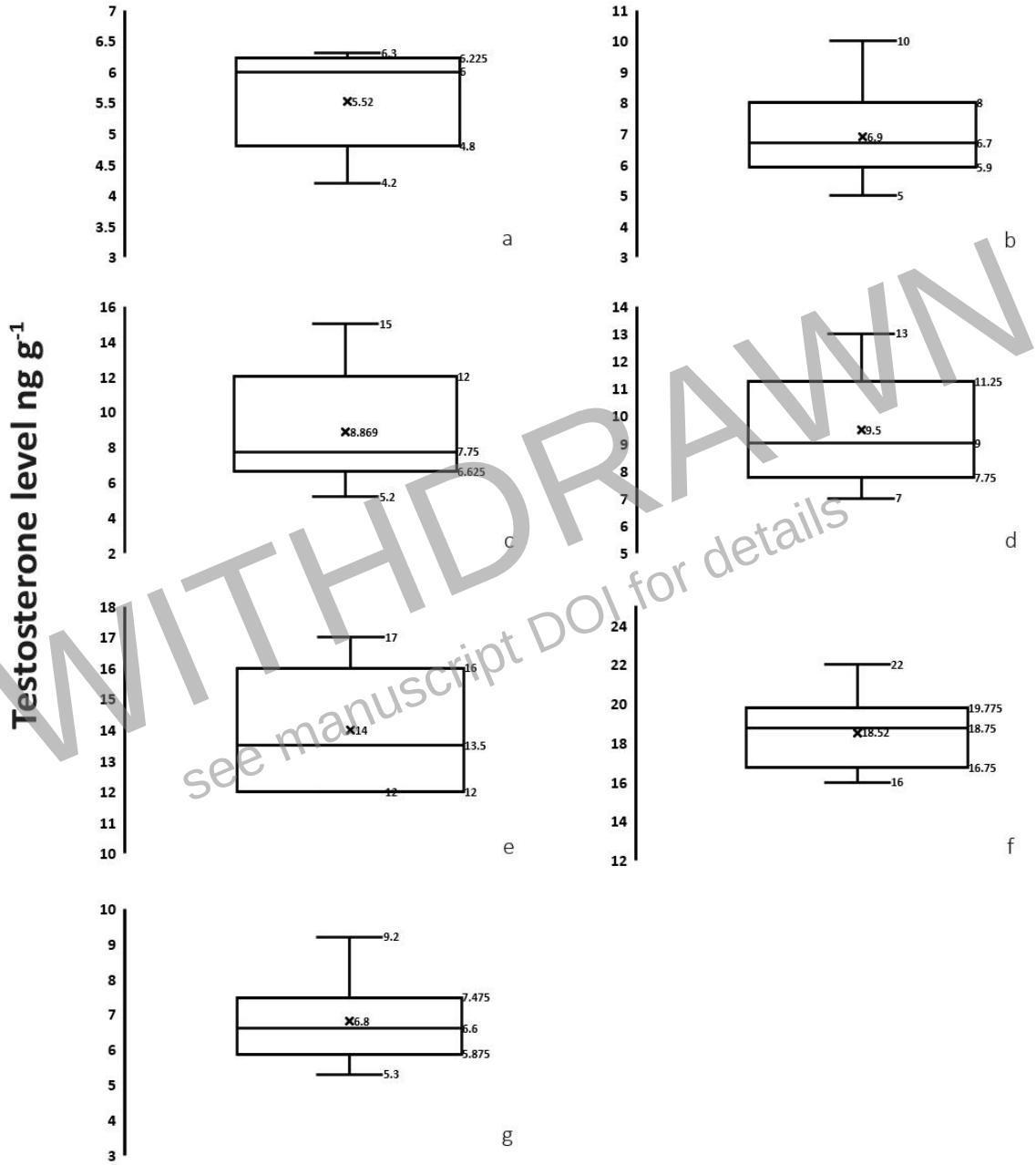
The antler cycle of sambar in HPNP

The sambar deer herds in HPNP selected for the study had been observed throughout the year following all identified antler phases (n=7). Any of the seven phases of the antler cycle could be observed at any given point of time during the year, indicating no marked seasonality in antler cycle. Shipka et al., (2002) suggested that deer sustenance near the equator, where seasonal day length differs only slightly, tend to be asynchronous in both their antler and sexual cycles. Seasonal relation in the antler cycle in a herd of Red deer by Wilson (1991) and Loudon et al., (1992) and Curlewis et al., (1988) are also supportive of the above findings on sambar deer stags in the HPNP. This observation is also supported by Heckeberg et al., (2017) who reported that most of the cervids in equatorial region are seasonal.

Examination of the male sambar deer in HPNP before the commencement of this study revealed that the sambar deer antlers were in velvet growth for a duration of seven to eight months. The hard antlers start displaying for the whole rutting season which lasted for about four to five months. This was followed by the antler casting stage, where the deer cast their antlers either in a fight, or hitting against a tree or any other hard structure (Chapman & Harris, 1991; Loudon & Curlewis, 1988; Wilson, Walker, Bond, Middleberg, & Staples, 1991).

Kierdorfet al. (2012) noted that antlers were casted after the rutting / mating season, while Price et al. (20 05) noted that deer cast antlers for combat and show during the prior mating season in each spring. However, it was observed that most of the stags lost both the antlers on the same day while some stags were observed to cast their antlers with a gap of 2-3 days (J. S. Price, Allen, Faucheux, Althnaian, & Mount, 2005). This stage did not last long, as antler growth is a rapid continuous cycle, and the beginning of the next pair of antlers set in without a long interval. The phases of the antler growth of sambar deer identified from our observations in HPNP are shown in the Figure 1. A more detailed description of the antler cycle of sambar in HPNP will be published elsewhere with further observations, as it is not the primary objective of this paper.

Concentration of fecal Testosterone is clearly associated with the phases of antler development in Cervids (Bubenik, Pomerantz, Schams, & Smith, 1987; Lincoln, Fraser, & Fletcher, 1982). Male sambars in HPNP with cast antler phase maintained a mean testosterone level of 05.52± 0.84 ng g^{-1} of dry feces. The maximum Testosterone level of 06.30ng g^{-1} of dry feces was recorded in the cast antler phase while the minimum testosterone level of 04.20ng g^{-1}during the same phase, together with a median value of 06ng g^{-1} and ranging for ±2.1ng g^{-1} (Figure 2 a). In white-tailed deer (*Odocoileus virginianus*) the Testosterone exhibits the highest congregation during the mating season (Bubenik et al., 1987). In red deer (*Cervuselaphus)*, testosterone exhibits the highest concentration during the rutting season (J. Price, Faucheux, & Allen, 2005).



143

144 **Figure 2**. Box and whisker plots for mean fecal testosterone levels in ng g-1 during different antler phases
145 of sambar in HPNP.Testosterone was obtained and measured from RIA using methanol. Fecal matter
146 analyzed belongs to either of the antler phases; (a) Cast, (b) Growing 1 – Single Bud, (c) Growing 2 -
147 Antlers Fork into a Y as First Tine Appears, (d) Growing 3 - Velvet Begins to Harden as Third Tine
148 Appears, (e) Growth Completed - Velvet Shedding Begins, (f) Hard Antler and (g) Casting.

149

The mean testosterone level of male sambar deer in growing antler phase were 06.190 ± 01.46 ng g^{-1} of dry feces with a median of 06.70ng g^{-1}. The maximum level of 10ng $g^{-1}$and minimum of 05ng g^{-1} was recorded in the dried feces in the stags passing growing phase (Figure 2).

Indistinguishable findings have been noted on red deer stags, which reveals that mean serum testosterone concentrations rose during the breeding season until about one to two weeks before the peak of the rut. (Gaspar-Lopez et al., 2010). Stags in growing antler fork into a Y as first tine appear phase showing an elevation in mean testosterone level as 08.87±03.13ng g-1 of dry feces with maximum level of testosterone in dry feces was 15ng g-1,and the minimum level of testosterone in the same phase was 05.20ng g -1 with a median value of 07.75ng g-1with a range of 09.80ng g-1(Figure 2 c). The casting is promoted by testosterone levels of 9.56±1.94 ng g-1 of dry feces for the rest of the study period. Ditchkoff et al (2001) discovered that Testosterone showed the largest concentration during the rut in white-tailed deer. Mean Testosterone concentration of Sambar deer Stags in Growing Third Tine - Velvet Begins to Harden phase was 09.50±2.01ng g -1, with maximum level of 13ng g -1 and minimum of 07 ng g-1 testosterone concentration. During the Growing Third Tine - Velvet Begins to Harden phase, the median testosterone level in dried feces was 09 ng g -1 and the range was 06 ng g-1 (Figure 2 d).

Median Testosterone concentration of male sambar deer of HPNP in Growing Finished - Velvet Shedding Begins phase was 13.50ng g-1of dry feces and the mean Testosterone level was 14 ± 01.89ng g-1 with a maximum concentration of 17ng g -1 and minimum of 12ng g-1 of Testosterone level, ranging from 05ng g-1(Figure 2). Stags in Hard Antler phase maintained a mean Testosterone levels of 18.52± 01.87 ng g-1 of dry feces and the median testosterone level was 18.75 ng g-1. The maximum Testosterone level of stags in Hard Antler phase was 22ng g-1 and the minimum was16 ng g-1 with a range of 06 ng g-1(Figure 2). This was a well-marked increase to the maximum mean Testosterone level in dried fecal matter compared to other phases (Figure 3).

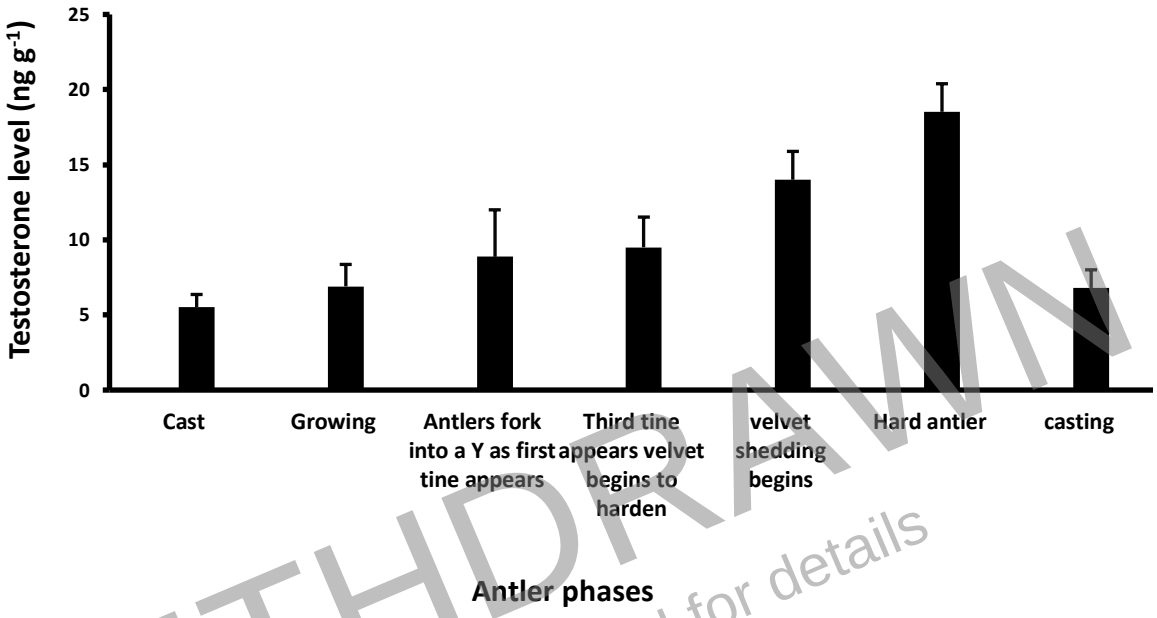
Mean testosterone level in dried fecal matter of male sambar deer in casting phase was maintained at 06.80± 01.20 ng g-1 of dry feces and the median testosterone level in dried fecal matter was 06.60 ng g^{-1}. The maximum level of testosterone in dried fecal matter of stags in Casting phase was09.20ng g^{-1} and the minimum level was 05.30ng g^{-1} with a range of 03.90ng g^{-1} (Figure 2 g). Uvira et al., (2018) and Antler development cycles are strongly linked to staggered sexual cycles and can be ascribed directly to d ifferences in seasonal photoperiod affecting gonadal steroidogenic activity in temperate regions. Stewart

et al., (2018) the largest concentration of testosterone observed at the beginning of the rutting season and a continuous decrease thereafter, which fully supported the outcomes of this research. Testosterone levels peak immediately before rut and it is the rapid decline in its level that causes Antler Casting. Antler Growth occurs at a low testosterone concentration and is seen increasing when the Antler Growth nears completion. Velvet Shedding and Antler Hardening is a consequence of high testosterone levels (Lincoln, Fraser, & Fletcher, 1984; Suttie, Lincoln, & Kay, 1984).

DISCUSSION

The fecal testosterone levels of the seven antler phases of male sambar deer in HPNP evaluated by RIA kit display that testosterone strive a powerful constraint on the phases in antler cycle. The sambar in hard antler phase reported to have a more elevated mean testosterone concentration in relation to the in velvet phase of the antler cycle. The results obtained in the present study were absolutely in agreement with identical research work carried out in other deer species which were predominantly with temperate ancestry. The variations of testosterone level observed in this research work streamlined with the findings of many other researchers (Bubenik, Brown, & Schams, 1991; Bubenik & Schams, 1986; Muir, Sykes, & Barrell, 1988; Ventrella et al., 2018). These studies also admit that, it is the immediate drop in the testosterone concentration which element the Casting of Antler (Figure 1). Velvet shedding was preceded by an increase the testosterone level highlighting its role (Figure 3). Nevertheless, the study could not conclude a clear seasonality in the antler cycle of sambar in HPNP as reported for many Cervids with temperate ancestry.

198



199 **Figure 3.** Phases in antler cycleplotted against the fecal testosterone concentrations in the dried fecal matter

200 of the Sambar deer monitored from January 2018 to January 2019 in Horton Plains National Park.

201

202

203

204

205

206

207

208

209

210

211

212

REFERENCES

Ajith Kumar, K. G., Ravindran, R., Johns, J., Chandy, G., Rajagopal, K., Chandrasekhar, L., . . . Ghosh, S. (2018). Ixodid Tick Vectors of Wild Mammals and Reptiles of Southern India. *J Arthropod Borne Dis, 12*(3), 276-285.

Bhattacharjee, K. K., & Franzmann, A. W. (1986). Intestinal rupture in a free-living sambar deer (Cervus unicolor) in India. *J Wildl Dis, 22*(1), 136.

Brodie, J. F., Giordano, A. J., Dickson, B., Hebblewhite, M., Bernard, H., Mohd-Azlan, J., . . . Ambu, L. (2015). Evaluating multispecies landscape connectivity in a threatened tropical mammal community. *Conserv Biol, 29*(1), 122-132. doi:10.1111/cobi.12337

Brodie, J. F., Helmy, O. E., Brockelman, W. Y., & Maron, J. L. (2009). Functional differences within a guild of tropical mammalian frugivores. *Ecology, 90*(3), 688-698.

Bubenik, G. A., Brown, R. D., & Schams, D. (1991). Antler cycle and endocrine parameters in male axis deer (Axis axis): seasonal levels of LH, FSH, testosterone, and prolactin and results of GnRH and ACTH challenge tests. *Comp Biochem Physiol A Comp Physiol, 99*(4), 645-650.

Bubenik, G. A., Pomerantz, D. K., Schams, D., & Smith, P. S. (1987). The role of androstenedione and testosterone in the reproduction and antler growth of a male white-tailed deer. *Acta Endocrinol (Copenh), 114*(1), 147-152.

Bubenik, G. A., & Schams, D. (1986). Relationship of age to seasonal levels of LH, FSH, prolactin and testosterone in male, white-tailed deer. *Comp Biochem Physiol A Comp Physiol, 83*(1), 179-183.

Chan, J. P., Tsai, H. Y., Chen, C. F., Tung, K. C., & Chang, C. C. (2009). The reproductive performance of female Formosan sambar deer (Cervus unicolor swinhoei) in semi-domesticated herds. *Theriogenology, 71*(7), 1156-1161. doi:10.1016/j.theriogenology.2008.12.007

Chang, A. M., Chen, C. C., Chang, C. D., Huang, Y. L., Ke, G. M., & Walther, B. A. (2018). Encephalitis induced by a newly discovered ruminant rhadinovirus in a free-living Formosan sambar deer (Rusa unicolor swinhoei). *J Vet Med Sci, 80*(5), 810-813. doi:10.1292/jvms.17-0477

Chapman, N. G., & Harris, S. (1991). Evidence that the seasonal antler cycle of adult Reeves' muntjac (Muntiacus reevesi) is not associated with reproductive quiescence. *J Reprod Fertil, 92*(2), 361-369.

Chellappandian, M., Pandikumar, P., Mutheeswaran, S., Gabriel Paulraj, M., Prabakaran, S., Duraipandiyan, V., . . . Al-Dhabi, N. A. (2014). Documentation and quantitative analysis of local ethnozoological knowledge among traditional healers of Theni district, Tamil Nadu, India. *J Ethnopharmacol, 154*(1), 116-130. doi:10.1016/j.jep.2014.03.028

Chen, R., Cui, Y., Zhang, X., Zhang, Y., Chen, M., Zhou, T., . . . Pan, C. (2018). Chlorpyrifos Induction of Testicular-Cell Apoptosis through Generation of Reactive Oxygen Species and Phosphorylation of AMPK. *J Agric Food Chem, 66*(47), 12455-12470. doi:10.1021/acs.jafc.8b03407

Dahlan, I., & Dawend, J. (2013). Growth and reproductive performance of sambar deer in Sabal Forest Reserve of Sarawak, Malaysia. *Trop Anim Health Prod, 45*(7), 1469-1476. doi:10.1007/s11250-013-0383-6

Forsyth, D. M., Woodford, L., Moloney, P. D., Hampton, J. O., Woolnough, A. P., & Tucker, M. (2014). How does a carnivore guild utilise a substantial but unpredictable anthropogenic food source? Scavenging on hunter-shot ungulate carcasses by wild dogs/dingoes, red foxes and feral cats in south-eastern Australia revealed by camera traps. *PLoS One, 9*(2), e97937. doi:10.1371/journal.pone.0097937

Gupta, S. K., Kumar, A., Angom, S., Singh, B., Ghazi, M. G. U., Tuboi, C., & Hussain, S. A. (2018). Genetic analysis of endangered hog deer (Axis porcinus) reveals two distinct lineages from the Indian subcontinent. *Sci Rep, 8*(1), 16308. doi:10.1038/s41598-018-34482-9

Hoogstraal, H., & Wassef, H. Y. (1982). Haemaphysalis (Garnhamphysalis) mjoebergi: identity, structural variation and biosystematic implications, deer hosts, and distribution in Borneo and Sumatra (Ixodoidea: Ixodidae). *J Parasitol, 68*(1), 138-144.

Hu, P. F., Xu, J. P., Ai, C., Shao, X. J., Wang, H. L., Dong, Y. M., . . . Xiumei, X. (2017). Screening weight related genes of velvet antlers by whole genome re-sequencing. *Yi Chuan, 39*(11), 1090-1101. doi:10.16288/j.yczz.17-116

Ismail, D., & Jiwan, D. (2015). Browsing preference and ecological carrying capacity of sambar deer (Cervus unicolor brookei) on secondary vegetation in forest plantation. *Anim Sci J, 86*(2), 225-237. doi:10.1111/asj.12271

Jerrett, I. V., Slee, K. J., & Robertson, B. I. (1990). Yersiniosis in farmed deer. *Aust Vet J, 67*(6), 212-214.

Lemaitre, J. F., Cheynel, L., Douhard, F., Bourgoin, G., Debias, F., Ferte, H., . . . Gaillard, J. M. (2018). The influence of early-life allocation to antlers on male performance during adulthood: Evidence from contrasted populations of a large herbivore. *J Anim Ecol, 87*(4), 921-932. doi:10.1111/1365-2656.12833

Li, Y., Ba, H., & Yang, F. (2016). Complete mitochondrial genome of Cervus elaphus songaricus (Cetartiodactyla: Cervinae) and a phylogenetic analysis with related species. *Mitochondrial DNA A DNA Mapp Seq Anal, 27*(1), 620-621. doi:10.3109/19401736.2014.908373

Lin, C. S., Pei, K. J., Wang, P. S., & Liu, B. T. (2014). Evidence of higher levels of testosterone during the velvet period in muntjac than in other cervids. *Theriogenology, 81*(3), 403-406. doi:10.1016/j.theriogenology.2013.10.011

Lincoln, G. A., Fraser, H. M., & Fletcher, T. J. (1982). Antler growth in male red deer (Cervus elaphus) after active immunization against LH-RH. *J Reprod Fertil, 66*(2), 703-708.

Lincoln, G. A., Fraser, H. M., & Fletcher, T. J. (1984). Induction of early rutting in male red deer (Cervus elaphus) by melatonin and its dependence on LHRH. *J Reprod Fertil, 72*(2), 339-343.

Loudon, A. S., & Curlewis, J. D. (1988). Cycles of antler and testicular growth in an aseasonal tropical deer (Axis axis). *J Reprod Fertil, 83*(2), 729-738.

Meng, S., Zhang, X., Xu, M., Heng, B. C., Dai, X., Mo, X., . . . Deng, X. (2015). Effects of deer age on the physicochemical properties of deproteinized antler cancellous bone: an approach to optimize osteoconductivity of bone graft. *Biomed Mater, 10*(3), 035006. doi:10.1088/1748-6041/10/3/035006

Muir, P. D., Semiadi, G., Asher, G. W., Broad, T. E., Tate, M. L., & Barry, T. N. (1997). Sambar deer (Cervus unicolor) x red deer (C. elaphus) interspecies hybrids. *J Hered, 88*(5), 366-372.

Muir, P. D., Sykes, A. R., & Barrell, G. K. (1988). Changes in blood content and histology during growth of antlers in red deer (Cervus elaphus) and their relationship to plasma testosterone levels. *J Anat, 158*, 31-42.

Munk, B. A., Garrison, E., Clemons, B., & Keel, M. K. (2015). Antleroma in a free-ranging white-tailed deer (Odocoileus virginianus). *Vet Pathol, 52*(1), 213-216. doi:10.1177/0300985814528216

Oria, A. P., Gomes Junior, D. C., Oliveira, A. V., Curvelo, V. P., Estrela-Lima, A., Pinna, M. H., . . . Ofri, R. (2015). Selected ophthalmic diagnostic tests, bony orbit anatomy, and ocular histology in sambar deer (Rusa unicolor). *Vet Ophthalmol, 18 Suppl 1*, 125-131. doi:10.1111/vop.12221

Pereira, R. J., Duarte, J. M., & Negrao, J. A. (2005). Seasonal changes in fecal testosterone concentrations and their relationship to the reproductive behavior, antler cycle and grouping patterns in free-ranging male Pampas deer (Ozotoceros bezoarticus bezoarticus). *Theriogenology, 63*(8), 2113-2125. doi:10.1016/j.theriogenology.2004.08.014

Pothana, L., Devi, L., & Goel, S. (2017). Cryopreservation of adult cervid testes. *Cryobiology, 74*, 103-109. doi:10.1016/j.cryobiol.2016.11.008

Price, J., Faucheux, C., & Allen, S. (2005). Deer antlers as a model of Mammalian regeneration. *Curr Top Dev Biol, 67*, 1-48. doi:10.1016/S0070-2153(05)67001-9

Price, J. S., Allen, S., Faucheux, C., Althnaian, T., & Mount, J. G. (2005). Deer antlers: a zoological curiosity or the key to understanding organ regeneration in mammals? *J Anat, 207*(5), 603-618. doi:10.1111/j.1469-7580.2005.00478.x

Quispe, R., Yohannes, E., & Gahr, M. (2018). Seasonality at the equator: isotope signatures and hormonal correlates of molt phenology in a non-migratory Amazonian songbird. *Front Zool, 15*, 39. doi:10.1186/s12983-018-0284-7

Radhakrishnan, S., Gopalan, A. K., Ravindran, R., Rajagopal, K., Sooryadas, S., & Promod, K. (2012). First record of Chrysomya albiceps Wiedemann, 1819 (Diptera: Calliphoridae) maggots from a sambar deer (Rusa unicolor) in Kerala, South India. *J Parasit Dis, 36*(2), 280-282. doi:10.1007/s12639-012-0110-7

Rajapaksha, W. R., Thilakaratne, I. D., Chandrasiri, A. D., & Niroshan, T. D. (2002). Development of PCR assay for differentiation of some important wild animal meat of Sri Lanka. *J Vet Med B Infect Dis Vet Public Health, 49*(7), 322-324.

Somma, A. T., Moura, C. M., Lange, R. R., Medeiros, R. S., & Montiani-Ferreira, F. (2016). Congenital cataract associated with persistent hyperplastic primary vitreous and persistent tunica vasculosa lentis in a sambar deer (Rusa unicolor) - clinical, ultrasonographic, and histological findings. *Clin Case Rep, 4*(7), 636-642. doi:10.1002/ccr3.500

Spratt, K. S., Spratt, J. S., Bauman, J. E., & Chandler, C. R. (2018). Behavioral and endocrine correlates of dominance in captive female Jackson's hartebeest (Alcelaphus buselaphus). *Zoo Biol*. doi:10.1002/zoo.21462

Suttie, J. M., Fennessy, P. F., Lapwood, K. R., & Corson, I. D. (1995). Role of steroids in antler growth of red deer stags. *J Exp Zool, 271*(2), 120-130. doi:10.1002/jez.1402710207

Suttie, J. M., Lincoln, G. A., & Kay, R. N. (1984). Endocrine control of antler growth in red deer stags. *J Reprod Fertil, 71*(1), 7-15.

Thapa, K., & Kelly, M. J. (2017). Density and carrying capacity in the forgotten tigerland: Tigers in the understudied Nepalese Churia. *Integr Zool, 12*(3), 211-227. doi:10.1111/1749-4877.12240

Ventrella, D., Elmi, A., Barone, F., Carnevali, G., Govoni, N., & Bacci, M. L. (2018). Hair Testosterone and Cortisol Concentrations in Pre- and Post-Rut Roe Deer Bucks: Correlations with Blood Levels and Testicular Morphometric Parameters. *Animals (Basel), 8*(7). doi:10.3390/ani8070113

Vongpralub, T., Chinchiyanond, W., Hongkuntod, P., Sanchaisuriya, P., Liangpaiboon, S., Thongprayoon, A., & Somphol, N. (2015). Cryopreservation of Sambar deer semen in Thailand. *Zoo Biol, 34*(4), 335-344. doi:10.1002/zoo.21214

Wilson, P. R., Walker, I. H., Bond, D. B., Middleberg, A., & Staples, L. D. (1991). Field evaluation of melatonin implants to advance the breeding season in 1-year-old farmed red deer hinds. *N Z Vet J, 39*(1), 23-28. doi:10.1080/00480169.1991.35651