CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Variation of blood plasma and fecal hormones during antler cycle of Pere David's deer (*Elaphurus davidianus*)

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Declaration

I hereby confirm that I wrote diploma thesis entitled: **,,Variation of blood plasma and fecal hormones during antler cycle of Pere David's deer (*Elaphurus davidianus*) "** myself and used only references cited in the text and reported in bibliography.

In Dobříkov of the 20th of August 2015 …….....

Zuzana Klapalová

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Content

Abstract

The objectives of this study were to to evaluate endocrinology of antler cycle in the Pere David's deer through fecal sampling and blood plasma. Evaluation of the effects of seasonal and antler cycle to hormonal changes in males Pere David‘s deer.

For evaluate endocrinology of antler cycle and evaluation of the effects of seasonal and antler cycle was use 660 samples from 10 males Père David's deer from four Zoological gardens and experimental farm at Institute of Animal Science-Uhříněves. For evaluation were monitoring these indicators fecal samples, blood samples, hierarchy, seasonal moult, antler info, cleaning, casting dates, growth, start of rut, (measurement of antler growth and neck circumference) and evaluation of hard antler chemical composition and mechanical properties.

Key words: antler cycle, *Elaphurus davidianus*, Enzyme Immunoassay, metabolites of hormones

Abstrakt

Cílem této diplomové práce bylo zhodnotit endokrinologii parožího cyklu u Jelen milu přes odběr vzorků trusu. Dále vyhodnocení hormonálních změn u samců Jelena milu při působení sezónnosti a parožním cyklu.

Pro vyhodnocení endokrinologie parožního cyklu a působení vlivů sezónnosti a paroží ho cyklu bylo použití 660 vzorků od 10 samců ze čtyř zoologických zahrada a VÚŽV - Uhříněvsi. Pro hodnocení byly sledovány tyto ukazatele vzorky stolice, vzorky krve, hierarchie, sezónní línání srsti, parožení, čištění, výběr dat, růst, začátek říje, (měření růstu paroží a obvodu krku) a vyhodnocení chemického složení parohu a mechanických vlastností parohu.

Klíčová slova: parožní cyklus, *Elaphurus davidianus*, Enzyme Immunoassay, metabolity hormonů

1. Introduction

Pere David's deer became extinct in the wild about 1000 years ago (Laidler and Laidler, 1992). Today, the deer is found widely in deer parks, Texas hunting ranches and zoos. It's even been returned to small reserves in its native China. But it doesn't roam freely in the wild (Miller, 2013). Typical for Pere David's deer males grow their antlers over the winter, shedding them in December or January. They're also known to grow two sets of antler in a year, but only when extensive hand-feeding is provided (Naish, 2011). This species has antlers with a main branched anterior segment, with the points extending backwards (Macdonald, 2001).

This work focuses on review of evaluation of the effects of seasonal and antler cycle to hormonal changes through fecal sampling and blood plasma in males Pere David's deer (*Elaphurus davidianus*).

Seasonal establishment changes in the levels of sex hormones deal with already several studies.The male deer seasonally polyhedral it is that the sexual cycle is impressed with photoperiod (Stella and Havlíček, 2004). The hormones and nervous system are elements key physiological processes and synchronize the internal functions with the external environment. Hormones are release into the blood by endocrine glands and are transported into susceptible cells, tissues or organs (Janský and Novotny, 1981). In the final phase are detected metabolites, which vary considerably. These metabolites and their quantities are species specific (Palme et al., 2005). To determine hormone in a liquid can be used specific enzyme immunoassay (Muir et al., 2001, Palm, 2005). The advantage of these methods is the ease of proceduremanagement , readiness for low cost and analysis. The main principle of this immunoassay is measured in known hormone binding to polyclonal antiboby place. Starting medium for analysis can be blood, saliva , urine, feces You can use invasive or noninvasive. For this thesis was use non-invasive methods. Non invasive method use mainly on the ground of that the analyse take place on surface of hormones and sample collection for metering happens to no physical interference with

animals bodies. The range of application of these methods is very wide for studying animals in the wild (Strier et al., 1999).

Each of techniques of ELISA can be used for a qualitative and quantitative purpose. Enzyme immunoassay (EIA) is used as diagnostic tool in medicine and as quality control measure in various industries is also used as analytical tool in biomedical research for the detection and quantification of specific antigens or antibodies in a given sample (Stephanie et al., 2013).

2. Literature review

2.1 Pere David's deer (*Elaphurus davidianus*)

2.1.1 Origin Pere David's deer

The modern species of *Elaphurus* sp. Père David's deer (Milu in Chinese) evolved in the Pliocene period of the Tertiary, according to fossils excavated in southern Japan (Hofmann, 2007). The Père David's deer were probably found in the lowlands of China, swampy areas and reed-covered marshlands (Nowak, 1999). Population declined due to hunting and land reclamation in the swamp areas as human population expanded (Jiang and Li, 1999) and the last wild animal was shot near the Yellow Sea in 1939 (Bedford, 1951). During the first decade of the 20th century, the 11th Duke of Bedford in the United Kingdom gathered the last 18 Père David's deer, but only 11 of these deer were capable of reproducing (Bedford, 1951).

Hoffman features that the first reintroduction back to China went a head in the year 1985, when was launched into park Beijing Milu. In 1998 the first group of deer was released from the paddocks into the wider reserve (Hu and Jiang, 2002). This kind affect genic, strangulation that is why lost big part his genic difference. On this account it is possible that the first generation brood, born in Woburn, descend from only male. It results in close relationship present Père David's deer (Jones et al., 1983).

2.1.2 Description Pere David's deer

Harper (1945) features that the Chinese call this deer "sze pu shiang" which means something to the effect of 'none of the four'. This old name refers to this deer's supposed

ownership of the neck of a camel, the hoofs of a cow, the tail of a donkey and the antlers of a deer though it is not completely like any one of these animals.

Père David's deer has reddish to deep reddish brown summer pelage with a medial black stripe down the shoulders. Winter pelage is grayish brown with darker areas on flanks and throat. The skin between the hooves is naked (Nowak, 1999). The unusually long and slender head has large, expressive eyes and small, pointed ears. The skin around the eye and the lips are light grey and the neck has a throat mane in males. The legs are long and the hooves are relatively long and slender and adaptation to walking on soft marshy ground. The donkey like tail ends in a black tuft (Butzler, 1990).

2.1.3 Antlers

The antlers is model for basic research in bones biology and animal science, because they are the only animal bone that is accessible without the intrusiveness of surgical procedures and the potentially adverse effects of such procedures, because antlers grow and are cast every year. The antlers are costly sexual secondary characters (Harvey and Bradbury, 1991). For their growth, require a partial demineralisation of the skeleton because the diet cannot supply the enormous amount of minerals required for their rapid growth (Meister, 1956, Muiret et al., 1987), and this growth can reach 2-4 cm per day (Goss, 1983). Antler size might not only be an index of male quality, but also of habitat or of diet quality is inversely related toconditions that exert stress on the physiology of the animal (Ullrey, 1983).

Unique among deer the antlers have a main branched anterior segment, with the tines extending backwards. Another strange feature of the antlers is that there may be two pairs per year. The summer antlers are the larger set and are dropped in November, after the June-August rut. The second set, if they appear are fully grown by January, and are dropped a few weeks later (Butzler, 1990). By Nowak (1999) features that the has antlers only male and dumps is in December or January. New antlers begins growth at once after his dismount and his development be finished on May (Huffman, 2001). After completing development antler (May), animals silences prickly feelings erosion bast from antlers.

Soon after knock out will stop inner circulation of blood and antler happen until dismount extinct bone (Nowak, 1999). Harper (1945) features span longitude stems from 55 into 80 cm. Main stalk antler faces upright up and from her go out two to three long jura regia. These tine antlertakes aim all, to the back on the contrary near of others harts takes aim forward. Lower tine antler is longest at the end furcate. Rises impression, as though antlers was to pitched conversely, tail forward.

At Pere David's deer we can observe two different kinds of antlers cycle. According to a studies we observe one or two cycles of antlers per year. Together with the European roe deer can have exceptional growth of antlers in the winter (Geist, 1998). Chaplin (1977) describes dropping antlers in adult males from October, with subsequent growth of new antlers. Antlers cleaning velvet in May until June. Duke of Bedford (1951) describes in Woburn double antlers cycle. Summer antlers- growth from December / January to May and dropping the September / October. Winter antlers – growth to December and the dropping after a few weeks. Dual antlers occurred repeatedly in most adult males, but this phenomenon disappeared and it is rare.

One from documented examples of occurrence dual antler cycle (Chaplin, 1977) was occurrence in English zoo gardens. For male from Edinburgh Zoo with occurred dual antlers after transport to Calderparku. According to Chaplin (1977) in the time I spent at Woburn Park, I never saw any evidence of this double growth. I was therefore rather surprised, on going to Edinburgh Zoo,to find that their stag, now at Calderpark, regularly produced two sets of antlers in a year – just as described by the Duke of Bedford. I am not, however, sure that it is feeding.

2.1.4 Reproduction Pere David's deer

Among polygynous mammals, males differ markedly in their reproductive success and a great deal of effort has been made to understand how selective forces have shaped traits that enhance male competitiveness both before and after copulation (i.e., sperm competition) (Malo et al., 2004). In polygynous sexually dimorphic species, sexual

selection should be stronger in males than in females (Kruuk et al., 1999). Male reproductive success is determined by the ability of males to gain sexual access to females and by their ability to fertilize ova (Malo et al., 2004). Males uniquelythe penis horizontally from side to side with an upward swing at the end of the traverse (Geist, 1999). Deers are seasonal breeders and show cyclic variation in testicular volume and cellular differentiation within the tubular and interstitial testis compartment (Hombach-Klonisch et al., 2004).

Animals reach maturity during second year (Hu a Jiang, 2002). About two months before breeding season in June, males will leave the herd. They will rejoin a harem of females and fast during the rut. When fighting, males will use antlers, teeth, and will even rise up on hind legs and box with their front legs (Nowak, 1999). Gestation is 270-300 days (Hu and Jiang, 2002). Females have an approximately 20 day long estrous cycle and within a breeding season can have multiple cycles (Nowak, 1999). One rarely two young are born. These are weaned in 10-11 months and adults live up to 18 years (Hu and Jiang, 2002).

2.2 Steroid hormones

Steroid hormones are crucial substances for the proper function of the body. They mediate a wide variety of vital physiological functions ranging from anti-inflammatory agents to regulating events during pregnancy. They are synthesized and secreted into the bloodstream by endocrine glands such as the adrenal cortex and the gonads (ovary and testis). Steroid hormones are all characterized by the steroid nucleus which is composed of three six member rings and one five member ring (McGraw, 1992). A steroid hormones is a steroid that acts as a hormone. Steroid hormones can be grouped into five groups by the receptors to which they bind: glucocorticoids, mineralocorticoids, androgens, estrogens and progestogens. Vitamin D derivatives are a sixth closely related hormone system with homologous receptors. They have some of the characteristics of true steroids as receptor ligands (Funder et al., 1987).

Androgens are a group of chemically related sex steroid hormones. In humans and other vertebrates, androgens are made primarily in the male testes, female ovaries, and adrenal glands. Like all steroid hormones, androgens produce effects by docking with receptors on the cell's membrane surface or inside the cell in the liquid cytoplasm (Cato et al., 2002).

Estrogens are one of the two types of female sex hormones. They are secreted mainly by the ovaries and in smaller amounts by the adrenal glands. Functioning similarly to androgens, the estrogens promote the development of the primary and secondary female sex characteristics. Estradiol is the most potent of the estrogens. Progestins the other type of female sex hormone and are named for their role in maintaining pregnancy. The most important of which is progesterone. Estrogens and progestins are secreted cyclically during menstruation (Encyklopedie Britannica, 2015).

2.2.1 Testosterone

Testosterone is connected to production microgram (sperm) (Malo et al., 2009). Participates to the formation pedicle and controlling the growth of antlers, the same as in other species manages the development of secondary sexual characteristics (Li et al., 2003). In seasonally reproducing species is a large seasonal variability in testosterone levels. During the spring stay low levels and during summer soar levels. In the winter there is a rapid decrease (Lincoln 1972a). Development of antlers mass is associated with an increase concentration testorene in many species cervids (Bartos et al., 2012). According to study Bartoš et al., (2012), Gaspar-López et al., (2010), Malo et al., (2009), Suttie et al., (1995) and Suttie et al., (1984) during antlers growth rising levels testosterone at first stimulate growth of antlers and after exceeding value in stops antlers growth through mineralization and cervids antlers cleaning velvet. The growth new antlers connected with short-term testosterone pulse. Dominant specimen have earlier and higher increase testosterone levels during the period of levels low (Bartoš 1990). Cleaning velvet antlers arrive in a period when the testosterone levels increase seasonally (Lincoln 1992). The fact that the growth of antlers accompanied by low testosterone levels, confirmed by the study

conducted to castrate males. Males after castration able to achieve the same antlers as in conservation ability to reproduce. But antlers remains in velvet (Price et al., 2004, Li et al., 2003).

Testosterone has a direct effect on the aggressiveness of males. According to Lincoln et al., (1972b) they have deer with high levels of testosterone in engagements using antlers and not able aclashes apply forelimbs. Limbs are ordinarily used during antler growth or rut when are testosterone levels low.

2.3 Determination of hormones

For determination of hormones is several methods, that we can divide into two basic groups. These are invasive and noninvasive methods. Every group employs specific enzyme immunoassay, from which is possible given metabolite measure. Their separation is based on effect of each method have on physical state of studied animal (Kobelt et al., 2003).

2.3.1 Invasive and Non-Invasive methods

Invasive methods cover process of sample collection when physical interference of animal bodies is penetrated, such as for example cut, puncture, and so on. The blood collection is the most frequent invasive method with subsequent analysis of hormone‘s concentration from blood plasma (Kobelt et al., 2003). In wild animals or in some zoology gardens can be blood taking dangerous or impossible (Möstl and Palme, 2002). Advantage of those methods is the direct measurement of instantaneous hormones in blood, without influence of their conversions (Kobelt et al., 2003).

Among noninvasive methods belong such processes during which physical interference with bodies is not breaked. Hormones are not determined from blood plasma, but from alternative sources as are saliva (Greenwood and Shutt, 1992), urine (Bamberg et

al., 2001), feces (Pereira et al., 2005), milk (Rabiee et al., 2002a), feathers or coat (Bortolotti et al., 2008).

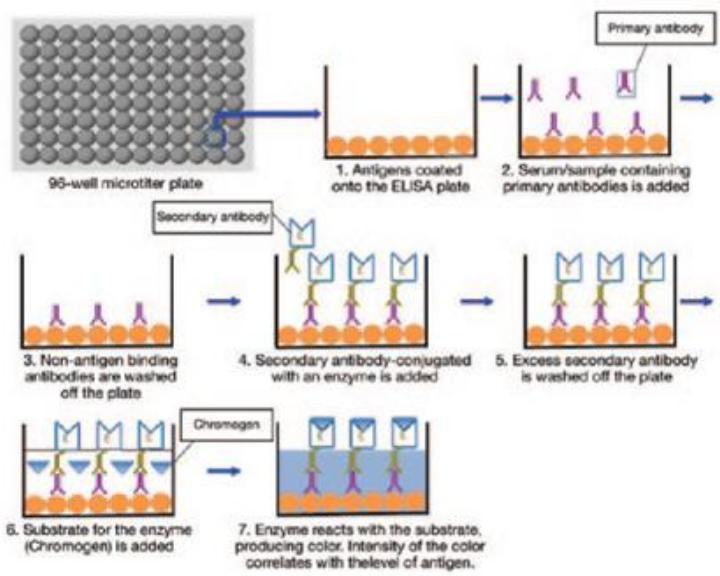
Nevertheless, also these media have some limitation. At analysis of milk or urine is yet necessary to define manipulation of animals for sample collection and application of these methods have one's limits that only on some individual animals. Using urine in free living animals, where experimenter has not suitable conditions to obtained samples, is nearly impossible. Similar limitation is for collection of saliva where the size of animal can be another difficulty. Using milk, it is possible to apply only to lactating female, therefore for analysis of hormone levels like optimal of all media seems to be feces (Möstl and Palme, 2002). Metabolites are extracted from feces preserved by freezing shortly after defaecation or after lyophilisation or cure in alcohol (Palme, 2005). Usage gives exhibits freshly and after cure, freshly are however more suitable because of simpler manipulation (Möstl and Palme, 2002). Advantage and preferred application of noninvasive methods of collection before invasive is reality that the designs of collection is more simple, repeatable and wihout necessary equipment or handling facilities (Palme, 2005). It means that after application of those methods will not get to influence level of measured hormones. In addition make possible to long - term observed one animal, without get to his physical detrimental effect (Hirschenhauser et al., 2005).

2.3.2 Enzyme Immunoassay and Enzyme-Linked Immunosorbent Assay

Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are both widely used as diagnostic tools in medicine and as quality control measures in various industries, they are also used as analytical tools in biomedical research for the detection and quantification of specific antigens or antibodies in a given sample. Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are derived from the radioimmunoassay (Stephanie et al., 2013). Radioimmunoassay (RIA) is an in vitro assay that measures the presence of an antigen with very high sensitivity. Basically any biological substance for which a specific antibody exists can be measured, even in minute concentrations. RIA has been the first immunoassay technique developed to

analyze nanomolar and picomolar concentrations of hormones in biological fluids. The target antigen is labeled radioactively and bound to its specific antibodies (a limited and known amount of the specific antibody has to be added) (Patron et al., 1987). Because of the safety concern regarding it is use of radioactivity, RIA assays were modified by replacing the radioisotope with an enzyme, thus creating the modern day EIA and ELISA.

EIA/ELISA uses the basic immunology concept of an antigen binding to its specific antibody, which allows detection of very small quantities of antigens such as proteins, peptides, hormones, or antibody in a fluid sample. EIA and ELISA utilize enzyme-labeled antigens and antibodies to detect the biological molecules, the most commonly used enzymes being alkaline phosphatase and glucose oxidase. The antigen in fluid phase is immobilized, usually in 96 well microtiter plates. The antigen is allowed to bind to a specific antibody, which is itself subsequently detected by a secondary enzyme-coupled antibody. A chromogenic substrate for the enzyme yields a visible color change or fluorescence indicating the presence of antigen. Quantitative or qualitative measures can be assessed based on such colorimetric reading. Fluorogenic substrates have higher sensitivity and can accurately measure levels of antigen concentrations in sample.



Enzyme-linked immunosorbent assay (ELISA) technique used to detect an antigen in a given sample. The

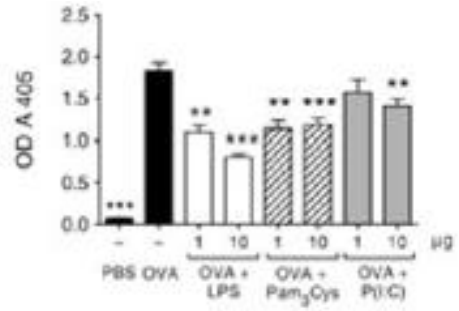
antigen (in liquid phase) is added to the wells, where it adheres to the walls. Primary antibody binds specifically to the antigen. An enzyme-linked secondary antibody is added that reacts with a chromogen, producing a color change to quantitatively or qualitatively detect the antigen) (Stephanie et al., 2013).

The key step in the ELISA assay is the direct or indirect detection of antigen by adhering or immobilizing the antigen or antigen-specific capture antibody, respectively, directly onto the well surface. For sensitive and robust measurements, the antigen can be specifically selected out from a sample of mixed antigens via a "capture" antibody. The antigen is thus "sandwiched" between such capture antibody and a detection antibody. If the antigen to be measured is small in size or has only one epitope for antibody binding, a competitive method is used in which either the antigen is labeled and competes for the unlabeled antigen /antibody complex formation or the antibody is labeled and competes for the bound antigen and antigen in the sample. Each of these modified techniques of ELISA can be used for a qualitative and quantitative purpose (Stephanie et al., 2013).

2.3.2.1 Types of ELISA

2.3.2.1.1 Indirect ELISA

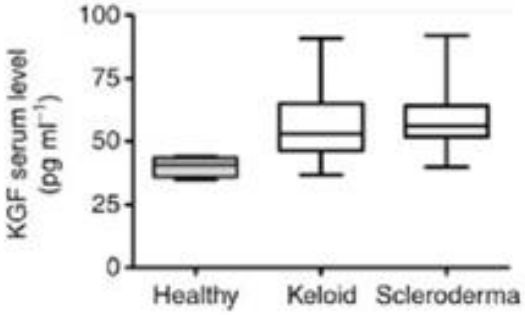
A sample that must be analyzed for a specific antigen is adhered to the wells of a microtiter plate, followed by a solution of nonreacting protein such as bovine serum albumin to block any areas of the wells not coated with the antigen. A main disadvantage of indirect ELISA is that the method of antigen immobilization is not specific. When serum is used as the test antigen, all proteins in the sample may adhere to the wells of a microtiter plate (Stephanie et al., 2013).



Indirect enzyme-linked immunosorbent assay (ELISA). Dermal exposure to Toll-like receptor ligands (lipopolysaccharide, Pam3Cys, P (I:C)) was demonstrated to downregulate ovalbumin-specific IgE antibodies in serum, as measured by the indirect ELISA technique. Reprinted from Haapakoski et al., (2013).

2.3.2.1.2 Sandwich ELISA

The sandwich technique is used to identify a specific sample antigen. The well surface is prepared with a known quantity of bound antibody to capture the desired antigen. After nonspecific binding sites are blocked using bovine serum albumin, the antigen-containing sample is applied to the plate. A specific primary antibody is then added that "sandwiches" the antigen (Canady et al., 2013).



Sandwich enzyme - linked immunosorbent assay (ELISA). " Sandwich " was used to detect levels increase keratinocyte growth factor (KP) in the serum of keloid and scleroderma patients compared to healthy controls quantify human KGF . Taken from Canady et al., (2013).

2.3.2.1.3 Competitive ELISA

The key event of competitive ELISA is the process of competitive reaction between the sample antigen and antigen bound to the wells of a microtiter plate with the primary antibody. The main advantage of competition ELISA is its high sensitivity to compositional differences in complex antigen mixtures, even when the specific detecting antibody is present in relatively small amounts (Dobrovolskaia et al., 2006).

2.3.2.1.4 Multiple and portable ELISA

Multiple and portable ELISA is a new technique that uses a multicatcher device with 8 or 12 immunosorbent protruding pins on a central stick that can be immersed in a collected sample. The main advantage of these ready-to-use lab kits is that they are relatively inexpensive, can be used for large population screening and do not require skilled personnel or laboratory equipment, making them an ideal tool for low-resource settings (Balsam et al., 2013).

3. Aims of the Thesis

The objectives of this study were to evaluate seasonal dynamics of antler cycle in Pere David's deer through concentrations of fecal metabolites of anrogens. Evaluation of the effects of seasonal and antler cycle to hormonal changes in males of Pere David‘s deer.

4. Hypothesis

We hypothesised that concentrations of fecal metabolites of testosterone and epiandrosterone will differ during stages of antler or reproductive cycle, with the highest levels before cleaning velvet and casting

5. Material and methods

We monitored adult males in five zoological garden, namelyOstrava Zoo, Brno Zoo, Bratislava Zoo, Chomutov Zoo, Prague Zoo and at Institute of Animal Science in Prague-Uhříněves (IAS).

For evaluation of IAS were monitoring these indicators:

- fecal samples
- blood samples
- hierarchy
- seasonal moult
- antler info, cleaning, casting dates, growth
- start of rut
- measurement of antler growth and neck circumference
- evaluation of hard antler chemical composition and mechanical properties

For evaluation Zoological garden were monitore these indicators:

- fecal samples
- seasonal moult
- antler info, cleaning, casting dates, growth
- start of rut
- evaluation of hard antler chemical composition and mechanical properties

a) Photo by Zuzana Klaplová

5.1 Characteristics of farming at Institute of Animal Science-Prague- Uhříněves

In 1953 the Institute has been moved to the premises of former Training Farm of the University of Agricultural and Forest Engineering in Uhrineves, which provided both suitable conditions for experimental work and demonstration facility for practical training of students.

We have a 4 hectar large deer facility (divided into 6 pens + equipment for fixing and handling the animals) with the capacity of 50 red deer (*Cervus elaphus*) and 6 Pere David's stags (*Elaphurus davidianus).* Sex ratio and age structure is variable and fully dependent on actual experimental needs.

The department took part in establishing deer farming in the country since eighties of the last century. Location VÚŽV-Uhříněvsi is southeast the edge Prague in urban district Prague 10 this part is now called Prague 22. Farming lie 50°1'50.302"N, 14°36'18.802"E.

5.2 Methodological procedure

Within work was collect 660 samples. The collection took place from 2011 to 2012. In the VÚŽV-Uhříněves was collect to six males, every deer was mark color:

- blue
- yellow
- red
- green
- white
- brown

The samples was collect to especially plastic tube, every tube was mark sign, dates and place. Then samples of each day put in a bag and stored in the freezer.
In the Zoological garden was to also available four males, every male was mark sign:

- Ostrava zoo – 62
- Prague zoo – Prague
- Bratislava zoo – Jozo
- Brno zoo – 1.0

The collection samples, marking samples and stowage samples was the same.
The whole processing was realize in the University of Veterinary Medicine, Vienna at the Faculty of Chemistry. For the evaluation of the samples was patent method procedure.

It was collected the freshly voided fecal samples (usually within 15 minutes of the defecation). The fecal samples were kept in sealed plastic bags and labeled tags (day-month-year). The fecal samples were stored in cooler instantly and the fecal samples throughout were stored at – 20 °C within 30 minutes until the laboratory analyses.
Before weighing were removed all foreign materials from the original fecal samples. For measuring the dry matter content of the fecal samples, were dried a proportion of 50% of each sample at 120 °C to constant weight. The dry substance content of each sample was calculated as follows: $\alpha = C/G$ where G represents the gross weight of the sample and C

the constant weight of the sample. It was used the index of $\beta = 1 / \alpha$ as a correction factor to transfer wet fecal sample steroid concentration into dry fecal sample steroid concentration (Li et al., 2001). According to Wasser et al., (1991) who described the technique of choice to extract fecal testosterone, estradiol and progesterone from the wet fecal samples.

5.2.1 Testosterone and progesterone

We placed 0,5 g wet fecal samples into a tube added 4 ml of a mixture of analytically pure methanol and distilled H_2O and homogenized and vibrated the tube for 1,5 minutes. For lipid extraxtion we added 2,5 ml analytically pure petroleum ether and vibrated the tube for 0,5 after centrifugation at 1500 g for 10 minutes at room temperature we transferred the 2 ml methanol layer to another tube and dried it at 70 °C. For future analysis we redissolved the dried sample with 2 ml phosphate buffer solution (0,1 M, pH 7,0) to from the last samples (Li et al., 2001).

b) Photo by Zuzana Klapalová

5.2.2 Estradiol

We placed 0,5 g wet fecal sample into a tube added 3,5 ml of analytically pure dichloromethane and 1,5 ml KOH (O,1 N) homogenized and vibrated the mixture for 3 minutes and then centrifuged the mixture at 1500 g for 10 minutes. We then discarded the water layer and extracted 3 ml dichloromethane into another tube. We washed the dichloromethane solution with distilled H_2O twice and then extracted 1 ml of dichlormethane solution and evaporated 1 ml of dichlorrmethane solution and evaporated it at 45 °C. For estradiol determination we redissolved the dried sample with 0,6 ml gelatin phospate buffer solution (0,1%) to from the last sample (Li et al., 2001).

c) Photo by Zuzana Klapalová

5.2.3. Statistical procedure

All analyses were performed using statistical software SAS System V 9.4 (SAS Inst. Inc., Cary, NC). To fit the skewed concentrations of hormones‘ metabolites into a normal distribution, log transformation was applied. Data normality was assessed by plotting histograms and normal probability plots;. Four different tests were performed (Shapiro–Wilk, Kolmogorov–Smirnov, Cramer–von Mises and Anderson–Darling). Afterwards, the transformed concentrations of hormonetestosterone and epiandrosterone metabolites were included as a dependent variable in analyses. Each was analyzed separately using the Generalized Linear Mixed Model (GLMM). The stage of antler growth or reproductive period was then used as level of explanatory variable including ‚antler growth‘; ‚cleaning

velvet‘; ‚rut‘; ‚after rut‘ and ‚antler casting‘. To account for repeated measures on the same animals over the experimental period, analyses were performed with PROC MIXED, using the individual animal as a random factor. Differences between the effects were tested using the F-test. For multiple comparisons, we used the Tukey-Kramer adjustment.

6. Results

We proved that different stage of antler cycle, or reproductive period resulted in different level of concenration of fecal metabolite of testosterone ($F_{(4,489)} = 13.64$, $p < 0.0001$), see Figure AA and epiandrosterone group ($F_{(4,489)} = 35.46$, $p < 0.0001$), see Figure BB.

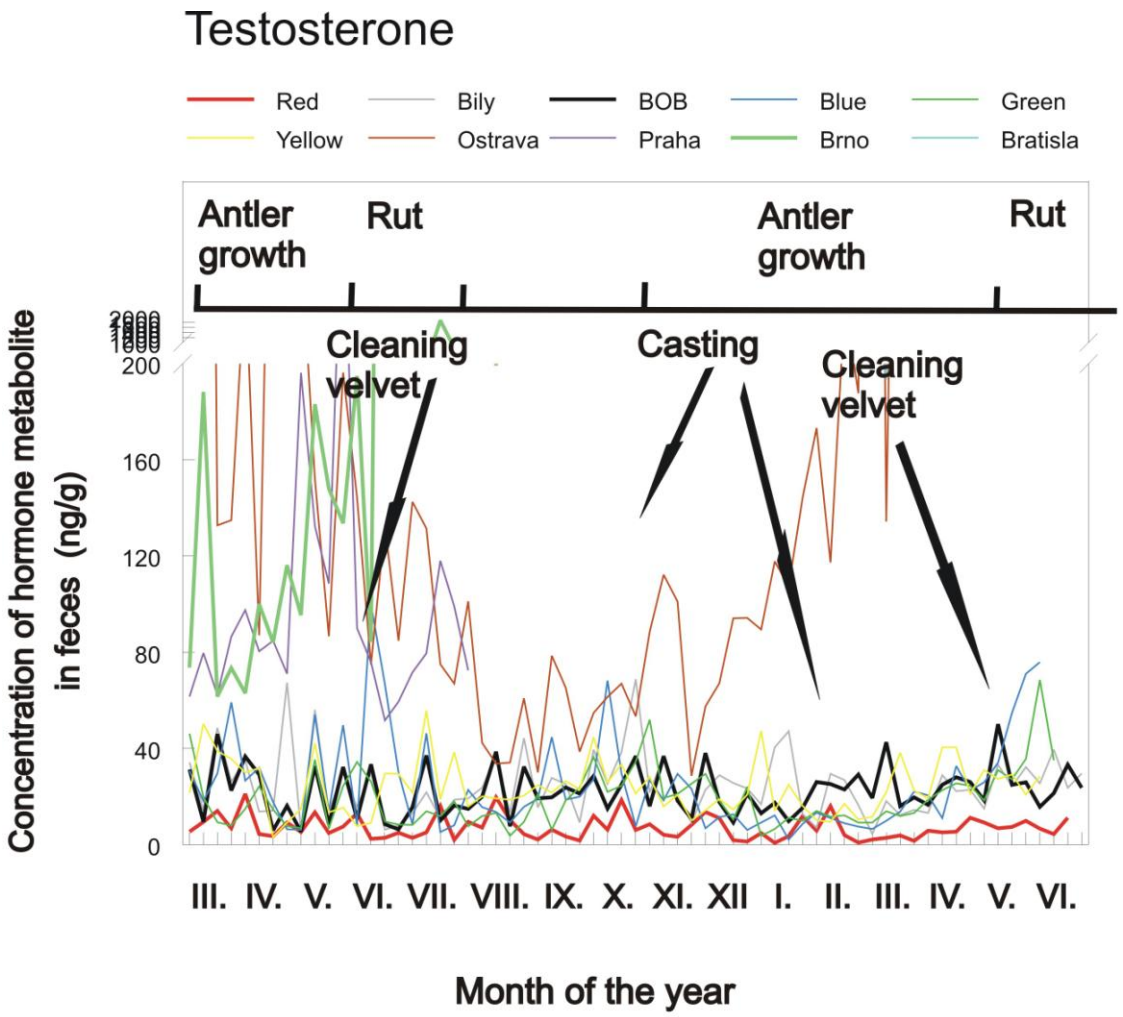


Figure A.) Resulted in different level of concenration of fecal metabolite of testosterone

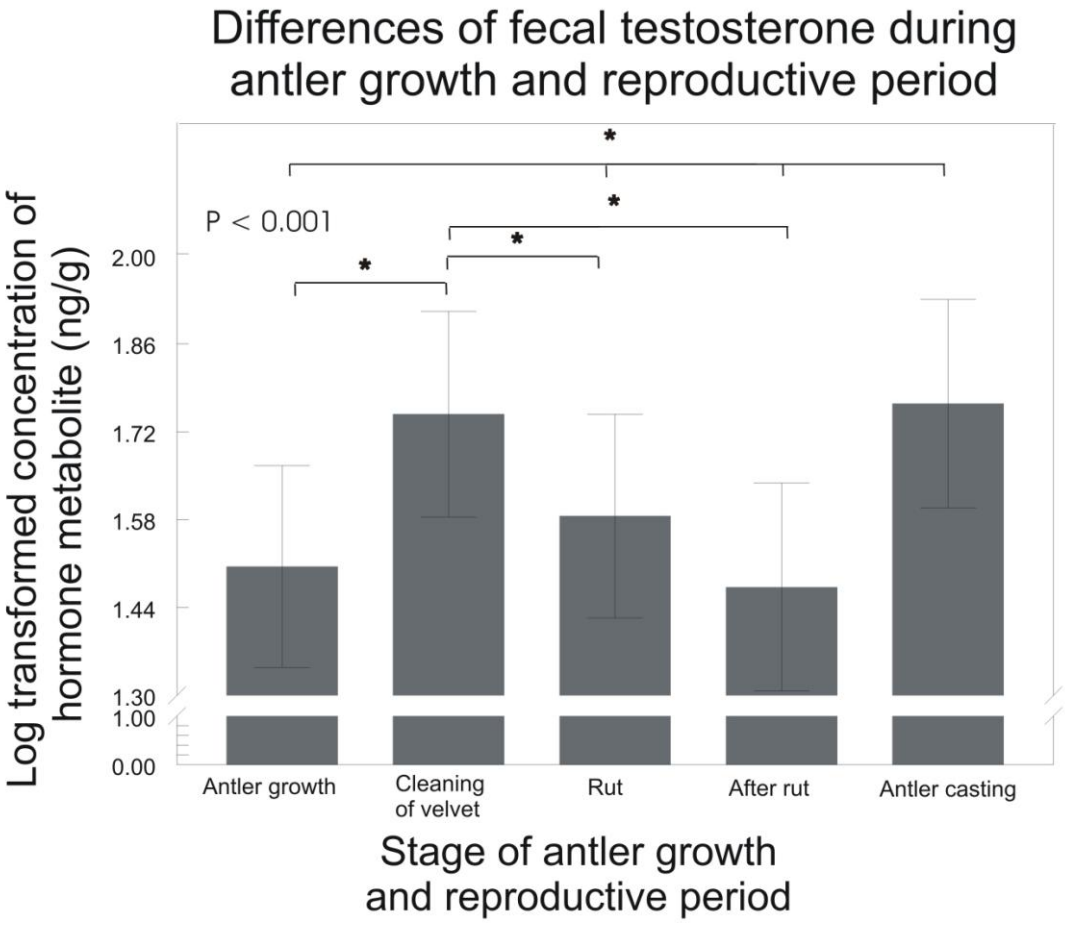


Figure a.) Differnces of fecal testosterone during antler growth and reproductive period

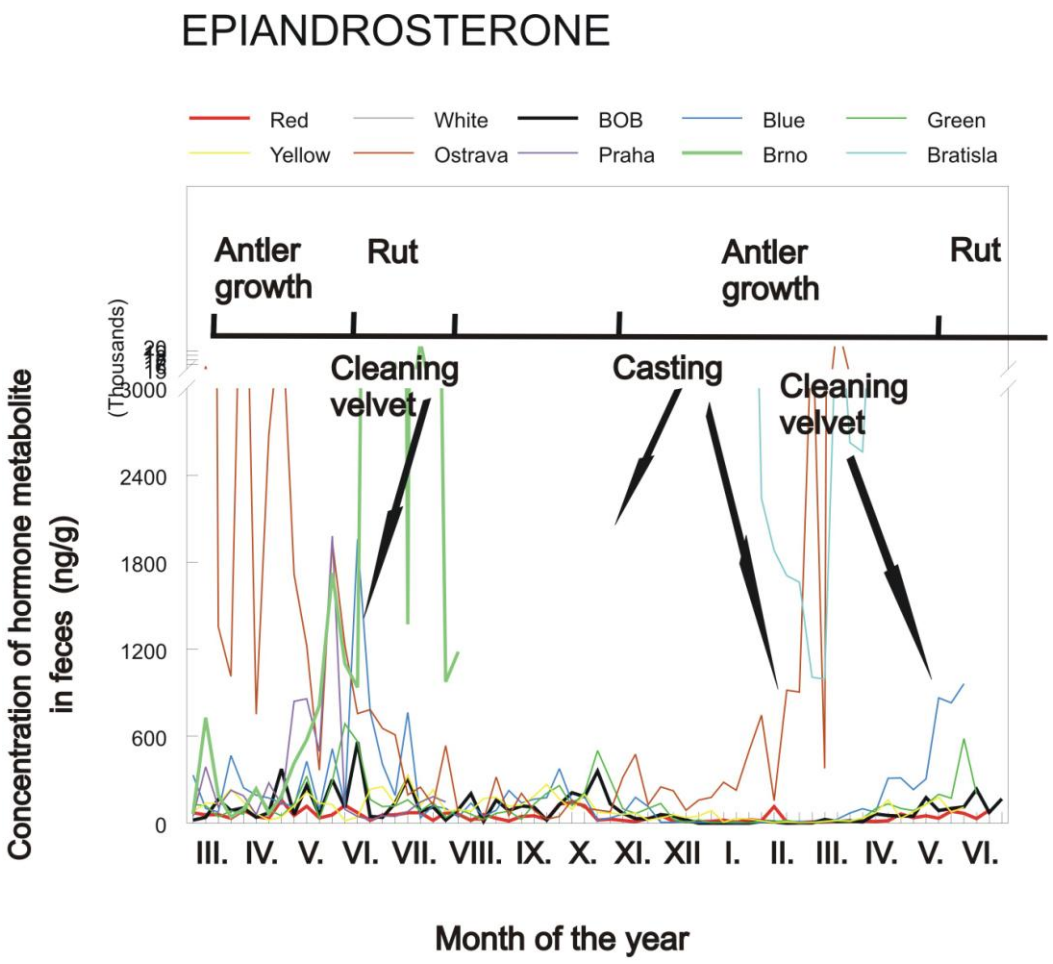


Figure B.) Resulted in different level of concenration of fecal metabolite of epiandrosterone

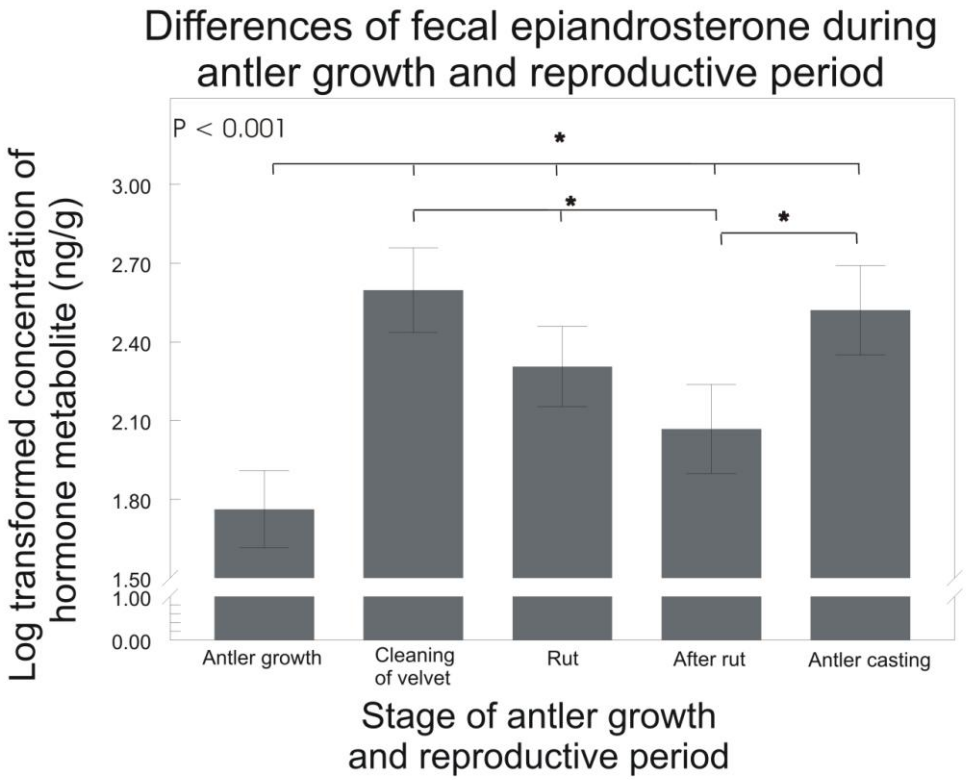


Figure b.) Differnces of fecal epiandrosterone during antler growth and reproductive period

7. Discusion

At Pere David's deer as one of the few cervids it was observed double antlers cycle. According to a studies we observe one or two cycles of antlers per year because can have exceptional growth of antlers in the winter (Geist, 1998). According to the studies Chaplin (1977) describes dropping antlers in adult males from October, with subsequent growth of new antlers. According to a studies Mr. Bedford (1951) describes the summer and winter antlers cycle of Pere David's deer. Summer antlers begins growth from December - January to May and dropping the September – October and winter antlers begins growth to December and the dropping after a few weeks. Dual antlers occurred repeatedly in most adult males, but this phenomenon disappeared and it is rare. However, it is recorded cases of double cycle in English zoo gardens. Local male Pere David's deer from Edinburgh Zoo with occurred dual antlers after transport to Calderparku.

Antlers cycle is influenced by steroid hormones. In this work are described two steroid hormones Testodterone and Epiandrosterone. Testosterone participates to the formation pedicle and controlling the growth of antlers, the same as in other species manages the development of secondary sexual characteristics (Li et al., 2003). In seasonally reproducing species is a large seasonal variability in testosterone levels. During the spring stay low levels and during summer soar levels. In the winter there is a rapid decrease (Lincoln 1972a). Development of antlers mass is associated with an increase concentration testorene in many species cervids (Bartos et al., 2012). According to study Bartoš et al., (2012), Gaspar-López et al., (2010), Malo et al., (2009), Suttie et al., (1995) and Suttie et al., (1984) during antlers growth rising levels testosterone at first stimulate growth of antlers and after exceeding value in stops antlers growth through mineralization and cervids antlers cleaning velvet. The growth new antlers connected with short-term testosterone pulse. Dominant specimen have earlier and higher increase testosterone levels during the period of levels low (Bartoš 1990). Cleaning velvet antlers arrive in a period when the testosterone levels increase seasonally (Lincoln 1992).

Epiandrosterone or it is one of the terminal metabolites of T. Despite the fact in literature iz is labeled as a weak andrgen (Martin, 1976), 5 –androsterone given

intramuscularly to castrated whitw-tailed buck a transformation of previously rapidly proliferating trabeculae of woven bone into more mature lamellar bone, characterized by little osteoblastic activity in between numerous islands of concentrically buil osteons (Morris and Bubenik, 1982). 5 Androsterone this other terminal metabolite of androgens had very little effect on the antler bone of castrated white-tailed deer (Morris and Bubenik, 1982). Epiandrosterone or 5 α–androsterone, 5 β-Androsterone it is one of the terminal metabolites of T. Despite in literature iz is labeled as a weak androgen (Martin, 1976), 5 α–androsterone given intramuscularly to castrated white-tailed buck a transformation of previously rapidly proliferating trabeculae of woven bone into more mature lamellar bone, characterized by little osteoblastic activity in between numerous islands of concentrically built osteons (Morris and Bubenik, 1982). This other terminal metabolite is 5 β-Androsterone this other terminal metabolite of androgens had very little effect on the antler bone of castrated white-tailed deer (Morris and Bubenik, 1982).

8. Conclusion

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