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Non-invasive monitoring of cortisol metabolites level in farmed brown hare (*Lepus europaeus*)

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ABSTRACT

Cage breeding technology of hares (*Lepus europaeus*) was established on some mini farms in Croatia in order to produce sufficient number of hares for hunting as well as for repopulation purposes. Conditions in hare's cage breeding cause certain level of stress. Influence of stress on reproduction and health status caused by daily manipulation was previously undetectable. Measuring of corticosteroid metabolite 11,17-dioxoandrostan (11,17 DOA) in hare faeces was tested as non-invasive method of stress detection. Therefore we analyzed stress status due to daily manipulation in mating couple in the beginning of reproduction season and after it. Samples were not collected as individual but as average to represent couple sample. The 11,17 DOA level showed less significant difference between couples in the reproductive season and more significant difference of couple value during and after mating season.

Key words: hare, cage breeding, stress, 11,17-dioxoandrostan

Introduction

Significant decrease of brown hare (*Lepus europaeus*) population in Croatia and Europe (KLANSEK, 1996) in past decades, caused establishment of several mini farms in order to produce sufficient number of hares for hunting as well as for repopulation purposes. Secretion of glucocorticoids (often used as stress indicators) increases metabolism rate,

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providing energy necessary to cope with negative environmental conditions (SAPOLSKY, 1992). Applied technology of cage breeding presumed keeping of mating couple of hare in the same cage during reproductive season. Among other negative effects, handling procedure cause additional stress with negative influence on reproduction (BOONSTRA et. al., 1998). Therefore, estimation of stress status of animals in reproduction might be an important parameter in modifying applied breeding technology. As stress increases the glucocorticoid production in the adrenal cortex (AXELROD and REISINE, 1984) (following the release of adrenocorticotropic hormone–ACTH) rise, giving us the opportunity to use glucocorticoid concentrations as a parameter in stress monitoring. However, blood glucocorticoid concentration as indicator of adrenocortical activity provides non-objective results in wildlife, as sampling itself will induce secretion of glucocorticoids (LEMAHO et al., 1992; BOONSTRA and SINGLETON, 1993). Therefore, a non-invasive method using cortisol metabolite (11,17-dioxoandrostan) from hare feces was established (TESKEY-GERSTL et al., 2000). Metabolite 11,17 DOA reaches peak concentration 12 to 24 hours after stress event (TESKEY-GERSTL et. al., 2000). Faecal sample collection is easy and without any feedback that affect results interpretation (HUBER et al., 2003). The presented study was aimed to investigate level of 11,17 DOA in mating couple as indicator of stress during and after reproductive season.

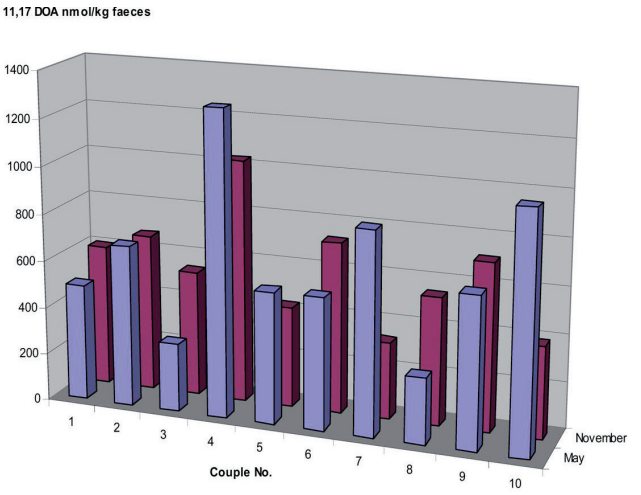
Materials and methods

The study was performed on hare breeding mini farm located near Zagreb. Object of study were cage maintained adult hares as mating couples. Hare couples were kept in the cages at open area of farm, each couple in their own cage. Cages (166 cm in length, 80 cm in width and 100 cm in height) were made of wood and wire gratings on ground. They were south-north oriented and aligned in rows. Each cage was placed on (60 cm height) stand. In study period samples were collected twice. Due to feeding, inspection and other manipulation in according to applied breeding technology all animals were treated equally. A total of 10 couples (out of 16) at age from 36 to 48 months were chosen. Other couples (6 of them) were not taken in study because of previously irregular reproduction in that season. Those irregularities (absence of copulation, still birth etc.) were of unknown cause. First collecting of samples were taken at the middle of May (the beginning of mating season) and second at the end of November (after mating season). May was chosen as starting point of study as period when reproduction physiology of male should be well established and regular. During first sample collection females were at different stage of pregnancy, but sampling itself was prior to removing of offspring's to separate cages to exclude that manipulation as additional stress cause. Samples of faeces were collected as average sample (to represent medial couple value) in dawn as fresh

sample from plate beneath cage. Immediately after collection samples were stored into marked plastic bags on -20 ºC. Total numbers of collected samples (No. 20) were divided in two series, as 10 samples in each season. All samples were transported to Institute of Biochemistry and Ludwig Boltzmann Institute of Veterinary Endocrinology, University of Veterinary Medicine, Vienna, Austria. Samples were analyzed on concentration of group-specific enzyme immunoassay (EIA) for 11,17 - dioxoandrostanes (DOA) by methodology established by PALME and MÖSTL (1997) and validated on hare (*Lepus europaeus*) species by TESKEY-GERSTL et al. (2000).

Results

As shown in the Fig. 1 and Table 1, minimum and maximum concentrations of 11,17 DOA values ranged from 278 to 1290 nmolkg^{-1} / faeces through the mating season. Such range might be result of a specific individual reaction of hare on certain breeding conditions. Despite that fact, the average couple value did not differ significantly between May and November samples (P>0.5). Remarkable standard deviation range was obvious (Fig. 2).



	1	2	3	4	5	6	7	8	9	10
May	493	683	289	1290	558	561	858	278	642	1007
November	600	669	532	1029	428	725	327	542	710	389

Fig. 1. Concentration of 11,17 DOA of mating hare couples in May/November

Table 1. Statistical data derived from 11,17 DOA concentrations in faeces; a comparison between May and November samples.

	May samples	November samples
Average	665,9	595,1
Max	1290	1029
Min	278	327
T TEST (p>0,5)	0,558524615	
SD	314,2322	203,554224

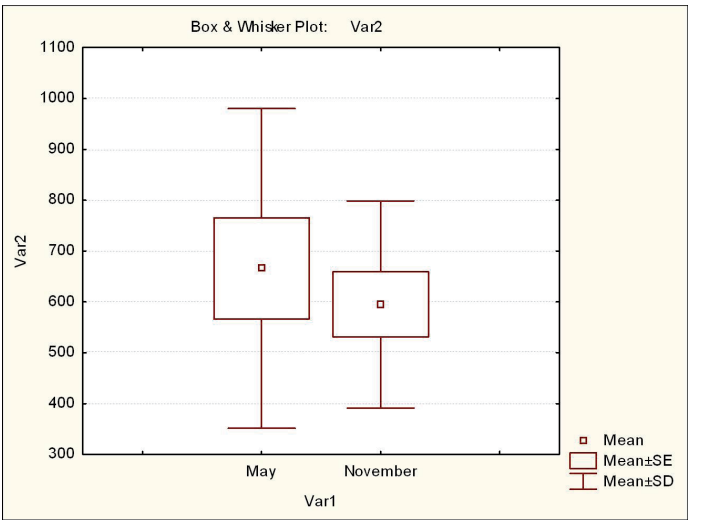


Fig. 2. Parameters of 11,17 DOA from hare faeces, note the remarkable SD range.

Discussion

For non-invasive monitoring of disturbance by measuring of 11,17 DOA sampling procedure might influence the final result and correct interpretation. Effect of the time period past defecation occur if faeces samples collected 6 hours after defecation. Those samples contained significantly lower concentration of cortisol metabolites than fresh faeces (HUBER et al., 2003). Depending on conditions, in droppings exposed to sun time

past defecation might be overestimated and in droppings stored in shade underestimated. Sample collection of night droppings early in dawn ensures that time past defecation is less then 6 hours, with minimal influence of sun light and less possible evaporation rate. On the other hand collecting of average couple faeces sample might as well as collecting of anonymous faeces lead to overrepresentation of particular individual in estimating mean value of 11, 17 DOA. It is difficult to cope with this risk due to study on wildlife species in open area as well as in specific hare farm conditions. Therefore we took the risk of possible overrepresentation due to practical value of sample collecting methodology aimed to be eligible for breeders. In addition sex and reproduction state in female may have an influence on glucocorticoid secretion. Sex induced specificity of glucocorticoid concentration in various species have been documented (WINGFIELD and FARNER, 1993; RUIS et al., 1997; BOONASTRA et al., 2001;). Despite that, in some cervidae species, i.e. red deer and reindeer (BUBENIK et al., 1998; HUBER et al., 2003), a faecal cortisol metabolite levels did not differ between females and males and neither gestation, lactation nor rut affected faecal cortisol excretion. The best way to eliminate effect of overrepresentation would be genotyping of fecal samples, but it would require a lot of time and money (TABERLET et al., 1999), which would certainly affect the applicability of proposed non-invasive method of stress monitoring. According to our results there was no significant difference in value of 11,17 DOA between May and November sampling. Small number of total samples (no.20) probably results with remarkable standard deviation range. On the other hand such deviation might be result of specific individual reaction of hares to same source of stress. There is no significant difference between May and November samples ($P>0.5$) probably due to standard and routine manipulation procedure through all seasons. This could mean that adaptation period is completed during previous reproduction seasons (third reproductive cycle) and no further gravidity, delivery nor weaning in cage conditions caused additional stress. As the literature on glucocorticoid metabolism and excretion of their metabolites in hare is rather scarce, it is difficult to describe all possible circumstances and their side effects in this study. However, documented inter-species differences due to the metabolism and to excretion of glucocorticoids and their metabolites do not enable possible analogous conclusion on other studies results (TAYLOR, 1971; PALME et al., 1996). Comparing to results of hares in metabolic cages TESKEY-GERSTL et al. (2000) difference in 11,17 DOA concentration in hare faeces prior and after stress could be fivefold in maximum peak value. In their study median value prior to stress was 100 $nmolkg^{-1}$ / faeces of 11,17 DOA (with range from 43 $nmolkg^{-1}$ / faeces to 274 $nmolkg^{-1}$ / faeces) and maximum value after stress did not exceed 600 $nmolkg^{-1}$ / faeces of 11,17 DOA. According to same study, difference between levels of faecal cortisol metabolites in non pregnant females (higher) and males was considered statistically non-significant. As our results showed significantly higher average concentrations of 655.9/595.1 nmolkg-1

(May/November - not related to season) possible explanation could be in species atypical cohabitation of both sex through all seasons and continuous mutual disturbance. Such results might suggest that cage breeding induce certain amount of constant stress for adult hares, which is however insufficient to be considered as significant influence on reproduction efficiency and health status. However, proposed non-invasive methodology of monitoring stress status due to applied hare cage breeding technology represent useful and applicable method to evaluate stress induced by technology. Furthermore, it is possible to evaluate annual-dependant stress status related to physiological status of hare couples.

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SAŽETAK

Kavezni uzgoj zečeva (*Lepus europaeus*) započet je na pojedinim malim farmama u Hrvatskoj s ciljem proizvodnje dostatnog broja zečeva za potrebe lova i napučivanja otvorenih staništa. Samo držanje zečeva u kaveznom sistemu uzrokuje određenu razinu stresa. Učinak stresa uzrokovanog svakodnevnim zahvatima na rasplodnu sposobnost i zdravstveni status zečeva do sada nije bilo moguće pouzdano utvrditi. Zbog toga je ustanovljena i provjerena neinvazivna metoda određivanja stresa mjerenjem količine metabolita kortizola 11,17 – dioksoandrostana (11,17-DOA) u izmetu zečeva. Prema tome analizirali smo razinu stresa u rasplodnih parova uzrokovan svakodnevnim zahvatima u uzgoju u početku i nakon rasplodne sezone. Uzorci su prikupljani po paru, a ne zasebno od svake životinje. Razina 11,17-DOA metabolita je pokazala manje razlike među parovima tijekom rasplodne sezone i značajnije razlike između vrijednosti tijekom rasplodne sezone u usporedbi s vrijednostima nakon sezone parenja.

Ključne riječi: zec, kavezni uzgoj, stres, 11,17-dioksoandrostan

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