The Effect of Transport Stress on Corticosterone Metabolites in Pheasant Droppings

Martina Volfova (✉ volfovam@vfu.cz)

University of Veterinary and Pharmaceutical Sciences Brno: Veterinarni a farmaceuticka univerzita Brno
<https://orcid.org/0000-0002-6069-1769>

Zuzana Machovcova

University of Veterinary and Pharmaceutical Sciences Brno: Veterinarni a farmaceuticka univerzita Brno

Pavel Forejtek

University of Veterinary and Pharmaceutical Sciences Brno: Veterinarni a farmaceuticka univerzita Brno

Eva Voslarova

University of Veterinary and Pharmaceutical Sciences Brno: Veterinarni a farmaceuticka univerzita Brno

Iveta Bedanova

University of Veterinary and Pharmaceutical Sciences Brno: Veterinarni a farmaceuticka univerzita Brno

Vladimir Vecerek

University of Veterinary and Pharmaceutical Sciences Brno: Veterinarni a farmaceuticka univerzita Brno

Research article

Keywords: non-invasive, monitoring, adrenocortical activity, EIA, Phasianus colchicus

Posted Date: December 10th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-123018/v1>

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Abstract

Background

Transport has been proven to represent a significant stressor for animals. In view of the fact that pheasants are frequently reared in captivity in hatcheries and are then transported for the purpose of their sale or release, it is essential from the viewpoint of welfare and health protection to obtain as much information as possible about their response to this stressor. The aim of this study was to assess changes in corticosterone metabolite (CM) levels related to transport in common pheasants (*Phasianus colchicus*).

Methods

Sixteen birds aged 17 weeks were crated and transported for 3.5 hours (distance travelled 140 km) to the target customer. Individual droppings were collected during the 2 days prior to transport and 3 days after transport. Concentrations of faecal CMs were determined with a non-commercial EIA (enzyme-immunoassay) kit, cortisone was used as a standard and values were expressed as cortisone equivalents in ng per g of droppings.

Results

Transport represents a major stressor for animals and this was also reflected in increased CM concentrations in the droppings in our study. CM concentrations increased within 24 hours of transport ($P < 0.01$) as compared to pre-transport levels (533.82 ng/g vs. 228.85 ng/g) and continued to rise for another 24 hours (745.35 ng/g). Three days after transport CM concentrations fell back to baseline levels (297.97 ng/g).

Conclusions

The EIA used in this study proved to be sensitive enough to detect biologically meaningful alterations in the adrenocortical activity of pheasants exposed to procedures related to their transport.

Background

Increasing attention has been devoted to animal welfare in recent years on all types of farms. While there are many definitions of welfare, the majority of authors agree that animal welfare is comprised of two elements – the physical health of the animals and their emotional state. The first of these, physical health, includes the absence of disease and injury, adequate feeding, and physical and thermal comfort. The second element of welfare, the emotional state, includes both the absence of negative emotions and the presence of positive ones [1]. One important indicator of good welfare is the absence of stress. Stress, and the action of chronic stress in particular, may have a negative effect on the immune system, reproduction and animal behaviour [2].

Stressors of various kinds may be responsible for the activation of the hypothalamic-pituitary-adrenal axis (HPA), thereby initiating the secretion of the corresponding hormones [3]. The main secreted hormones produced by the adrenal glands are cortisol and corticosterone, the latter being secreted on a predominant basis in birds [3, 4]. Monitoring endocrine markers is a well-known method for evaluating stress. Blood sampling has considerable limitations. In birds, capture and subsequent blood collection is extremely stressful and has a noticeable influence on the concentration of glucocorticoids [5]. Determination of hormonal stress indicators from droppings, however, minimises the effect of sample collection on hormone levels and enables frequent collection [6]. Non-invasive methods of monitoring adrenocortical activity, however, require validation for specific species of monitored animals [7–10].

Non-invasive evaluation of stress levels in birds has been conducted in a number of studies. The relationship between levels of corticosterone in plasma and droppings in three species of bird, specifically the chicken (*Gallus domesticus*), great cormorant (*Phalacrocorax carbo*) and northern goshawk (*Accipiter gentilis*) was assessed, with the use of high-performance liquid chromatography (HPLC) following administration of ACTH (adrenocorticotrophic hormone) [7]. Further, a method for measuring corticosterone metabolites in chicken droppings developed and validated [5]. Changes in the concentrations of a corticosterone metabolite have also been observed in the droppings of the western capercaillie (*Tetrao urogallus*) [11] and similarly in the snow goose (*Chen caerulescens*) [12]. The effect of transport stress has been monitored in laying hens transported for a period of one hour [13]. Validation of a non-invasive method of measuring stress has also been performed on African penguins (*Spheniscus demersus*) held in captivity [14]. The most effective method of analysis was determined in the northern spotted owl (*Strix occidentalis caurina*) following administration of ACTH [15]. No such study has, as yet, been performed on the common pheasant (*Phasianus colchicus*). Studies monitoring stress in captive-reared pheasants in connection with various periods of handling [16] and in connection with transport [17, 18] have consisted of traditional analysis of the concentration of glucocorticoids and other stress markers (lactate and glucose) in the blood plasma.

The aim of this study was to assess changes in the levels of corticosterone metabolites (CM) in the droppings of the common pheasant (*Phasianus colchicus*) in connection with transport. Transport has been proven to represent a significant stressor for animals [19]. In view of the fact that pheasants are frequently reared in captivity in hatcheries and are then transported for the purpose of their sale or release, it is essential from the viewpoint of welfare and health protection to obtain as much information as possible about their response to this stressor.

Results

The mean concentrations of CMs measured in 8 males and 8 females over 6 days of monitoring are recorded in Table 1.

An increased concentration of CM was seen after the transport of the pheasants in the droppings of all 16 individuals. The mean CM concentrations increased significantly ($P < 0.01$) over the 24 hours

following transport in comparison with the pre-transport levels (533.82 ng/g vs. 228.85 ng/g), as can be seen in Fig. 1.

The increase in mean CM concentrations continued for another 24 hours, with the peak being recorded on the second day after the transport of the pheasants (D5 = 745.35 ng/g). CM concentrations approached their initial values again 3 days after transport (297.97 ng/g). CM levels before transport and 3 days after transport did not differ statistically.

When the groups of male and female common pheasants were compared, higher mean CM concentrations were recorded in absolute values for males, with the mean peak values amounting to as much as 876.30 ng/g, while the corresponding figure for females was 614.30 ng/g of droppings (Fig. 2). Statistically, however, no significant differences were confirmed ($p < 0.05$), and the progression of the changes in CM levels was similar in both sexes (Fig. 2).

Discussion

The transport of pheasants from rearing facilities to the final customer is common practice in European countries [18, 20]. The birds are transported over short and long distances in various age categories, and are confronted by a number of stressors during transport, such as capture, placement in crates, loading, transport, and unloading in a new environment. Moreover, the welfare of the animals during transport itself is affected by a large number of other factors, such as temperature, humidity, density of occupation of the transport box and length of transport, as well as time of day and season of the year [2, 19]. The handling of the animal itself can be considerably stressing, as has been shown by a number of studies [16, 21, 22]. This has been confirmed in our study, in which the transport of common pheasants for around three hours resulted in CM levels higher than those before transport. While the pre-transport CM levels ranged from 203.00 ng/g to 275.59 ng/g, their mean post-transport levels increased to as much as 745.35 ng/g in the droppings. A more than threefold increase has also been demonstrated in laying hens transported for one hour [13]. Like the results of our study, another study in laying hens has also shown that increased CM levels lasted more than 36 hours after transport [13].

Sex is commonly given as a factor affecting the resultant concentration of glucocorticoid metabolites in the droppings [23]. For example, the differences exist in CM levels between male and female laying hens⁵ and between male and female black grouse (*Tetrao tetrix*) [24]. In our case, differences were recorded in absolute values, with the levels of the CMs measured being higher in males. Statistically significant differences were not, however, demonstrated. Levels of glucocorticoids in the droppings of males and females are influenced by different physiological and behavioural aspects. Higher levels have been shown both in males (e.g. in the domestic chicken) [5] and in females (e.g. in certain mammals) [25]. The resultant faecal CM levels can be affected in certain males with an increased level of androgens circulating in the blood (in elephants during the “musth” period). In females, in contrast, a considerable quantity of circulating glucocorticoids in the blood is connected with “globulin binding sex steroids”, for which reason the overall levels of glucocorticoids may be higher than in males [9, 10]. There are, however,

also studies that have, like our study, not recorded any differences in CM levels (such as in the mourning dove) [26]. This may be explained by the fact that the pheasants in our study were 17 weeks old and were not sexually mature (pheasants reach sexual maturity at the age of between 8 and 10 months) and therefore their CM levels were not affected.

The EIA method (first described by Rettenbacher et al. [5]), targeting the antibody against 4-pregnene-17 α ,21-diol-3,11,20-trione-21-HS, was used to determine the degree of transport stress in common pheasants in our study. This analysis can be used on various species of bird from the gamebird genus (*Galliformes*) and involves non-commercial EIA analysis developed in the laboratory. Certain studies state that it is also possible to use commercially produced ELISA kits that measure corticosterone for the determination of CMs in bird droppings. Corticosterone itself is not excreted in bird droppings, though the kits are also capable of recording the levels of its metabolites on the basis of cross-reactivity [5]. The entire spectrum of these metabolites has not yet been thoroughly researched. As some studies state, the use of analysis targeting cortisone, as in our study, is the most appropriate as it captures more kinds of metabolites present in bird droppings [5, 7].

Conclusions

Our results indicate that the measurement of corticosterone metabolites using a non-commercial EIA kit with the 4-pregnene-17 α ,21-diol-3,11,20-trione as a standard is suitable for the measurement of adrenocortical activity in common pheasants, and demonstrated that the CM concentrations remained elevated for more than 36 hours following the action of the stressor. This method can, then, be used for the further assessment of stress in pheasants instead of the traditional invasive methods. From the viewpoint of animal welfare, it would be appropriate for future research to focus on the role played by age in transported pheasants. Pheasants are commonly transported at an age of between 7 and 21 weeks. We studied pheasants at the age of 17 weeks for the purposes of this study. It would be appropriate for the sake of completeness to assess the response to transport stress in other age categories and to recommend the appropriate period for their transport to their final destination on the basis of the results determined in order to reduce transport stress as much as possible. The results of previous studies indicate that individuals are more sensitive to the stress action of various factors, such as capture, placement in transport crates and transport itself, with increasing age [27]. Similar results have also been published in common pheasants. The level of plasma corticosterone in birds aged 8 and 16 weeks was determined, with older pheasants showing a significantly higher corticosterone level than the younger birds [28].

Methods

Monitoring was performed before and after transport at a pheasant rearing facility. Subjects of the study were pheasants owned by the owner of the facility. Sixteen individuals (eight of each sex) were selected at random for the purposes of the study and placed individually in cages for a period of two weeks before transport. All the birds were, however, in visual, vocal and olfactory contact with each other for the entire

observation period. Each individual was also fitted with a different coloured ring on the leg for the purposes of differentiation. Food and water were provided *ad libitum* for the entire period. The pheasants were loaded into transport crates at the age of 17 weeks and transported for a period of 3.5 hours over a distance of 140 km to the final customer. Samples of droppings were collected over a period of 2 days before (for the determination of basal concentrations) and 3 days after transport. Plastic trays were installed beneath the cages, from which droppings were simply collected, for the purposes of problem-free collection. A total of 464 samples were collected (29 samples from 16 birds). All the samples were collected within an hour of defecation and placed in sealable plastic bags, before being labelled and frozen at -20 °C. Processing of the samples and analysis of glucocorticoid hormones in the droppings were performed at the University of Veterinary Medicine in Vienna, Austria. The samples were defrosted, individually homogenised and then weighed into test tubes at a weight of 0.480–0.520 g. An amount of 5 ml of 60% methanol was added to the weighed samples. Each sample with extraction agent was then mixed for 30 minutes on a shaker and then centrifuged at 4 °C (3,750 g, 10 minutes). Concentrations of CM in the droppings were determined using a non-commercial EIA test developed at the laboratory. Cortisone (4-pregnene-17 α ,21-diol-3,11,20-trione) was used as a standard and the values were expressed as equivalent cortisone in ng per g of droppings.

The mean CM value for each day was calculated for the individual birds. The mean levels of CM were then calculated separately for males and females, which were compared with one another with the use of the statistical program Unistat 5.6. (Unistat Ltd., London, England). As the CM concentrations did not differ significantly between males and females, combined data from both sexes were used for further analysis. The normality of the data was tested with the use of the Shapiro-Wilk test and, in view of the fact that the data had a Gaussian distribution, the statistical significance of differences in the levels of CM concentrations was determined by means of a parametric ANOVA test. The Tukey-HSD test was subsequently used for determination of significant differences in CM concentrations in the droppings between all the sampling days. The value of $P < 0.05$ was considered statistically significant.

Abbreviations

CM

corticosterone metabolite

EIA

enzyme immunoassay

HPLC

high-performance liquid chromatography

ACTH

adrenocorticotrophic hormone

ANOVA

analysis of variance

Declarations

Ethics approval and consent to participate

This study was carried out in strict accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and Czech national legislation, i.e. Act no. 246/1992 Coll., on the protection of animals against cruelty, as amended. All samples were collected non-invasively and animals did not undergo any experimental procedures. Since no handling of animals related to research was carried out, a formal ethics approval of the Animal Welfare Body of the University of Veterinary and Pharmaceutical Sciences Brno having regard to the EU Directive 2010/63/EU was not required. Written informed consent to use the animals in the study was obtained from the owner of the animals.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

Funding

This study was supported by the Internal Creative Agency of the University of Veterinary and Pharmaceutical Sciences Brno (Project No. FVHE/Vecerek/ITA2020).

Authors' contributions

MV, ZM, and PF carried out the experiment and oversaw data curation and analysis. MV drafted the manuscript. IB and EV assisted with experimental design and methodology. EV and VV edited the manuscript. All authors approved the final manuscript.

Acknowledgements

We wish to acknowledge the advice of Prof. Rupert Palme, of the University of Veterinary Medicine, Vienna. Furthermore, we would like to thank Edith Klobetz Rassam for technical assistance with the EIA analysis.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Mean concentrations of corticosterone metabolites (CMs) in common pheasants measured before and after transport.

Sampling days 1-6 (the arrow indicates the transport day - D3)

Figure 2

Mean concentrations of corticosterone metabolites (CMs) in female (F) and male (M) common pheasants before and after transport.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.pdf](#)