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Level of welfare of male farmed fallow deer kept indoors during winter

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Summary

Farmed cervids are not fully domesticated animals, usually kept on large open pastures. However, in intensive breeding farms, animals are often kept indoors during the winter in order to obtain desirable weight gain or avoid deaths. The aim of the study was to assess the stress level in farmed fallow deer (*Dama dama*) kept indoors during the winter with a changed photoperiod, as well as to analyze the effect of the cortisol level on the animals depending on their age and body mass. Two groups of adult fallow deer males, 15 individuals each, with an average age of 5.9 years, were used in the study. The experimental group was kept in a utility room with a changed photoperiod and a temporary access to an enclosure, while the control group spent the entire time in an outdoor enclosure. Blood samples were collected from all animals three times, at monthly intervals, to determine the cortisol concentration. No differences were found between the study groups. The average cortisol level ranged from 2.86 to 3.92 µg/100 ml in the experimental group and from 3.13 to 3.46 µg/100 ml in the control group. The average cortisol concentration in older stags was similar to that in younger males (3.48 µg/100 ml and 3.42 µg/100 ml, respectively). Simple manipulation procedures do not affect the welfare of farmed fallow deer. Cervids tolerate natural winter conditions well both on the run and in utility rooms with appropriate space.

Keywords: ***Dama dama*, welfare, stress hormone, wintering conditions, utility room**

The homeostasis of the animal may be disturbed by the occurrence of short-term or long-term stress. The former is usually a quick response to an acting stimulus, e.g. hunting, road accident or presence of large predators. Long-term stress may be due to climate change, overpopulation of animals in a given area, human activity, such as tourism or sports, or changes in the body's behavior and physiology, e.g. during the mating season or antler growth (11, 12, 19, 28, 42, 46). Because of the relatively short period of farm use, cervids are still not considered as fully domesticated animals (13, 30). Ensuring their welfare on the farm requires specific breeding activities that gradually modify their natural behavior. This applies primarily to the way animals are kept, handling procedures, and technical equipment on farms. An important factor determining the type of activities undertaken is the significant variability in the behavior of deer related to their highly seasonal reproductive period. This is especially true of males, whose behavior is greatly modified by the testosterone level rising rapidly in that period. After the mating season ends, in late autumn, it is necessary to perform a number of zootechnical and veterinary procedures, involving the separation of stags, hinds and fawns from the herd into separate groups, so as to prevent competition for food between individuals with different positions in the herd hierarchy. Slowing down the metabolic rate in winter and limiting the volume of feed consumed make uninterrupted access to food extremely important for animals to survive the winter well. It is at that time that antlers, which are already dead tissue, are cut. This procedure is extremely important to ensure the safety of both animals and staff. At the same time, necessary veterinary treatments are performed, such as antiparasitic prophylaxis or mineral and vitamin supplementation. Some zootechnical and veterinary treatments are repeated in spring, before releasing the animals to pastures (8, 26, 33).

Effective animal management on a deer farm also includes efficient and safe movement of groups of deer between summer pens and the selection of stags for breeding. Manipulation of animals on the farm throughout the year requires a number of activities that put them in close contact with the staff, which creates potentially stressful situations. Observations indicate that even manipulations performed calmly can be a stress factor with a significant impact on the body (51). Stress can affect the functioning of the body, including metabolic processes. This may be reflected in the results of fawn rearing, body weight gains and, ultimately, the final carcass mass (3, 12). On farms where maximizing the weight of stags' antlers is an important goal, stress that reduces the efficiency of metabolism may also reduce their mass (11, 45). One of the methods of regulating antler growth is changing the photoperiod, which affects the hormonal balance of males (2, 24, 35, 43, 45). According to Goss (17), the acceleration of antler cycles can be induced by increasing the frequency of annual daylight cycles and obtaining a maximum of four full antler growth cycles in 12 months. This, however, may be accompanied by disturbances in the welfare of deer. Stress caused by improper handling of animals may affect the economic results of the farm. It is, therefore, extremely important to develop methods for an objective assessment of procedures aimed at ensuring animal welfare. Cortisol (CORT) is a commonly used indicator of stress levels (25, 29, 37). Gentsch et al. (16) clearly indicate that negative factors may accumulate and increase CORT release, which may determine the strength of the endocrine response. The concentration of CORT in hair, faeces, urine, saliva and blood is often measured to assess the level of stress in animals (7). The level of CORT in blood is widely used to monitor stress reactions, while the level of CORT in excretions (faeces, urine) is more useful as an indicator of chronic, long-term stress reactions. The obvious advantage of measuring the level of glucocorticoids in faeces is the possibility to collect samples without the need to catch animals, which eliminates unnecessary stress associated with immobilizing them or puncturing veins to collect blood. This method, however, despite its advantages, also has some limitations, as it is often impossible to attribute faeces to specific individuals, especially those living in large areas. In red deer, peak levels of corticosterone metabolites in fecal samples are typically observed approximately 18 hours after exposure to stress (22). CORT can also be determined from animal hair samples as an indicator of stress conditions. However, it should be remembered that the level of glucocorticoid metabolites in hair increases fairly slowly, i.e. over several weeks or months, depending on the species (5, 15, 22, 31, 38, 51). Therefore, due to the above limitations, in farmed deer, the best method seems to be the sampling of blood during routine immobilization, in which the CORT level reflects the intensity of stress and its variability over time (2, 9, 21, 34, 36).

A decrease in the level of welfare is noted by an increase in CORT concentration, which may have a negative effect. The glucocorticosteroids have a broad spectrum of action, influencing the carbohydrate, protein, fat, water-electrolyte and calcium-phosphate metabolism of organisms. Additionally, these hormones act on the immune and hematopoietic systems (32). Maintaining an appropriate level of welfare of farm animals is becoming increasingly important in modern animal production. One way to achieve this objective is avoiding chronic stress. In accordance with regulations in force in Poland, deer on farms are generally kept in an open system – without the use of utility rooms – although it is permissible to keep deer and fallow deer indoors outside the grazing season (13, 30). However, so far the impact of keeping deer in rooms of appropriate size with an altered photoperiod on their welfare has not been demonstrated. A consequence of keeping animals indoors is also a reduction in space, compared to groups kept in open pastures. This factor can also have a stressful effect. Therefore, the aim of the study was to assess the impact of periodically keeping male farm fallow deer (*Dama dama*) in a utility room with a changed photoperiod on their welfare, as well as to analyze the cortisol level depending on the age and body mass of the animals.

Material and methods

Experimental design. The research was carried out at the Research Station of the Institute of Parasitology, Polish Academy of Sciences, Kosewo Górne (Poland; N: 53°48'; E: 21°23'). The research included 30 male farmed fallow deer (*Dama dama*) divided into two groups of equal size ($n = 15$), receiving the same food and having different daily photoperiods during the winter months (from December 15, 2021 to April 30, 2022). Each control and experimental group consisted of eight older stags aged 6-8 year and seven younger ones aged 4-5 years.

The experimental group was housed in a utility room with an area of 103 m^2. In the case of fallow deer over 1 year of age, the area per individual must be at least 1.5 m^2 (1). The room used in this research provided each animal with an area more than 4 times as large, that is approximately 6.9 m^2 per animal. A total area of windows was 8 m^2. A hay feeder, concentrate feeding troughs and water containers were also installed inside. Lighting was provided by 18 electric lamps with LED bulbs having a nominal power of 20 W and a declared luminous flux of 1850 lumens. The light was cold white (color temperature 6000 K). The light intensity in the shelter and outside was measured with an Abatronic AB-8809A luxometer. Previous studies have shown that the light intensity obtained in this way is sufficient to simulate natural conditions (45). The lighting in the room was turned on and off automatically by an OR-PRE-433 controller (Orno-Logistic, Poland). The length of the lighting period was adjusted to the current length of the light day in first year research described by Tajchman et al. (45).

The control group (CG) was kept under natural daylight conditions without additional lighting and used a pen with an area of 2,950 m^2 24 hours a day.

The troughs and water containers were cleaned and concentrate feed and water were provided while the animals were in the enclosure in order to reduce stress resulting from close contact with the staff. The animals from the control group were fed with concentrate feed without the staff entering the pen, i.e. the troughs were filled from feeding corridors. Nutrition was identical in both groups. They were fed in the standard way on the farm, i.e. using roughage produced in areas belonging to the station, as described in detail by Tajchman et al. (47). Grass silage or hay was used *ad libitum*. Hay silage was served mainly during periods of negative air temperatures because, due to the freezing of water in watering containers, it was necessary to provide feed with increased moisture compared to the usually used hay. Because of the shorter access time to the feeder located in the enclosure, animals from the groups locked in the experimental room at night had access to hay in the feeder inside. All groups of animals always received feed from the same current batch in order to eliminate the influence of potential differences in the type or quality of feed. Mineral supplements were administered in the form of ready-made Josera Phosphor mineralizer licks in buckets placed in such a way that the animals had access to them 24 hours a day (47). Therefore, the consumption of mineral supplements (*ad libitum*) took place regardless of the intake of concentrate feed.

The animals were marked using subcutaneous microchips compliant with the ISO 11784/11785 standard and the FDX veterinary standard (MPMS Electronics reader, Poland). To make it easier to identify the animals in the monitoring recordings, they had ear tags with different markings.

Body weight was measured with a set of MP 800 sensors coupled with a Tru-Test DR 3000 weight reader LNB (Tru-Test Group, Auckland, New Zealand). As declared by the manufacturer, the accuracy of this set was ± 1% and the minimum resolution was 100 g.

Monitoring of the animals. In order to assess the animals' unusual behavior in the rooms, 24-hour monitoring was carried out. The rooms are equipped with three LBM24AD200FE cameras, also working in infrared, and an XVRDA2004D recorder. The cameras covered the entire area of the utility room. They were equipped with a motion detection sensor, which significantly reduced the time of recorded events and, consequently, improved the reading of data stored on the recorder's disk. The period directly related to animal feeding was analyzed in detail, and 2-3 minute sequences were selected for analysis every 30 minutes of the animals' stay in the room. Particular attention was paid to the possible occurrence in animals of antagonistic behavior or other types of aggressive situations, as well as the position adopted by the fallow deer (standing, lying, moving). Analysis were also paid to whether the animals were in groups or individually.

Sampling. Blood samples were collected during the experiment to determine CORT levels. The determinations were performed three times, at monthly intervals (1^{st} – January 20, 2^{nd} – February 20 and 3^{rd} – March 20, 2022). The samples were collected while the fallow deer were standing inside a small handling box (2 m × 2 m × 0.6 m) described by Tajchman et al. (45) with no need for sedation. Blood samples were collected from vena jugularis externa always at the same time (1 to 3 h after dawn) to avoid variations associated with circadian rhythms as in research by Gáspar-López et al. (15). Blood was collected into 5 ml tubes without anticoagulant for the so-called clot (BD Vacutainer System, Ref. No. 367525, Becton Dickinson, Poland, Warsaw). The samples were set aside for 30 minutes, and then serum was obtained by centrifugation of whole blood at 3000 rpm for 10 min in a laboratory centrifuge MPW350R (MPW Medical Instruments, Warsaw, Poland) and cooled at a temperature of 4°C. CORT concentration in serum was measured using an Atellica IM Analyzer (Siemens Healthineers, Germany) according to the manufacturer's protocols.

The experiment was performed with consent from the Local Ethics Committee for Animal Experiments in Olsztyn (Regulations of the Animal Welfare Committee, University of Warmia and Mazury resolution no. 24/2021).

Statistical analysis. The normality of distribution was tested using the Kolmogorov-Smirnov test. The statistical analysis used GLM and CORR procedures of the SAS software (Statistical Analysis System, 9.4, 2013). A 3-factor analysis of variance was performed (factors: animal age – 2 levels, group – 2 levels and subsequent measurement – 3 levels) along with the interaction of all factors. In analyses of variance, Tukey's post-hoc test was used with a P-value ≤ 0.05. Additionally, basic age and body mass characteristics of the groups of animals were presented.

Results and discussion

Cortisol level analysis. The fallow deer had relatively similar body mass ranging from 79.0 to 86.0 kg. The interaction of group, age and sampling time was not significant (Tab. 1). The interactions of group and age (Pr. > F = 0.1212), group and sampling time (Pr. > F = 0.5074), and age and sampling time (Pr. > F = 0.9528) were also insignificant and are not presented in the table. As a consequence of the lack of interaction, an analysis of the influence of the main factors was carried out. The influence of the group and age of the animals was insignificant, but the CORT level was significantly influenced by the time of sampling. Significantly the highest CORT concentration was recorded during the second sampling, and significantly the lowest CORT level was recorded during the first sampling. The CORT concentration during the third sampling did not differ significantly from those during the first and second samplings.

Tab. 1. Relationship between group, age, and sampling time effect in farmed fallow deer ($\bar{x} \pm$ se – means ± standard error)

Group	Age	Subsequent measurement	Effect: group*age*sampling time $\bar{x} \pm$ se	Group effect $\bar{x} \pm$ se	Age effect $\bar{x} \pm$ se	Sampling time $\bar{x} \pm$ se
EG	older	1	2.69 ± 0.34	3.41 ± 0.15		
		2	3.83 ± 0.58			
		3	3.26 ± 0.43			
	younger	1	3.05 ± 0.12			
		2	4.01 ± 0.24			
		3	3.58 ± 0.11			
CG	older	1	3.56 ± 0.40	3.49 ± 0.15		
		2	3.88 ± 0.50			
		3	3.63 ± 0.36			
	younger	1	2.98 ± 0.23			
		2	3.52 ± 0.42			
		3	3.39 ± 0.26			
	older				3.48 ± 0.15	
	younger				3.42 ± 0.16	
		1				$3.07^{b} \pm 0.18$
		2				$3.81^{a} \pm 0.18$
		3				$3.47^{ab} \pm 0.18$
Pr. > F			0.9160	0.6852	0.7983	0.0239

Explanations: a, b – means in the columns differ significantly at $p \leq 0.05$ (Tukey test); EG – experimental group; CG – control group

Monitoring analysis. The analysis of the recordings revealed no behaviors that were clearly different from those of other animals in the group. Particular attention was paid to the behavior of the fallow deer when they were provided with concentrated feed. It was found that from the day they were placed in the room until the end of the study, the male fallow deer showed no aggressive or antagonistic behavior related to food intake. Thus it was excluded that the animals might push each other away from troughs with concentrated feed, which could prevent some of them from taking food and negatively affect their welfare. The analysis of the recordings also showed that males placed in the utility room formed groups of 3-8 individuals throughout the entire period of the experiments. The composition of these groups changed, but no individual(s) were observed remaining outside a group or being driven away from a group. It should be noted, however, that separate groups were formed only approximately 7-10 days after the animals were introduced to the experimental room. In the first week, the animals stayed in one group consisting of all experimental animals. During that time, male fallow deer usually lay or stood in the corner of the building, and their activity in the room was limited to taking water and feed and leaving and entering the enclosure. Such limited physical activity and close grouping were probably a stress reaction to being locked up and isolated from the rest of the animals in neighboring enclosures. Moreover, it was found that the animals became used to going outside very quickly, already on the 3rd or 4th day.

During the observation period, male fallow deer living in specialized farm rooms did not show any behavior or interactions between them that could negatively affect their welfare.

The reaction of the organism to a stress factor, regardless of its type, is a hormonal response. During stress, various hormonal reactions necessary to improve the body's performance are initiated. Although the hormonal mechanism in response to stress is common to most mammals, there are differences in the level of hormonal response between individual species (8). The type of the stress factor may influence the strength of this response measured by the CORT level (16). In animals harvested by stalking hunts, the average CORT concentrations were similar in stags and hinds of wild red deer (*Cervus elaphus*) (20.2 and 21.5 ng/ml, respectively) (12), slightly higher (27.694 ng/ml) in female roe deer (*Capreolus capreolus*) and almost three times as high (58.066 ng/ml) in males (10). These values differ from those obtained in our study. It is difficult, however, to relate them directly to other species, such as fallow deer living on farms, which are kept differently and were tested at a different time of the year.

Observations of the effects of moving red deer to another pen lead to the conclusion that the resulting increase in the CORT level is rapidly reduced (18). The authors of the cited studies conclude that this type of manipulation does not significantly impair the required animal welfare, which is also confirmed by research conducted on farmed fallow deer.

Studies performed on a group of 8-year-old fallow deer showed that the average CORT level was 1-2 μg/100 ml at rest and reached a maximum of approximately 8 μg/100 ml 60 minutes after administration of adrenocorticotropic hormone at a dose of 10 or 40 IU to induce a hormonal reaction imitating stress (41). At the same time, strong inter-individual differences in the CORT level were detected. In studies conducted on fallow deer kept in a zoo, its level in males after chemical immobilization was similar and ranged from 0.83 to 1.32 μg/dl (14). In our studies, similarly to those cited above, the CORT level varied significantly between individuals. It ranged from 1.62 to 5.55 in the first sampling, from 1.81 to 7.26 in the second sampling, and from 1.72 to 5.86 μg/100 ml in the third. It should be noted that the highest maximum results were found in the second sampling, and in the third they returned to the level from the first sampling. These results suggest that the daily routine of locking the animals in a utility room at the same time of day and simultaneously administering concentrate, after an initial slight increase in stress levels, quickly led the animals to form positive associations and adapt to the new housing conditions. The results of this study showed a tendency for the serum CORT concentration to decrease during the stimulus period from January to April. A decrease in CORT concentration after regular and frequent occurrence of a stressor is often a sign of acclimatization (14), as observed in some studies on Brahman cattle (*Bos taurus indicus*) and Magellanic penguins (*Spheniscus magellanicus*) (48, 51), as well as in cervids (20). Belgian researchers reached similar conclusions. Studies on service dogs regarding, among others, their response to the sound of a gunshot showed that regular training activities ceased to induce an increase in CORT concentration, which eventually stabilized (4, 6). According to these authors, the low level of this hormone at the beginning and end of the training indicated the animals' quick and good adaptation to the exercise environment.

The influence of changes in the social structure of the herd on the CORT level in male red deer has also been investigated (49). Observations were carried out on two groups of males in the period from mid-April to the end of August, i.e. throughout the entire period of new antler formation until the beginning of the breeding season. It was found that the CORT concentration increased in the group of animals in which there was a change in the social structure during the study resulting from the introduction of new, younger animals. In the control group, in which the social structure was stable, there was no increase in CORT levels in the blood during the experiment. Similar results were obtained in the present study. It was also found that the appearance of younger stags in the herd resulted in an increased CORT concentration in older deer with a higher social position (40). This means that maintaining a stable social structure with an established hierarchy is an important stabilizing factor that reduces the level of stress. For deer males, this factor is likely to be more significant than others. This is particularly important under farm breeding conditions because animals are kept in utility rooms with a limited area, which prevents weaker or younger animals from escaping. In our research, no changes were made by introducing new individuals into particular groups. Under these conditions, even a relatively small area of the room proved to be sufficient for an appropriate level of welfare. In current farm practice, however, cases of a sudden increase in aggression towards stags that entered the area of a neighboring pen have been observed. Such situations occurred even though individuals from neighboring groups previously had close contact with each other because the neighboring pens were separated only by a wire mesh fence. A bull's entering a neighboring pen was apparently treated as a violation of the territorial boundary. Research conducted on wild animals has shown that young age and lack of life experience, including the stage of learning needed skills in adult life by young animals, influence the level of CORT and the position in the herd (49). The results of our study did not confirm the occurrence of such a phenomenon in fallow deer. Statistical analysis did not reveal any significant differences in CORT concentration depending on age or body weight. Previous studies have not confirmed the relationship between the CORT level and the age of cervids (3, 27, 40). Previous studies on farmed fallow deer (*Dama dama*) have shown that older males have higher levels of this hormone in the blood, which is generally known that CORT increases with age, however in cervids it is accompanied by large changes in CORT levels between the analyzed periods and lower body weight gain (44), which is confirmed by studies on wild red deer (10). However should be emphasized, that in studies on wild red deer it was shown that an increased CORT concentration resulted in a decrease in carcass mass, which may lead to a deterioration of the animals' physical condition (12). However, the special characteristics of the species and the environment in which a given animal lives may affect the level of CORT and thus the strength of the hormonal response (16, 23), which may explain the discrepancies in results obtained by other authors (10, 50).

In summary, the use of correct procedures for dealing with fallow deer on farms makes it possible to ensure their welfare. Based on both the analysis of monitoring records and the comparison of cortisol levels in blood serum, it can be concluded that keeping fallow deer stags in groups in closed rooms (meeting the appropriate requirements) with temporary access to an enclosure did not increase the level of stress or the frequency of antagonistic behavior. The level of stress hormone had no effect on the body mass of male farm fallow deer and did not depend on their age.

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