ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Review Derleme DOI: 10.17094/vetsci.1471401

Measuring Stress in Animals By Noninvasive Methods

Hayvanlarda Stresin Noninvasiv Metotlarla Ölçülmesi

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ABSTRACT

Stress is a biological response in the form of physiological, biochemical, hematological, and behavioral changes to internal or external stimuli that threaten the homeostasis of living beings. Effects that activate the defense system in living things are defined as stressors, and it is possible to talk about many different stress factors. Factors that cause stress can be divided into environmental, physical, social or emotional. Stress reactions, which begin with the effect of the stressor, vary according to the duration and severity of exposure to stress. In animal welfare, stress has many negative effects on organism. These negative effects may cause many problems and, shape future by adding problems such as stress and chain links in animals. Just as well-being is for humans, well-being is very important for animals. To determination of glucocorticoids or metabolites in the bloodstream of an organism under stress, noninvasive methods that provide reliable stress measurement without interfering with the organism have recently become increasingly popular. In this review article study, nineteen articles from various parts of the world were examined. In this review, measures of stress by non-invasive methods by looking at GlucoCorticoid Metabolites (GCM) and the latest developments in this field are discussed. In this review article study, nineteen articles from various parts of the world were examined. As a result of the articles reviewed, non-invasive methods for measuring stress may aid and improve our understanding of stress biology and animal welfare. Applying this method to many animal species and biological materials will provide accurate results and support animal welfare.

Keywords: Animal welfare, cortisol, glucocorticoid metabolites, non-invasive, stress.

ÖZ

Stres, canlıların homeostasisini tehdit eden iç veya dış uyarılara karşı, fizyolojik, biyokimyasal, hematolojik ve davranışsal değişiklikler şeklinde verilen biyolojik bir cevaptır. Canlılarda savunma sistemini harekete geçiren etkiler stresör olarak tanımlanmakta olup bir çok farklı stres faktörlerinden bahsetmek mümkündür. Strese neden olan faktörler ise çevresel, fiziksel, sosyal veya duygusal olarak ayrılabilir. Stresörün etkisiyle başlayan stres reaksiyonları, strese maruz kalma süresine ve şiddetine göre değişir. Hayvan refahında stresin organizma üzerinde birçok olumsuz etkisi bulunmaktadır. Bu olumsuz etkiler birçok soruna neden olabilmekte ve hayvanlarda stres, zincir halkaları gibi sorunları da ekleyerek geleceği şekillendirebilmektedir. İnsanlar için olduğu gibi hayvanlar için de refah çok önemlidir. Stres altındaki bir canlının kan dolaşımındaki glukokortikoidlerinin veya metabolitlerinin belirlenmesi için canlıya müdahale etmeden, güvenilir bir şekilde stres ölçümü sunan noninvazif yöntemler son zamanlarda giderek daha popüler bir hale gelmiştir. Bu derlemede stresin noninvasiv metotlarla GlikoCorticoid Metabolitleri (GCM) bakılarak yapılan ölçümleri ve bu alandaki son gelişmeler ele alınmıştır. Yapılan bu derleme makalesi çalışmasında dünyanın çeşitli yerlerinden on dokuz makale ele alınarak incelenmiştir. İncelenen makaleler sonucunda stresi ölçmeye yönelik invazif olmayan yöntemler, stres biyolojisi ve hayvan refahı konusundaki anlayışımıza yardımcı olabilir ve bunu geliştirebilir. Bu yöntemin birçok hayvan türü ve biyolojik materyalde uygulanması hem doğru sonuçlar verecek hem de hayvan refahını destekleyecektir.

Anahtar Kelimeler: Glikokortikoid metabolitleri, hayvan refahı, kortizol, non-invaziv, stres.

Received/Geliş Tarihi: 04.07.2023
Accepted/Kabul Tarihi: 25.01.2024
Publication Date/Yayın Tarihi:26.04.2024

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Cite this article: Serim Balcı E, Sabuncuoğlu Çoban N. Measuring stress by noninvasive methods. *Vet Sci Pract*. 2024; 19(1):52-58.

Atıf: Serim Balcı E, Sabuncuoğlu Çoban N. Stresin noninvasiv metotlarla ölçülmesi. *Vet Sci Pract*. 2024;19(1):52-58.

INTRODUCTION

Stress is a biological response in the form of anatomical, physiological, and behavioral changes to internal or external stimuli that threaten the homeostasis of living things.[1] In animal husbandry, stress is the sum of the organism's responses to unsuitable environmental conditions, which can lead to many undesirable consequences, from discomfort to death.[2] Stress; it can be determined by health, yield, behavior, and physiological parameters.[3]

In animal welfare, stress has many negative effects on organism. These negative effects may cause many problems and, shape future by adding problems such as stress and chain links in animals. Just as well-being is for humans, well-being is very important for animals.

The definition of animal welfare has been defined as "If an animal is healthy, comfortable, well fed, safe, can exhibit behavior patterns specific to its species, if it is not afraid, painless, and not under stress, that animal is well-off."[4]

Welfare refers to the long-term wellness of an animal, which is the result of its acquired experiences of the living conditions that it copes with. It is very important to optimize physical and climatic environmental conditions to keep health, welfare and production of the livestock.[5,6]

There has been an increased interest in animal welfare recently, but assessing animal welfare is both a difficult and complex subject. The definition of well-being is more than the absence of stress, but stress also plays an important role in good well-being. Stressors are stimuli that cause an imbalance of homeostasis. An animal's defense responses to stimuli, on the other hand, are defined as stress responses. The brain, on the other hand, has a central role in linking stressors to stress responses.[7]

In order to overcome stressful situations, glucocorticoids secreted from the adrenal glands are at the forefront of the war. They are usually measured in plasma samples as parameters of adrenal activity, but the collection of blood samples is uncomfortable and further stresses the animal. Therefore, non-invasive methods for the determination of glucocorticoids or their metabolites are better for animal welfare and stress.[7]

Stress Factors

The effects that activate the defense system in living things are defined as stressors. In this sense, it is possible to talk about quite different stress factors. Factors causing stress can be environmental, physical, social, or emotional.[8]

Extremely hot and cold conditions, precipitation, toxins in feed, poor care conditions, uncomfortable bedding, use of inappropriate litter, errors during transportation, some infections and chemicals, hunger, thirst, fear, noise, injury, diseases, social interactions between species or animals, frequency of settlement, social status, psychological stress, trauma, new or foreign environment, social isolation, punishment, problems in human-animal relations, alarm vocalizations, social disturbances and handling, social isolation, etc. are stressors.[1,8,9]

Effect of Stress

Stress in animals changes physiological (respiration, pulse, blood pressure, body temperature, heart rate), biochemical (hormone, enzyme, electrolyte levels, fatty acids, cortisol), hematological (heterophil, lymphocyte, hematocrit values, and rates), and behavioral (aggression, restlessness, nervousness, feed, and water consumption) parameters.[1]

Stress Response

Stress reactions begin with the influence of the stressor. This period is defined as the alarm phase. It continues with the adaptation phase against stress. If the adaptation phase is successfully passed, the creature returns to normal life. If the stressor cannot be dealt with, the organism enters the exhaustion phase.[8]

Changes in biological activities during stress are defined as the biological cost of stress. The organism has limited energy resources. If the biological cost is not at a level that will affect the resources necessary for the continuation of physiological functions, the animal is not at risk. However, if the stress is severe and long-lasting enough to consume the necessary resources for physiological functions, it results in a pathophysiological condition.[8]

Once the central nervous system perceives a threat, it devolops a biological responce or defence that consists of a combination of the four general biological defnece responses: the behavioural responce, the autonomic nervous system responses, the neuroendecorine system response or the immune response (Figure 1).[10]

Stress factors reveal neural and hormonal activities. Exposed stimuli affect the hypothalamus and turn into a neural hormonal factor. Corticotropin releasing hormone (CRF) secreted from the hypothalamus stimulates the anterior pituitary and releases adrenocorticotropic hormone (ACTH).

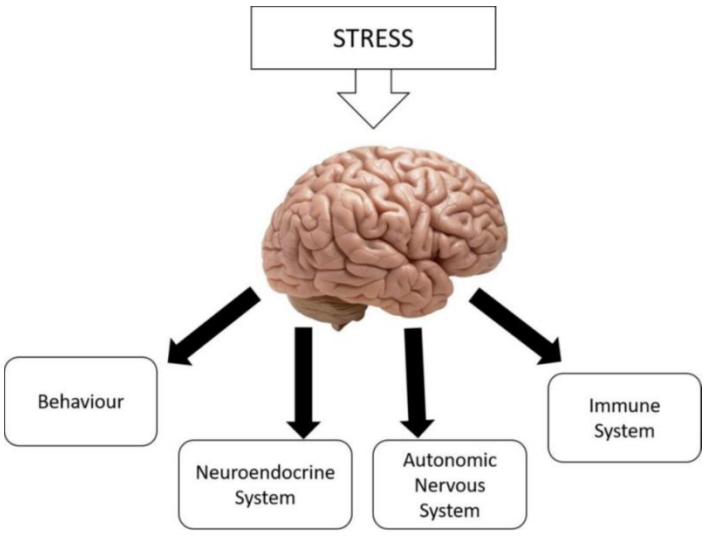

Figure 1. The general types of biological responses available to the animal for coping with stress.[10]

ACTH comes to the adrenal glands through the bloodstream and further increases the secretion of glucocorticoids. As a result of interaction with the stressor for a certain period of time, the first response in the organism is usually to fight. The body prefers to fight first rather than adapting to the stressor. This chain of events is called the fight-or-flight mechanism.[11]

While the sudden release of adrenaline or noradrenaline from the adrenal medulla continues this chain of events, an increase in energy production is inevitable. In addition, more energy production is required for the series of events in the nervous system. For this reason, neurogenic amines activate the hepatic adenylcyclase enzyme, which is necessary and effective in energy reactions, enabling the conversion of glycogen to glucose in the liver.[11]

In the alarm circuit, which starts with the effect of the stressor, hypochloremia occurs and blood density increases. Adrenaline secreted from the adrenal medulla and noradrenaline released from sympathetic nerve endings increase pulse, blood pressure and respiratory rate.[12,13]

In cases where the effect of the stressor is very long and continuous, it reduces the animal's relationship with the environment and even suspends its reproductive functions in order to maintain homeostasis and meet the increased metabolic needs. This passive and vulnerable response is called “protection or withdrawal” or “adaptation”.[14] At this stage, while the release of corticoids from the adrenal cortex becomes active; activated corticoids increase the metabolic effects of catecholamines and prolong the duration of action.[15-17] After the release of ACTH from the anterior pituitary lobe, the production of corticosterone increases, while the thymus, spleen, and peripheral lymph nodes shrink. At the same time, the pituitary lobe enlarges and the weight of the adrenal glands increases. While the number of lymphocytes in the circulation decreases, the number of heterophiles increases.[11] Glucocorticoids that occur in the adrenal cortex tissue and pass into the adrenal medulla regulate the conversion of noradrenaline to adrenaline by activating the phenyletolamine N methyl transferase enzyme.[12] Continuous stimulation of the adrenal cortex causes corticosteroids to remain in the circulation in high concentration and continuously.

Cardiovascular and gastrointestinal diseases, hypercholesterolemia, metabolic disorders, and changes in immunological functions may occur in animals. As a result of these, inflammatory events are suppressed, defense reactions related to lymphocytes are slowed down and antibody production is prevented. The magnitude of the effect on the immune system is greatly influenced by genetic factors and nutrition.[10] If the effect of the stressor continues for a long time and in this case, the defense mechanisms are insufficient, the "exhaustion" in the body of the animal. Then pathological changes may occur as a result of adrenal insufficiency and death comes at the end.[18,19]

Stress causes some rapid and temporary changes in the body then permanent and irreversible changes happen as a result of the continuation of stress. In addition, the growth, yield and reproduction of the sick animal comes to a standstill and the animal struggles to protect its health. Allostasis is the body's process of preventing problems that may occur against stimuli and maintaining homeostasis. In order to be successful in this process, both general and specific physiological systems and behavioral coping mechanisms are activated to a large extent.[20]

Why is Stress Important?

Stress affects animal growth rate, offspring mortality, disease resistance, meat and milk production, and reproductive ability by reducing the animals' ability to cope with the environment. Animals under stress get sick more easily, as a result, more drugs are used, so drug residues in animal products increase and this threatens public health.[1]

Stress factors such as transport and pre-slaughter conditions cause a decrease in muscle glycogen level and an increase in meat pH. Thus, dark, hard, dry meat and economic losses take place.[21] Animal welfare standards ensure animal health. Only healthy products are obtained from healthy animals.

Why is Stress Measured?

The glucocorticoid (cortisol) secreted by the adrenal glands is the frontline of the battle for both overcoming and measuring the stress. Cortisol levels can provide information about a stressed organism and can help to protect the animal from stressors and manage the environmental issues.

Cortisol, which is frequently used as a stress indicator in animal behavior researchs, is a defense hormone that protects the organism against any changes in physiological balances by affecting electrolyte, carbohydrate, protein, and lipid metabolism. Cortisol is a two-carbon steroid glucocorticoid found in plasma-bound to protein.[22]

Cortisol is commonly measured in plasma, saliva, feces, eggs, hair, and feathers. The welfare level of animals can be increased by environmental improvement with the information obtained from the animal.

How is Stress Measured?

Stress can be measured by invasive and non-invasive methods. Many of the invasive techniques can induce a stress response and this may affect the results. Recently, non-invasive methods have become increasingly popular for the determination of glucocorticoids themselves or their metabolites in the stressed animal. They offer a reliable measurement of stress without interfering with the animal.

Some of the studies, searching GCM (GlycoCorticoidMetabolites) usage potential in stress animals by non-invasive methods are summarized as folllows:

In a study conducted in New Zealand, fecal GCM was measured after dairy cattle exposed to stress factors (transport and adaptation to a new an environment). GCM was found to be significantly higher in the animal exposed to the transport than the control group ones; and peaked two days after they were moved to the new environment.[23] In Canadian deer and wolves, the fecal GCM values were significantly increased after sledding activity on the snow.[24]

The fecal GCM of the rabbits which smelt fox feces was found to be significantly higher than the control group and males had more GCM levels than females.[25]

In another study, red-footed partridges were caught, captived and exposed to various stress series. It was determined that the amount of GCM observed in feathers, was significantly correlated with plasma corticosteroid level.[26]

Laying hens were exposed to high environmental temperatures and moved to new cages, then GCM accumulation in egg albümin and plasma corticosterone levels were investigated. As a result of this study indicated that GCM levels in eggs were useful indicators of stress in laying hens.[27]

In a research performed in wild-living orangutans showed that, fecal GCM levels increased significantly after tourist visits when compared to the pre-visit.[28]

Fecal GCM levels were measured in captive bengal and sumatran tigers at Dreamworld Theme Park and Zoo in Australia. As a result of the study, no significant difference was found between bengal and sumatran tigers. However, the mean fecal GCM level was higher in females and sick animals than males and healthy ones, respectively.[29]

It was concluded that cats living in the crowd had significantly higher fecal GCM levels than those singles. Besides, older cats showed higher fecal GCM levels than youngers.[30]

In a study carried out in broilers which were exposed to heat stress for two hours, fecal GCM levels increased significantly when compared to control animals.[31]

Guinea pigs were intermittently brought together in a new social environment by applying regroubing. Stress levels were measured by looking at fecal and salivary GCM; then a significantly higher increase in fecal and salivary GCM levels was detected.[32]

A significant increase in both plasma cortisol and fecal GCM levels was observed during the inflammatory disease outbreak in Atlantic Salmon.[33]

In a previous study, male green lizards were exposed to hierarchy and regrouping stressors. Fecal GCM levels were found to be high in stressed groups.[34]

Fecal GCM levels of otters were investigated both in a man-made environment and natural environment. As a result; fecal GCM levels of otters which were living in a man-made environment were found to be significantly higher than the others.[35]

In a study conducted in African wild dogs, fecal GCM levels of captive dogs were found to be higher than those of free dogs.[36]

It was observed that hair GCM levels of the snowshoe rabbits increased when they encountered their predators, the lynx.[37]

Fecal GCM levels were investigated in polar bears before and after transportation of them to the zoos. The transportation process effected the levels of GCM significantly.[38]

In a study conducted on a farm in South America, cows were divided into 3 groups. The cows in the 1st group were constantly listened to classical music. The cows in the second group were listened to classical music for a limited time. The cows in the 3rd group were not allowed to listen to any music. Faecal glucocorticoid metabolites of cows were examined and milk yields were measured. As a result, it was observed that the cows in the 1st group that constantly listened to classical music had higher milk yield and lower fecal glucocorticoid metabolites than the others. These findings showed that auditory stimuli such as classical music have a positive effect on cow welfare and milk yield. [39]
In a study, it was seen that the fecal GCM levels of whales showed a significant positive correlation between ship traffic and underwater ambient noise levels. This suggests that noise generated by ship traffic may be a causal factor for the increased fecal GCM. [40]

In another study in which stress experienced by weaning foals was determined through fecal GCM, foals showed a marked stress response to the weaning process through increased fecal GCM levels. [41]

CONCLUSION

Stress is defined as the biological response to any effect that disrupts the internal balance of the organisms. Although the animals have mechanisms to cope with stress, the long exposure to stress puts them on irreversible paths. The stress response can be detected by abnormal behavioral pattens, also biochemical changes due to the extent of the stress. Aggression, loss of appetite, decrease in growth and reproductive activities together with biochemical hormone secretions can be measured objectively. Glucocorticoid (cortisol), one of the most important hormone among other stress hormones, increases when the animal faced a stress factor. This incline in cortisol level shows us that the animal is under stress. Invasive applications to measure the stress level can cause extra stress and increase cortisol levels. This makes it difficult for the researchers to make a healthy measure of stress. Using non-invasive tools to measure stress can help alleviate this problem. Noninvasive methods for measuring stress can help and improve our understanding of stress biology and animal welfare. In the scientific literature, there are limited studies on the measurement of glucocorticoid metabolites in plasma, saliva, feces, hair and/or feathers. The application of this method in many animal species and biological materials will not only present accurate results, but also support animal welfare by adapting the environment.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - N.S.Ç., E.S.B.; Design - E.S.B.; Supervision - N.S.Ç., E.S.B.; Resources - N.S.Ç., E.S.B.; Data Collection and/or Processing - E.S.B.; Analysis and/or Interpretation - N.S.Ç.; Literature Search - N.S.Ç., E.S.B.; Writing Manuscript - E.S.B.; Critical Review - N.S.Ç.

Declaration of Interests: The authors declare that there is no conflict of interest.

Funding: The authors declared that they received no financial support for this study.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - N.S.Ç., E.S.B.; Tasarım - E.S.B.; Denetleme - N.S.Ç., E.S.B.; Kaynaklar - N.S.Ç., E.S.B.; Veri Toplanması ve/veya İşlemesi - E.S.B.; Analiz ve/veya Yorum - N.S.Ç.; Literatür Taraması - N.S.Ç., E.S.B.; Yazıyı Yazan - E.S.B.; Eleştirel İnceleme - N.S.Ç.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar, bu çalışma için finansal destek almadığını beyan etmiştir.

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