# The impact of traumatic limb injuries resulting from operations related to transport for slaughter on biochemical indices in end-of-lay hens

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**Abstract**

The study focused on the welfare of end-of-lay hens during their transportation for slaughter from the viewpoint of the stress load on hens resulting from unsatisfactory treatment that leads to traumatic injury to the limbs of hens detected during the veterinary examination of hens at the slaughterhouse. Blood samples were taken during bleeding after slaughter at the slaughterhouse for the determination of corticosterone and other biochemical indices from 35 hens with traumatic limb injuries and 35 hens without traumatic limb injuries. The stress load during the transportation of hens for slaughter potentiated by traumatic injuries was found to increase ($P < 0.01$) the plasma corticosterone concentration as an indicator of stress in birds (6381 pg/ml vs. 3681 pg/ml) and affect the plasma concentration of some biochemical indices, in particular increasing ($P < 0.05$) the level of total protein and albumin and decreasing ($P < 0.05$) levels of triglycerides, calcium and alanine aminotransferase. These findings demonstrate that hens with traumatic limb injuries occurring during the course of transport (loading, transport, unloading) are burdened by a greater degree of stress than hens that have not suffered traumatic injuries. From the viewpoint of the protection and welfare of end-of-lay hens, this study unequivocally demonstrates the necessity of the corresponding careful handling of birds during their loading onto and unloading from means of transport during transportation to the slaughterhouse – the kind of handling that does not cause injuries and, thereby, an increased stress load on hens slaughtered at the slaughterhouse.

*Poultry, plasma corticosterone, stress, welfare, transport*

Operations occurring at the end of the laying cycle, namely catching, crating, and transport to the slaughterhouse can lead to injuries in laying hens. Due to their relative inactivity and an inherent high rate of laying, end-of-lay hens are likely to present a variety of skeletal problems resulting in increased bone fragility and susceptibility to fracture (Whitehead and Fleming 2000; Lay et al. 2011). Injuries may occur during removal from cages, when birds collide with cage equipment (feeders, perches, nest boxes) or the cage entrance. Collisions with pen furniture during hen catching have also been identified as a major source of fractures during the depopulation of aviary systems (Gerpe et al. 2021). Birds may also be injured when they are carried down the narrow aisles of a hen house (Weeks 2014). Another risk of injury is posed by crating during loading and removal from the crates during unloading at the slaughterhouse when hens can be injured while passing through the doors (Knowles and Wilkins 1998; Gerpe et al. 2021).

Gerpe et al. (2021) assessed the welfare of hens during depopulation on 15 farms and found approximately 8.1% of hens exhibiting skeletal injuries such as bone damage or dislocated joints that appeared to have resulted from depopulation. Gregory and Wilkins (1989) assessed the incidence of broken bones in end-of-lay hens at slaughter and found that 29% of the live birds had broken bones by the time they reached the waterbath stunner, with an average of 0.5 broken bones per bird. Večerek et al. (2019) and Večerková et al. (2019)

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compared patho-anatomic findings detected in poultry during postmortem slaughterhouse inspection and found traumatic findings to be more frequent in end-of-lay hens than in broiler chickens and turkeys. Similarly, Válková et al. (2021) reported the greatest incidence of traumatic injuries in end-of-lay hens (2.80%) in comparison with broiler chickens, turkeys, geese, ducks and rabbits. When comparing the location of traumatic injuries in end-of-lay hens, they found traumatic injuries to the limbs significantly more often than traumatic injuries to the trunk (2.79% and 0.01%, respectively).

Depopulation itself is extremely stressful for birds that are unaccustomed to being handled in commercial systems. Injured hens are likely to experience increased stress and discomfort due to pain until they are killed. The aim of this study was to determine whether traumatic limb injuries in end-of-lay hens resulting from operations related to their transportation for slaughter cause a greater level of stress in hens expressed in changes in the concentration of corticosterone in the blood plasma than the kind of handling of hens that does not itself lead to traumatic limb injuries. The study also aimed to determine whether traumatic limb injuries in end-of-lay hens cause internal changes in hens reflected in changes in the concentration of biochemical indices measured in the blood plasma.

### Materials and Methods

The study was conducted on end-of-lay hens transported in the early morning hours from a farm to a slaughterhouse 50 km away. Hens with traumatic damage to the limbs were differentiated from hens without traumatic limb injuries during examination at the slaughterhouse. Blood was taken for the determination of corticosterone and other biochemical indices during bleeding after the slaughter of the hens at the slaughterhouse from 35 laying hens with traumatic limb injuries and 35 laying hens without traumatic limb injuries.

Blood was taken at a volume of 4 ml from each hen into heparin test tubes, and was subsequently centrifuged (837 × *g* for 15 min) to obtain blood plasma. Samples of blood plasma were immediately frozen and stored for further analyses at a temperature of −80 °C.

The concentration of corticosterone in the blood plasma was determined with the use of a commercial kit and the ELISA method (Cayman Chemical, Ann Arbor, MI, USA). Plasma concentrations of total protein, albumin, uric acid, glucose, lactate, triglycerides, cholesterol, calcium, phosphorus, and activity of enzymes AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), LDH (lactate dehydrogenase) and creatine kinase were determined photometrically with the use of a Konelab 20i biochemical analyser and commercial kits (Biovendor, Brno, Czech Republic).

Statistical assessment of the differences in the monitored indices between the groups of hens with traumatic limb injuries and without traumatic limb injuries was performed in the program Unistat for Excel 5.6 (Unistat Ltd, London, UK). An evaluation of data normality was conducted in the initial phase of testing. Since all the data met the condition of normal distribution, unpaired *t*-test was selected for further testing. Evaluation was performed at a level of significance of $P < 0.05$.

## Results

The results of the analysis of the level of corticosterone and selected biochemical indices in end-of-lay hens in which the occurrence of traumatic limb injuries was determined following transportation for slaughter and in end-of-lay hens without traumatic injuries after transportation for slaughter are given in Table 1.

End-of-lay hens in which traumatic limb injuries were detected during examination at the slaughterhouse showed significantly ($P < 0.05$) higher concentrations of plasma corticosterone, total protein, and albumin and significantly ($P < 0.05$) lower concentrations of plasma glucose, triglycerides, and calcium and lower activity of ALT during bleeding.

## Discussion

Previous research has documented that end-of-lay hens are particularly susceptible to traumatic injuries (Whitehead and Fleming 2000; Lay et al. 2011), specifically injuries to the limbs (Válková et al. 2021). Careless handling of the birds during unloading,

Table 1. The impact of traumatic limb injuries resulting from operations related to transport to slaughter on concentrations of plasma corticosterone and other biochemical indices in end-of-lay hens (mean ± SEM).

| Indices | Group | | *P* |
|---|---|---|---|
| | Hens with traumatic injuries | Hens without traumatic injuries | |
| Corticosterone (ng/ml) | 6380.93 ± 698.45 | 3680.70 ± 372.63 | 0.001 |
| Lactate (mmol/l) | 4.08 ± 0.34 | 4.71 ± 0.32 | > 0.05 |
| Glucose (mmol/l) | 13.04 ± 0.19 | 13.56 ± 0.22 | 0.036 |
| Cholesterol (mmol/l) | 3.32 ± 0.18 | 3.77 ± 0.33 | > 0.05 |
| Triglycerides (mmol/l) | 4.83 ± 0.89 | 8.95 ± 1.62 | 0.015 |
| Total protein (g/l) | 55.24 ± 1.45 | 50.86 ± 1.33 | 0.015 |
| Albumin (g/l) | 20.05 ± 0.61 | 17.53 ± 0.51 | 0.001 |
| Uric acid (µmol/l) | 529.13 ± 84.26 | 446.19 ± 75.63 | > 0.05 |
| Creatine kinase (µkat/l) | 169.37 ± 16.28 | 154.03 ± 11.14 | > 0.05 |
| ALP (µkat/l) | 2.87 ± 0.32 | 3.34 ± 0.25 | > 0.05 |
| AST (µkat/l) | 8.15 ± 0.59 | 7.09 ± 0.44 | > 0.05 |
| ALT (µkat/l) | 0.18 ± 0.02 | 0.37 ± 0.06 | 0.002 |
| LDH (µkat/l) | 9.38 ± 1.15 | 8.51 ± 0.91 | > 0.05 |
| Calcium (mmol/l) | 3.94 ± 0.13 | 4.58 ± 0.24 | 0.012 |
| Phosphorus (mmol/l) | 2.12 ± 0.15 | 2.29 ± 0.16 | > 0.05 |

SEM – standard error of the mean; ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; LDH – lactate dehydrogenase

which may occur in view of their economically low value, may also contribute to this in addition to the fragility of the bones of end-of-lay hens (Petracci et al. 2006). The method of housing laying hens has also been shown to have an effect on the occurrence of injury. A small study comparing end-of-lay hen pathology with different housing systems found that injuries after transport, including fractures, were found almost exclusively in hens depopulated from cages rather than deep litter (barn) or free-range systems (Keutgen et al. 1999). In the Czech Republic, laying hens are predominantly housed in cage systems which may explain the high rates of traumatic injuries detected in end-of-lay hens during postmortem examination at Czech slaughterhouses (Večerek et al. 2019; Večerková et al. 2019; Válková et al. 2021; Ninčáková et al. 2022). Birds with traumatic injuries of the limbs were also frequently detected in our study by the researcher who observed birds during slaughter and collected blood samples for analysis.

The results of biochemical examination of blood samples showed significant differences between the values of the monitored indices in birds with and without traumatic injuries, namely those related to stress. Hens in which traumatic limb injuries were detected during slaughterhouse inspection showed higher plasma corticosterone concentrations during bleed-out in comparison with intact birds. Corticosterone is a major adrenocorticoid hormone in birds (Carsia and Harvey 2000) and its secretion increases in response to stress (Volfová et al. 2022). The release of corticosterone by the adrenal gland results from the activation of the hypothalamic-pituitary-adrenal axis in response to stress stimuli (Cockrem 2007). Plasma corticosterone measurement is considered the most appropriate method of assessing short-term stress in poultry (Mormède et al. 2007). The results of our study document increased stress in hens suffering from traumatic injuries of the limbs, probably resulting from pain caused by handling as birds are typically held by the legs.

Stress hormones activate metabolic processes that result in an increase in the level of glucose in the blood as an immediate source of energy. Monitoring of the concentration

of glucose is, for this reason, one of the most frequently used biochemical indicators of stress after the analysis of glucocorticoids (Zhang et al. 2009; Thrall et al. 2012). Elevated glucose concentrations are typically observed in poultry exposed to short-term stress (e.g. Bedáňová et al. 2007). During longer periods of stress, the body's stores of glucose are exhausted and the body is unable to produce them fast enough, for which reason the concentration of glucose in the blood declines, as has been documented by e.g., Freeman et al. (1984), Nijdam et al. (2005), Chloupek et al. (2008) and Vošmerová et al. (2010). This may explain the lower level of glucose in the blood of end-of-lay hens with traumatic injuries of the limbs in our study in comparison with hens without traumatic injuries. The origin of the traumatic injuries detected in the hens in our study was unknown. Evidence suggests, however, that the predominant origin of injuries is seen during catching and crating operations (Weeks 2014; Gerpe et al. 2021). Injured birds were, therefore, likely to experience pain and suffering for a prolonged time throughout the subsequent stages of transport and slaughter operations until they were killed. Depending on the transport and handling conditions, including their duration, the welfare consequences may have increased with time and longer periods of stress may have been involved. This is corroborated by the lower triglyceride concentrations found in the injured hens in our study. The plasma concentration of triglycerides as lipids in fat deposits is not affected by short-term stress, and triglycerides decline only after a longer period of time, as has been documented by e.g., Chloupek et al. (2008) and Voslářová et al. (2011).

The metabolism of saccharides, lipids, and proteins is interconnected under physiological conditions and even more so if the animal organism is exposed to stress or suffering. Glucocorticoids exert a number of effects in chickens, including increases in the circulating concentration of glucose and shifts in lipid and protein metabolism (Scanes 2016). Consequently, higher concentrations of total protein and albumin were also found in the blood plasma of injured hens in our study in addition to lower concentrations of plasma glucose and triglycerides.

The activity of enzymes can also be studied during the evaluation of stress. Of the enzymes monitored in our study, a difference in activity was determined between injured and intact hens only for ALT, i.e. injured hens showed a lower activity of this enzyme. In birds, ALT activity has been described in the liver, skeletal muscle, and many other tissues and it leaks into the blood when these tissues are injured. Although plasma ALT activity is generally not considered a specific or sensitive test for hepatocellular damage in birds, its activity is increased by significant liver or muscle damage. In contrast, a decrease in ALT activity usually has no diagnostic significance (Pijarska et al. 2006; Thrall et al. 2012; Gattani et al. 2016).

A lower concentration of calcium in the blood plasma was also determined in end-of-lay hens with traumatic limb injuries in comparison with hens without traumatic injuries. In this case, this can be expected to be the cause of the occurrence of traumatic injuries rather than the consequence of them. Demineralisation and related increased bone fragility and brittleness lead to fractures of the limbs (Whitehead 2004).

The results of the study indicate that the handling of end-of-lay hens during transportation to the slaughterhouse leading to traumatic limb injuries and the action of the traumatic injury itself cause a higher degree of stress load on hens than handling of the hens that does not lead to traumatic limb injuries. The results also indicate that the handling of hens during transportation to the slaughterhouse leading to traumatic limb injuries and the action of the traumatic injury itself cause internal changes that are reflected in a change in some biochemical indices in the blood plasma. The method of handling of end-of-lay hens related to their transportation for slaughter should ensure that it will not cause traumatic injury and, thereby, not increase the already high level of stress caused by transportation itself.

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