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Distribution and elimination of radiolabelled corticosterone in chicken broilers

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Summary

The critical evaluation and reduction of stressful processes involved in farm animal husbandry, transport and slaughter represent serious issues in animal welfare discussion. Elevated concentrations of adrenocortical hormones are potential indicators of various forms of stress, however, the negative effects of the blood sampling procedure pose serious limitations to their measurement in the plasma. For non-invasive stress-assessment, profound knowledge about the metabolism of stress hormones is required. The present study aimed to monitor the distribution of corticosterone in the body of chickens, and to investigate if and to which extent, corticosterone is metabolised to other substances. Therefore, radiolabelled (^{3}H-) corticosterone was administered intravenously to 24 broilers. Each 6 broilers were killed 1, 2, 4 or 8 h after hormone injection and radioactivity was measured in blood, tissue and faecal samples. Within 8 hours, the main part of the radiolabelled steroids was voided via the droppings. Only trace amounts were found in tissue samples at any time. Reversed-phase high-performance liquid-chromatographic (RP-HPLC) separations were performed on samples of the bile. They revealed that 1 hour after injection, ^{3}H-corticosterone was completely metabolised into more polar substances. Furthermore, the results showed that the faecal corticosterone metabolites (CM) were already present in the bile and that a cortisone enzyme immunoassay (EIA) was more suited for the measurement of immunoreactive CM in the chicken than a corticosterone EIA. The findings from this study underline the suitability of recently developed non-invasive methods, which by evaluating stressful conditions can help to improve animal welfare.

Abbreviations: CM = corticosterone metabolites; D1-D4 = divisions of the gut (see methods section); EIA = enzyme immunoassay; RP-HPLC = reversed-phase high-performance liquid-chromatography

Schlüsselwörter: Masthühner, Schlachtkörper, Kortikosteroide, Stress.

Zusammenfassung

Verteilung von Kortikosteronmetaboliten im Schlachtkörper von Masthühnern

Eine kritische Betrachtung der in der Geflügelproduktion auftretenden Belastungen und das Bestreben nach deren Reduktion sind wichtige Anliegen des Tierschutzes. Die Konzentration von Nebennierenrindenhormonen (beim Huhn: Kortikosteron) steigt als Reaktion auf Belastungen im Plasma an. Die Bestimmung im Blut ist jedoch aufgrund der negativen Rückwirkungen der Probennahme problematisch. Um diesen Parameter mittels nicht-invasiver Stressmessung erheben zu können, ist Wissen über die Metabolisierung von Stresshormonen erforderlich. An 24 Masthühner wurde radioaktiv markiertes (^{3}H-)Kortikosteron intravenös verabreicht, um dessen Verteilung und Metabolisierung zu beobachten. Nach 1, 2, 4 und 8 h wurden jeweils 6 Tiere getötet und Blut-, Gewebe- und Kotproben entnommen. Innerhalb von 8 h wurde der Großteil der verabreichten Radioaktivität über den Kot ausgeschieden. In den Gewebeproben fand sich zu allen Untersuchungszeitpunkten nur ein geringer Anteil der radioaktiv markierten Steroide. Analysen mittels „reversed-phase" Hochleistungsflüssigkeitschromatographie (RP-HPLC) zeigten, dass in der Galle bereits nach einer Stunde das verabreichte Kortikosteron vollständig in Metaboliten mit höherer Polarität umgewandelt wurde. Die aus Galle und Kotproben extrahierten immunreaktiven Kortikosteronmetaboliten (CM) zeigten ein nahezu identisches Muster. Ein Kortison Enzymimmunoassay (EIA) war für die Bestimmung von CM beim Huhn besser geeignet als ein Kortikosteron EIA. Die Ergebnisse dieser Studie bestätigen, dass nicht-invasive Verfahren Belastungen erkennen und in weiterer Folge dazu dienen können, diese zu vermeiden.

Introduction

Animal welfare increasingly arouses public interest. Especially issues such as farm animal husbandry, meat production and livestock transport are a matter of general concern. Due to different motivations, certain lobbies (consumers, animal welfare activists and farmers) are interested in improved conditions for farm animals (BINDER et al., 2004). In drawing up scientific advice, it is necessary to rely on scientific data and principles to determine the welfare of animals (MOYNAGH, 2002). The lack of physiological signs of stress is an important indicator (BROOM and JOHNSON, 1993).

Commercial broiler chickens are exposed to a number of potential stressors prior to slaughter (KANNAN and

MENCH, 1997). These conditions not only affect welfare, but also result in economic losses due to carcass downgrading (reviewed by KNOWLES and BROOM, 1990). The processes of handling, transportation and slaughter are known to be stressful for the animals (KANNAN and MENCH, 1996). Stress can be caused by inappropriate temperatures during transport (too hot in summer, too cold in winter) and by "rough" handling before slaughter (NICOL and SCOTT, 1990; MITCHELL and CARLISLE, 1992; KANNAN et al., 1997a, b). A transport duration of 2 - 4 hours resulted in significantly elevated levels of plasma concentrations of glucocorticoids, free fatty acids and glucagon in chicken (FREEMAN et al., 1984). As a consequence of stress, the concentrations of glucocorticoids and catecholamines increase in blood, but as the blood sampling procedure itself is causing additional disturbance (BEUVING and VONDER, 1978; ROMERO, 2004), non-invasive sampling, e.g. by collecting faecal samples is becoming increasingly important for the evaluation of stressful conditions in animals (for review see PALME et al., 2005). To successfully establish non-invasive methods, profound knowledge about metabolism and excretion of stress hormones is required (EL-BAHR et al., 2005; PALME et al., 2005). Stress hormones are distributed in the body, metabolised in the liver and excreted via the kidney into the urine and via bile into the gut and the faeces (PALME et al., 2005). The application of non-invasive monitoring in chickens was reported for the first time by DEHNHARD et al. (2003) and the time course of excretion has been described by RETTENBACHER et al. (2004). However, basic knowledge about the distribution and metabolism of steroids within the different tissues of the body is lacking so far. In order to determine the distribution within the body, radioactive corticosterone was administered to chicken broilers. Radioactivity as well as the immunoreactive corticosterone metabolites (CM) were measured in the droppings and after slaughter in the carcass. The present CM were immunologically characterised.

With the conducted experiments, we wanted to investigate if and how long, the CM are present in edible tissues. Furthermore, detailed knowledge about metabolism and distribution of corticosteroids is essential for basic and applied research. The findings from this study might contribute to the successful implementation of new strategies to observe and avoid stressful situations and improve animal welfare.

Material and methods

Administration of radiolabelled corticosterone and sampling protocol

1 day old White Leghorn commercial broiler chickens were purchased from a local breeder (Geflügelhof Diglas, Feuersbrunn) and raised at the Clinic for Avian, Reptile and Fish Medicine until 6 weeks of age. The permission for performing the animal experiment was obtained from the Austrian Federal Ministry for Education, Science and Culture (GZ 68.205/101-BrGt/2002). Groups of 6 chickens (3 of each sex) were each administered 40 µCi of ^{3}H-corticosterone intravenously (v. brachialis) and killed after 1, 2, 4 and 8 hours, respectively. Dropping samples were collected every 30 min after injection, put into plastic freezer bags and immediately stored at -24 °C. At the time of slaughter, blood samples (3 ml) were drawn into a syringe, containing 1 ml of a citrate solution to avoid clotting. Tissue samples (10 g) were taken from the fat and from the thigh and breast muscle. Liver, gall bladder, 1 kidney and 6 sections of the gut were collected and deep frozen at -24 °C. The selection of the different sections of the gut (ligated at both ends) was done according to the following morphological criteria: D1 = beginning of the duodenum until the bend at the end of the pancreas; D2 = adjacent part approximately the same length as D1; D3 = until the diverticle; D4 = until the entrance of the caeca. Additionally, the contents of the caeca and cloaca were collected.

Extraction and measurement of the corticosterone metabolites (CM)

Blood samples (1 ml) were extracted with 2 x 5 ml diethyl ether which was dried down under a stream of nitrogen and re-dissolved in 0.5 ml absolute methanol to measure the unconjugated glucocorticoids. Thereafter, 5 ml acetone were added to the aequous phase to precipitate the protein (haemoglobin). After centrifugation, the supernatant was dried down under 60 °C and 6 ml scintillation fluid were added for determination of radioactivity. Tissue samples (0.5 g) were suspended in a glass tube with 5 ml of 60 % methanol, homogenised by 3 strokes of each 3 s with an Ultra-Turrax™ homogenizer (IKA, Labor Partner, Vienna, Austria), shaken for 20 min, centrifuged and the supernatant taken for the analysis. The contents of the gall bladder and of the various sections of the gut were squeezed out and mixed with the 10 fold volume of 60 % methanol.

From all extracts, aliquots (0.5 ml in duplicates) of the supernatants were mixed with 6 ml scintillation fluid (Quicksafe A™, No 100800, Zinsser Analytic, Maidenhead, UK). Radioactivity was measured in a liquid scintillation counter (Packard TriCarb 2100TR, Meriden, CT, USA). Quench correction was automatically performed by the counter. For the calculation of the total radioactivity in the respective sample, the relative activity (dpm/g or dpm/ml) measured in the samples was multiplied with the total mass or volume of that particular sample. For the calculation of the total volume of blood and the total mass of muscle and fat, their proportion of the carcass (according to its total mass) was derived from the tables of a handbook (SCHMIDHOFER, 1988). From the total sum of the recovered radioactivity of the particular animal, the percentage of radioactivity in the samples was calculated.

Characterisation of the CM

The concentration of the immunoreactive CM was determined in all samples by using 2 different enzyme immunoassays (EIAs). Aliquots of the supernatants (in blood only the ether extracts) were analysed as decribed in the respective publications (corticosterone EIA: PALME and MÖSTL, 1997; cortisone EIA: RETTENBACHER et al., 2004). To characterise the CM, reversed-phase high-performance liquid-chromatography (RP-HPLC) immunogramms were performed on 4 samples of bile extracted from the gallbladder of 2 males and 2 females, which were killed 1 or 8 h after administration. Details of the method used are described in previous publications (TESKEY-GERSTL et al., 2000; RETTENBACHER et al., 2004).

Tab. 1: Percentages (medians of 6 broilers) of radioactivity measured 1, 2, 4 and 8 h after i.v. administration of ^{3}H-corticosterone; different superscripts in the same line indicate significant differences (p<0.05) between the groups.

Tissue/Sample	1 h	2 h	4 h	8 h
blood	4.4^{a}	2.0^{ab}	0.7^{bc}	0.2^{c}
muscle	0.8^{a}	0.3^{b}	0.2^{bc}	0.1^{c}
fat	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
kidney	1.6^{a}	0.6^{ab}	0.3^{bc}	0.1^{c}
liver	6.5^{a}	2.2^{ab}	0.9^{bc}	0.5^{c}
bile	6.9^{a}	2.4^{ab}	1.9^{ab}	0.6^{b}
D1	3.3^{a}	1.6^{ab}	0.4^{bc}	0.2^{c}
D2	1.5^{a}	0.6^{ab}	0.3^{bc}	0.1^{c}
D3	43.0^{a}	17.5^{ab}	4.1^{bc}	2.0^{c}
D4	17.1^{a}	18.4^{a}	4.0^{ab}	1.2^{b}
caeca	1.2^{a}	6.0^{ab}	37.2^{c}	26.4^{bc}
cloaca	10.4^{ab}	20.3^{a}	18.6^{a}	4.2^{b}
faeces	8.0^{a}	32.9^{ab}	39.6^{ab}	72.4^{b}

Statistical analysis

As the data were not normally distributed, the medians are given (see Tab. 1). Within the 4 different groups (time of slaughter), Friedman tests were performed to compare the different tissues and sample sites. Groups were compared for significant differences by Kruskal-Wallis one way ANOVA and pairwise multiple comparison procedure (Tukey tests) using a statistical software package (SIGMASTAT® Version 3.0 for Windows, Jandel GmbH, Erkrath, Germany). Test rejection probability levels were set at 5 %.

Results

Excretion of radioactivity

The percentages of the radioactive CM in the samples are given in Tab. 1. Significant differences were found among the groups (see different superscripts) as well as between the different tissues/organs within each group (data not shown). Differences between males and females were not observed. The percentage of radiolabelled steroids, 1 h after administration, was highest in D3, followed by D4, and cloaca. After 2 h, the highest percentage was found in the droppings, followed by cloaca, D4 and D3. After 4 h, droppings, caecum and cloaca contained the highest percentages of radioactivity. There was a marked individual variation among the 6 animals of each group, especially regarding the concentration of the radioactive CM in the caeca and cloaca. More than 2 thirds of the injected corticosterone were voided via droppings within 8 h, the rest was found mainly in the caeca. In the blood, radioactivity (ether and acetone extracts combined) decreased rapidly. The acetone fraction contained the larger portion of radioactivity and the percentage increased over time (77-90 %). Radioactivity in D1, D2 and the kidneys showed a similar pattern. At any time, the lowest amounts were measured in fat and muscle, respectively. The total radioactivity in the droppings was calculated as the sum of the radioactivity in all dropping samples of that particular animal and therefore represents the cumulative value over the time of sampling.

Immunological characterisation of the CM

RP-HPLC separations of the CM in 4 bile samples revealed a number of different CM, however, no radioactive peaks were found at the elution position of corticosterone. The radiolabelled corticosterone was completely metabolised into more polar substances, most of the CM eluting as 2 close peaks between the fractions 30 and 40. Furthermore, the RP-HPLC immunograms showed that the main peaks of radioactivity were not identical with the main peaks of immunoreactivity, which could be measured by the EIAs. Differences between the 1 h and the 8 h sample concerning the elution pattern of metabolites were not observed. The concentrations of the CM in the samples measured by the 2 EIAs were different. In the gut and in the kidney, the cortisone EIA resulted in higher values than the corticosterone EIA, whereas in the samples of liver and bile this ratio was reverse. The highest concentrations of the immunoreactive CM were found in the bile 1 h after injection with the corticosterone EIA (197 ng/ml) and with the cortisone EIA (193 ng/ml), but also in the kidney (77 ng/g), 2 h after injection with the cortisone EIA. At the same time, the corticosterone EIA detected concentrations below 5 ng/g in the kidney. With both EIAs, the measured

concentrations of the CM in liver, fat and muscle were less than 4 ng/g at any time. In the blood, corticosterone concentrations ranged from 14 - 18 ng/ml, with no significant differences between the 4 groups. Sex differences were not found in any of the performed analyses.

Discussion

The aim of this study was to monitor distribution and excretion of injected ^{3}H-corticosterone in the chicken's body and to investigate if and to what extent, corticosterone is metabolised to other substances. Therefore, the endogenous metabolism of corticosterone was followed by administration of radiolabelled hormone. In chicken, the time course of the excretion of injected radioactive corticosterone has been established by RETTENBACHER et al. (2004). In mature laying hybrids, the authors found 2 main peaks, the first appearing at 1 and 1.5 h, the second after 3.4 and 4.7 h in females and males, respectively, representing urinary and faecal excretion. In the present study, sex differences were not observed, probably because the broilers were immature. To gain more information about the distribution and metabolism within the body, the contents of different parts of the gut, as well as blood and samples of various body tissues were analysed. From the received data it can be derived that there is a time dependent route of excretion, which starts in the liver and bile and ends in the droppings. The main part of the injected ^{3}H-corticosterone was excreted via the droppings within 8 h, the rest was retained in the caeca. 2 thirds of the administered radioactivity were already present in gut and droppings after 1 h. The percentage of radioactivity in the blood decreased rapidly. The increasing radioactivity in the acetone fraction indicates conversion of the injected corticosterone into more polar metabolites, which are then excreted via the kidneys. Radioactivity measured in the kidney samples also decreased rapidly. The observations of HELTON and HOLMES (1973), who performed similar experiments in ducks, go along with our findings in the chicken. The authors stated that after 1 hour, less than 10 % of the administered radioactivity was found in the blood, over 50 % was found in the gut, whereas only 5 % and 1.5 % were present in liver and kidney, respectively. In the present study, the low percentages of CM present in samples from muscles and fat indicate that the consumers do not have to be concerned about high levels of CM in edible tissues.

RP-HPLC separations of the bile samples resemble the findings of RETTENBACHER et al. (2004) in the droppings. The prominent peaks of ^{3}H-CM between fractions 30 and 40 were observed in both studies. The presence of more than 15 radioactive peaks indicates a large number of different metabolites, most of them more polar than the authentic corticosterone. As the immunogramms of the bile samples and the droppings are similar, we conclude that the faecal metabolites are already present in the bile and are formed in the liver. Additional metabolism by intestinal bacteria, as found in ruminants (MÖSTL et al., 1999), is of minor importance in the chicken. In bird species with different nutritional strategies, the situation might be different (KLASING, 2005), but research on that topic has not been performed so far. In the present and our previous study (RETTENBACHER et al., 2004), only an EIA, using cortisone as immunogen, showed crossreactions with the radiolabelled CM. It is important to notice that the mentioned antibody is group-specific and detects various metabolites having a 3,11-dione structure. The fact that cortisone itself is present in the droppings can be excluded. Although the cortisone EIA measured immunoreactive CM in the RP-HPLC fractions containing high concentrations of radioactivity, no crossreactions with the main peaks of radioactivity could be found. Further investigations are necessary to identify these CM and to develop suitable antibodies for their quantification. On a quantitative basis the immunoreactivity of CM cannot be directly related to the radioactivity, as different crossreactions of different CM are likely. Additionally, unknown amounts of endogenous CM might be present in the samples resulting from experimental stress imposed on the broilers. The corticosterone EIA detected CM in fractions, which contained only small amounts of radioactivity. Earlier investigations showed that corticosterone itself is not found in substantial amounts in chickens' droppings (RETTENBACHER et al., 2004). In this study, RP-HPLC separations revealed that authentic corticosterone was not present in the bile. In ducks, where bile, gut and cloacal fluid were analysed, authentic corticosterone was also absent, as radiolabelled CM eluting at the position of authentic corticosterone were not found (HELTON and HOLMES, 1973). This indicates that corticosterone is already completely metabolised in the liver. However, our corticosterone EIA measured high concentrations in the bile. An explanation for this might be the presence of different crossreacting metabolites, which remain to be characterised. Compared to the antibody against corticosterone, the antibody against cortisone measured higher concentrations of CM in the majority of samples and also showed crossreactions with some of the radiolabelled CM. Both EIAs measured high concentrations of immunoreactive CM in the bile, but only the cortisone assay detected significant CM concentrations in the kidney samples. These findings underline that the cortisone EIA is better suited for detection of CM than the corticosterone EIA (RETTENBACHER et al., 2004), because urine and faeces are excreted together in the chicken. In the blood, the measured concentrations of corticosterone did not differ significantly among the groups. As the blood samples were obtained during the slaughter procedure, it can be concluded that the whole manipulation during the experiment was stressful for the animals. The measured plasma corticosterone concentrations were consistent with findings in other studies, where similar levels (13-20 ng/ml) were found after severe handling, crating and heat stress (BEUVING and VONDER, 1978).

From the present study we gain basic information about the distribution and excretion of CM in broiler chickens. Furthermore, CM from other samples than droppings were characterised to some extent by RP-HPLC and 2 EIAs. The issue of animal welfare is becoming increasingly important, not only in the poultry industry, but also in public discussion. Both, consumers and producers will benefit from improved rearing conditions of farm animals. However, the appropriateness of stress hormones as indicators of animal welfare needs to be assessed carefully and in context (MOYNAGH, 2002). This study contributes further knowledge about their metabolism, which is necessary for the refinement of established techniques for non-invasive

stress-assessment. Elevated plasma concentrations of corticosterone in broilers do not imply a direct health risk for the consumer, because the possible maximum dietary uptake of steroids is negligible compared to endogenous production (FRITSCHE and STEINHART, 1999). Still, it should be an important concern to improve animal welfare by reducing stress during animal husbandry, transport and slaughter.

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References

BEUVING, G., VONDER, G.M. (1978): Effect of stressing factors on corticosterone levels in the plasma of laying hens. Gen. Comp. Endocrinol. **35**, 153-159.

BINDER, R., PALME, R., SOMMERFELD-STUR, I., BAUMGARTNER, J., NIEBUHR, K., ZALUDIK, K., BARTUSSEK, H., WAIBLINGER, S., REIFINGER, M., KÜBBER-HEISS, A., RABITSCH, A., GNEIST, M., TROXLER, J. (2004): Animal welfare in Austria: basic concepts of animal ethics, legal framework and animal welfare-related research. Vet. Med. Austria/Wien. Tierärztl. Mschr. **91**, 44-58.

BROOM, D.M., JOHNSON, K.G. (1993): Stress and animal welfare. Chapman and Hall, London, p. 87-144.

DEHNHARD, M., SCHREER, A., KRONE, O., JEWGENOW, K., KRAUSE, M., GROSSMANN, R. (2003): Measurement of plasma corticosterone and fecal glucocorticoid metabolites in the chicken *(Gallus domesticus)*, the great cormorant *(Phalacrocorax carbo)*, and the goshawk *(Accipiter gentilis)*. Gen. Comp. Endocrinol. **131**, 245-352.

EL-BAHR, S.M., KAHLBACHER, H., RAUSCH, W.D., PALME, R. (2005): Excretion of catecholamines (adrenaline and noradrenaline) in domestic livestock. Vet. Med. Austria/Wien. Tierärztl. Mschr. **92**, 207-213.

FREEMAN, B.M., KETTLEWELL, P.J., MANNING, A.C.C., BERRY, P.S. (1984): Stress of transportation for broilers. Vet. Rec. **114**, 286-287.

FRITSCHE, S., STEINHART, H. (1999): Occurrence of hormonally active compounds in food: a review. Eur. Food Res. Technol. **209**, 153-179.

HELTON, E.D., HOLMES, W.N. (1973): The distribution and metabolism of labelled corticosteroids in the duck *(Anas platyrhynchos)*. J. Endocrinol. **56**, 361-385.

KANNAN, G., MENCH, J.A. (1996): Influence of different handling methods and crating periods on plasma corticosterone concentrations in broilers. Br. Poult. Sci. **37**, 21-31.

KANNAN, G., MENCH, J.A. (1997): Prior handling does not significantly reduce the stress response to pre-slaughter handling in broiler chickens. Appl. Anim. Behav. Sci. **51**, 87-99.

KANNAN, G., HEATH, J.L., WABECK, C.J., MENCH, J.A. (1997a): Shackling of broilers: effects on stress responses and breast meat quality. Br. Poult. Sci. **38**, 323-332.

KANNAN, G., HEATH, J.L., WABECK, C.J., SOUZA, M.C., HOWE, J.C., MENCH, J.A. (1997b): Effects of crating and transport on stress and meat quality characteristics in broilers. Poult. Sci. **76**, 523-529.

KLASING, K.C. (2005): Potential impact of nutritional strategy on noninvasive measurements of hormones in birds. Ann. N. Y. Acad. Sci. **1046**, 5-16.

KNOWLES, T.G., BROOM, D.M. (1990): The handling and transport of broilers and spent hens. Appl. Anim. Behav. Sci. **28**, 75-91.

MITCHELL, M.A., CARLISLE, A.J. (1992): The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl *(Gallus domesticus)*. Comp. Biochem. Physiol. A **101**, 137-142.

MÖSTL, E., MESSMANN, S., BAGU, E., ROBIA, C., PALME, R. (1999): Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. J.Vet. Med. A. **46**, 621-631.

MOYNAGH, J. (2002): EU regulation and consumer demand for animal welfare. AgBioForum **3**, 107-114.

NICOL, C.J., SCOTT, G.B. (1990): Pre-slaughter handling and transport of broiler-chickens. Appl. Anim. Behav. Sci. **28**, 57-73.

PALME, R., MÖSTL, E. (1997): Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int. J. Mammal. Biol. **62** (Suppl. II), 192-197.

PALME, R., RETTENBACHER, S., TOUMA, C., EL BAHR, S.M., MÖSTL, E. (2005): Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Ann. N. Y. Acad. Sci. **1040**, 162-171.

RETTENBACHER, S., MÖSTL, E., HACKL, R., GHAREEB, K., PALME, R. (2004): Measurement of corticosterone metabolites in chicken droppings. Br. Poult. Sci. **45**, 704-711.

ROMERO, L.M. (2004): Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. **19**, 249-255.

SCHMIDHOFER, T. (1988): Geflügelschlachtung. In: PRÄNDL, O., FISCHER, A., SCHMIDHOFER, T., SINELL, H.-J. (Hrsg.): Handbuch der Lebensmitteltechnologie; Fleisch: Technologie und Hygiene der Gewinnung und Verarbeitung. Ulmer Verlag, Stuttgart, S. 147-172.

TESKEY-GERSTL, A., BAMBERG, E., STEINECK, T., PALME, R. (2000): Excretion of corticosteroids in urine and faeces of hares *(Lepus europaeus)*. J. Comp. Physiol. B **170**, 163-168.

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