

A Review: Hens Laying Welfare Indicators, A World View

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ABSTRACT

Animal Welfare is one of the most important goals for improvement the animal production. So many factors are believed to be involved for improving the animal welfare. Today, there are alternatives for hen housing in the world in order to have a better animal condition. For the other hand, hen welfare assessment has been used a number of parameters of well-being in order to use evaluation techniques to reduce the confounding influence of handling on stress responses. For this reason new insights with non-invasive methods are performed for it. The subject matter of this study is a review of the results obtained by different authors in relation of welfare assessment of corticosterone in albumen and yolk egg, droppings, feather, relation of plasma corticosterone and H/L ratio, image analysis and infrared thermography in hens. This article reviews the best and most relevant publications during the past 10 years to promote hen laying healthcare using non-invasive methods as welfare indicators.

Keywords: Animal Welfare, Hen Housing, Non-Invasive Method, Welfare Indicators.

I. INTRODUCTION

Hen Laying, World Importance

Projections from FAO suggest that global egg consumption will rise from 6.5 kg per person per year in 1997/99 to 8.9 kg per person per year in 2030 in developing countries. In industrial countries, egg consumption is projected to rise from 13.5 kg per person per year in 1997/99 to 13.8 kg per person per year in 2030. Additional data from FAO suggest that the world's production of eggs will reach 70.4 million metric tons in 2015 and 89.9 million metric tons in 2030, at a growth rate of 1.6 percent per year from 2015 through 2030. Of this global total, developing countries will produce 50.7 million metric tons of eggs in 2015 and 69 million metric tons of eggs in 2030 (FAO 2015). For the other hand, there are few cultures that do not consume eggs in large quantities. Egg production in a variety of systems has grown to meet the increased demand. Over the years, exchanges of scientific and technical information, increasingly on an international level, have greatly influenced the development of poultry systems. The World's Poultry Science Association (WPSA) has been influential in encouraging research and development and in promoting its results worldwide (Elson A *et al* 2011).

Also the economic and market implications of different production practices affect the egg industry's contribution to employment, nutrition, and consumer satisfaction. Besides affecting costs and relationships in egg markets, hen housing and other conditions affecting the flock may be of direct interest to members of society whether they consume eggs or not. Some members of society would likely be willing to pay for humane hen treatment or environmental sustainability if a market for these services could be established. Eggs from non-cage systems are generally more costly to produce than conventional eggs and must command significant market premiums to be competitive when buyers have a choice. It is important to know that the likely economic and market implications of shifting egg production from the current system, which relies primarily on caged housing for hens, to alternative non-cage systems (Holt P.S. *et al* 2011).

II. METHODS AND MATERIAL

A. Evolution of Animal Welfare

The welfare of animals used for food production is a major concern for society which stems from the recognition that animals are not only reactive to their

environments but also sentient. Consideration of the mental well-being of animals implies that animals have emotional capacities, such that they attempt to minimize negative emotions (e.g., fear and frustration) and to seek positive emotions (**Boisy Alan 2013**). Although stress-triggering stimuli are not necessarily painful but can also initiate psychological states, altogether known as emotions, such as fear or anxiety that activate physiological responses. In most cases, stress describes a condition that is detrimental to the welfare of the animal and should be avoided. Indeed, much effort has been invested in the adjustment of breeding practices to the animal needs and to avoid unnecessary challenges. However, stress responses are related not only to the nature and the intensity of the triggering stimulus, but also to individual response tendencies or temperament shaped by genetic factors, early environment and previous experiences. The influence of genetic factors on stress responses is well documented. Although most evidence for genetic variation in behavioural stress responses comes from laboratory animal studies, some information is also available in farm. Most papers describe differences between genotypes (breeds) and a few studies have evaluated genetic parameters from family studies (**Per Jensen A. 2008**). Also improvements regarding the welfare of laying hens can be achieved by development and enhancement of environmental factors such as production systems, management practices, procedures for transportation and slaughter, diet formulation and allocation of enrichment. Laying hen welfare may also be greatly affected by breeding goals and legislation. In order to evaluate how these factors affect laying hens, assessments of stress and welfare are needed. If stress and welfare can be monitored, this makes it possible to avoid specific stressful situations and reduce inevitable causes of poor welfare. There is a growing concern regarding the welfare of laying hens worldwide and there are both ethical and economic reasons for improving their welfare. Although several different welfare indicators are used today, their ability to accurately reflect welfare status is sometimes questioned (**Alm Malim 2015**). On-farm scoring of behavioural indicators of animal welfare is challenging but the increasing availability of low cost technology now makes automated monitoring of animal behaviour feasible (**Rushen J et al 2012**). Therefore diagnostics of stresses in poultry farming is an important issue, intended to solve some adjacent tasks. The main point of scientific application of the diagnostics of stress

state is study of stresses in hens under the conditions of industrial technologies and laboratorial physiological experiences and evaluating of the extent of the external factors' influence on the physiological state of the poultry. Monitoring physiological state of poultry while working out new methods of the stress prevention, working out and choosing of the optimal systems of poultry keeping and feeding. Productivity of hens is always an objective indicator, because compensating mechanisms of the organism allow keeping productivity and health on the certain level for some time. It has proved that as the resources of adaptation systems are depleting, increase of reactivity and resistance is observed and it is resulting in sharp drop of productivity, development of diseases and large-scale death of hens. High mortality can be an important indicator of acute and chronic stresses in poultry farming working out and choosing of the optimal systems of poultry keeping and feeding (**Tikhonov S and Miftakhutdinov A 2014**). Although the research was originally intended to resolve problems in confinement production systems, many of the scientific methods and findings have proven applicable to animals in a wider range of circumstances (Fraser David et al 2013). It is important to keep in mind that the assessment of hen welfare used a number of parameters of well-being. An objective is to use minimally invasive assessment techniques on live birds to reduce the confounding influence of handling on stress responses (**Cook N.J. 2011**). For this reason the interest has turned to non-invasive methods of measuring corticosterone concentrations in the animal. Currently the most recognized method to measure corticosterone is plasma corticosterone concentration, which involves blood sampling the animals. Blood sampling in itself is invasive because it involves handling an animal and drawing blood from the animal which can cause a stress response. If corticosterone can be measured non-invasively, the risk of a confounded result is decreased because handling and blood sampling the animal is not required. In the laying hen it is possible to measure corticosterone non-invasively through the egg or the excreta (**Engel Joana 2010**).

The present reviews the best and most relevant publications in relation of non-invasive techniques to evaluate animal welfare in hens from 2005 to 2015. We resumed all significative data about it and were embodied in tables.

B. Corticosterone in Droppings, Egg And Feather

In terms of correlations between the non-invasive measures, Total egg (albumin + yolk) and faecal corticosterone concentrations show a significant positive correlation for each of the three sampling days, but these are not correlated with plasma corticosterone concentration. It needs to be recognised that because of the small scale there are a number of factors limiting its ability to detect real relationships. It is therefore recommended that more extensive non-invasive sampling and more birds are required to comprehensively study the relationships between plasma and non-invasive measures of corticosterone. Another limitation is that plasma is taken as a single sample while egg and faecal corticosterone concentrations are likely to reflect plasma corticosterone concentrations over a period of time. Because of the pulsatile secretion of plasma corticosterone, multiple plasma samples throughout a day are required to estimate basal plasma corticosterone concentrations (Engel Joana 2009). Furthermore, investigators that have reported significant relationships studied more substantial variations in plasma corticosterone concentrations induced by imposing an acute stressor or an ACTH). It is possible non-invasive measures may be less predictive in measuring small differences between birds in basal corticosterone concentrations. It was reported no differences in faecal corticosterone concentration between treatments manipulating stocking density and flock size. Similarly, other authors were unable to measure differences in faecal corticosterone between free-range and conventional cage farms. Those that have investigated the relationship between plasma and egg corticosterone have also had difficulty measuring any significant relationships. For the other hand, other authors reported no significant difference between two housing systems. While a significant increase in egg albumen corticosterone concentration has been observed in response to a sharp drop in temperature in free-ranged laying hens. Also other studies found that on all other days of sampling, there was no difference in egg albumen between hens housed in free-range versus cage systems (Engel Joana 2009). Further information about yolk and albumen levels of corticosterone reflect measurements of corticosterone over the periods of time that yolk and albumen are deposited in the egg, i.e. approximately 10 days and 6 hours, respectively. Consequently, egg corticosterone

measurements are an attempt to assess the relative degree of chronic, or long-term, stress among birds housed in the different cage environments. Albumen corticosterone concentrations are lower in eggs collected at 60 weeks compared to 35 weeks of age. This observation is consistent with the results of comparative studies between caged and free-range birds. Albumen corticosterone levels of caged birds, but not free-range birds, are observed to decrease between 22 weeks and 45 weeks of age. Also albumen corticosterone levels are lower in the eggs of caged compared to free-range birds. Other authors suggested that the lower albumen levels observed over time in caged birds might be a reflection of habituation to the cage environment. Also it suggested that chronic stress may manifest itself as a reduction in egg corticosterone levels. In either case, the reported reduction in egg albumen corticosterone levels is probably not a function of age since the effect would have been noted in all housing conditions. The discrepancy in the observed responses of albumen and yolk levels may be a function of the relative sensitivity of the two measures to changes to the daily output of corticosterone. As a consequence, albumen may be the more sensitive measure to changes in daily adrenocortical output (Cook N.J. 2011). For the other hand, in eggs from floor pens, albumen corticosterone concentrations are also higher at the start of the laying period, indicating stress over the 6-h period of albumen deposition. The birds in floor pens are already acclimated to their environment when they began to lay (Singh R. 2009). Also the persistently low albumen corticosterone concentrations, low mortality, high egg production and large egg size, where hens were housed individually, serves to illustrate the importance of group dynamics and social adaptation in laying hens. In any flock there are likely to be some hens that perceive the challenges as more severe than others and have high corticosterone concentrations. The mean albumen corticosterone concentrations over the entire production cycle tended to be lower in flocks with lower mortality. However, further data is needed to establish a definitive relationship. These data suggest that the elevated albumen corticosterone concentrations in the early stages of the production cycle are likely correlated with reductions in performance. At this stage, the measurement of albumen corticosterone concentrations has highlighted the importance of early adaptation to housing system, which is likely to be improved with further knowledge and attention to management

(Downing Jeff 2012). For this reason egg steroid concentrations have been shown to reflect a laying hen's stress levels. The findings are consistent with behavioural observations that agree that battery hens are subjected to unusually high levels of physical stress and are unable to fully express their natural behaviours. Although, suggestive of chronic stress in battery hens, it would need to be combined with a number of parameters (health, production, behaviour) to get an encompassing measure of laying hen welfare and to confirm the chronic stress condition of battery hens (Bulmer Elena and Gil Diego 2008).

Analysis of feather corticosterone is the only method available to obtain a long-term and retrospective measure of stress. It appears that feather corticosterone is measurable and gives meaningful results even after years of storage as we showed with the analysis of flank feathers kept for nearly a decade. Another advantage of the technique is that experiments can be initiated at any time, as plucked feathers are soon replaced and the new growth can be related to any manipulated or natural source of variation in the bird's external environment or endogenous physiology. Collectively, these methodological advantages confirm that evaluation of feather corticosterone adds a powerful tool with novel insights in the study of stress – one of the most profound and pervasive factors influencing the well-being (Bortolotti Gary R *et al* 2009). For the other hand, if corticosterone is to be compared among feathers it must be shown that it does not degrade appreciably over time or after exposure to the environment. Also it is a considerably longer time line is possible by comparing among feathers grown at different times on an individual bird (Bortolotti Gary R *et al* 2008).

C. Relation of Plasma Corticosterone And H/L Ratio

The birds in floor pens are already acclimated to their environment when they began to lay. This short-term stress could be associated with the initiation of egg laying because it decreased as the hens grew older. The data on the H/L (heterophils and lymphocytes) ratio suggest that none of the laying hens are unduly stressed and that on

corticosterone in yolk and albumen support the suggestion that hens adapted to their environments with age. Although we could not directly associate the H/L ratio of a hen with specific eggs, it can make a general comparison. Both the H/L ratio and yolk corticosterone concentration are measurements of stress over a relatively long time period, suggesting that egg yolk corticosterone level can be used to measure stress in a fashion similar to the H/L ratio. In contrast, the measurements of the H/L ratio and the albumen corticosterone concentration in the cage environment disagree, possibly because the albumen is secreted over a short time. The albumen corticosterone level may indicate short-term stress in contrast to the yolk corticosterone level and the H/L ratio, which infer long-term stress. The results indicate that although the measurement of yolk corticosterone and the H/L ratio may be comparable, the corticosterone level in the albumen may differ because it is secreted over a short time period (Singh R. *et al*, 2009).

III. RESULTS AND DISCUSSION

Infrared Thermography and Image Analysis

Infrared thermography (IRT) provided a quantifiably accurate measure of heat losses that is capable of differentiating between loss of feather quantity and degradation to feather quality. As such, IRT has potential as an animal-based, outcome measure of the wellbeing of laying hens. Also feather quantity and quality can be accurately measured by IRT, which provides a quantifiable, animal-outcome based measure of bird well-being (Cook N.J. *et al*, 2009).

By analysing bird behaviours, could be observed the expression of natural comfort behaviours in breeding system with litters that certainly allowed better conditions of bird welfare when it was compared to conventional breeding system in cages, where comfort behaviours were not observed. Quality parameters of eggs were affected when birds were submitted to heat stress conditions, mainly in breeding system in cages, showing the importance of hen welfare aspects and the type of breeding system (Barbosa Filho A.D. *et al*, 2008).

Most of the advantages and disadvantages of animal welfare indicators above mentioned are showed in tables 1 and 2.

Table 1. Welfare Indicators (Corticosterone in droppings, egg and feather)

Reference	Indicator	Advantage	Disadvantage
G. R. Bortolotti <i>et al</i> 2008 G.R. Bortolotti <i>et al</i> 2009	Feather corticosterone concentration	Quantifiably accurate measure of heat losses was capable of differentiating between loss of feather quantity and degradation to feather quality. A considerably longer time line is possible by comparing among feathers grown at different times on for individual bird.	It doesn't used for acute stress evaluation.
Jeff Downing 2012	Albumen corticosterone concentration	The different patterns in albumen corticosterone concentrations could reflect the rate that the hens are able to adapt to the challenges in their environment. Albumen may be the more sensitive measure to changes in daily adrenocortical output, Measurement of albumen corticosterone concentrations could be correlated with a lice infestation and an outbreak of cannibalism.	The discrepancy in the observed responses of albumen and yolk levels may be a function of the relative sensitivity of the two measures to changes to the daily output of corticosterone.
N. J. Cook <i>et al</i> 2011	Albumen corticosterone concentration	Corticosterone is easily measured in albumen and sample processing is relatively inexpensive.	Albumen is deposited over periods of 5 to 6 hours, whereas yolk is deposited over 11 to 12 days.
Joanna Engel 2009	Droppings corticosterone concentration	The relationship between plasma corticosterone concentrations and non-invasive measures of corticosterone requires further examination under a huge range of situations, both when birds are at rest and under stress.	Egg and faecal corticosterone concentrations are likely to reflect plasma corticosterone concentration over a period of time.
Joanna Engel 2009	Relation between plasma and droppings corticosterone	It is important to clear the relationships between plasma corticosterone concentrations and non-invasive measures of corticosterone, studies used non-invasive measures of corticosterone.	Scientists using data on plasma corticosterone need to interpret the data cautiously particularly when based on single or infrequent samples, because of the pulsatile nature of corticosterone secretion.

Table 2. Other Welfare Indicators

Reference	Indicator	Advantage	Disadvantage
R. Singh <i>et al</i> 2009	Ratio H/L	Both the H/L ratio and yolk corticosterone concentration are measurements of stress over a relatively long time period.	H/L ratio and the albumen corticosterone concentration in the cage environment disagree, possibly because the albumen is secreted over a short time. The albumen corticosterone level may indicate short-term stress in contrast to the yolk corticosterone level and the H/L ratio, which infer long-term stress.
J. A. D. Barbosa <i>et al</i> 2008	Image Analysis	Quality parameters of eggs were affected when birds were submitted to heat stress conditions, mainly inbreeding system in cages, showing the importance of hen welfare aspects and the type of breeding system.	One problem was the impossibility of executing a complete individual evaluation of birds due to the distance of video cameras and to bird crowding in cages, beside the lack of effectiveness for distinguished birds by dorsal painting.
Cook <i>et al</i> 2012	Infrared Thermography	Infrared thermography provided a quantifiably accurate measure of heat losses that was capable of differentiating between loss of feather quantity and degradation to feather quality.	Most birds retaining good feather cover over the study period the differences measured between 35 and 60 weeks of age amounted to radiated temperature losses of approximately 4°C.

IV. CONCLUSION

Continued assessment of egg albumen corticosterone concentrations with particular interest to its correlation with production, body weight and mortality but also extend this to other measures of welfare such as plumage condition, health, feather pecking and cannibalism. **The comparison between production systems is not that useful because the variation between farms within a system can be large.** In a commercial context, the focus of welfare assessment should start at the flock level but eventually get to evaluating how individual hens cope within their environment. More thorough practical benchmarking of body weights, plumage condition, body injury, other measures of stress (H/L) and mortality is required at the farm level. The rearing management of pullets in assisting them to

adapt to new housing; group dynamics and social interactions requires more attention. The use of egg albumen corticosterone concentrations be promoted within the Poultry Science community as a technique that can further our understanding of bird welfare assessment (**Dowing Jeff 2012**). It is important to keep in mind that responses to stressors are complex and context dependent and therefore a combination of different measurements (eg. physiological and behavioural) for evaluating stress should be considered. Applied properly, non-invasive techniques for monitoring glucocorticoid metabolites in faecal samples are a useful tool for welfare assessment in various species, especially as they are easily applied at farm or group level. **Inter-disciplinary approaches using such methods** can advance our understanding of the biology of stress and related animal well-being (**Palme R. 2012**).

For the other hand, factors related to poultry welfare: disease, skeletal and foot health, nutrition, pest and parasite load, behaviour, stress, affective states, and genetics, all areas that provide specific challenges when managing flocks in the various housing systems. Hens can experience stress in all housing types and non-single housing system ranks high on all welfare parameters. Likewise, no single breed of laying hen is perfectly adapted to all type of housing systems (**Lay Jr. D.C. et al 2011**).

Until we better understand the relationships between plasma corticosterone concentrations and non-invasive measures of corticosterone, studies used non-invasive measures of corticosterone need to be interpreted cautiously. Similarly, scientists using data on plasma corticosterone need to interpret the data cautiously particularly when based on single or infrequent samples, because of the pulsatile nature of corticosterone secretion (**Engel Joana 2009**).

There are so many attempts to find better animal welfare indicators, but it goes far with one answer to explain what is going on with animal welfare in hens in different housing systems and using welfare indicators. It is a global answer and it depends of so many factors that include: genetic factors, breed hen, age, food, health, behaviour, geographical location, ammonia levels, specific characteristics of each housing system, etc. Also it is important to evaluate acute and chronic stress to keep in mind all the factors that could be participate to affect animal welfare in each hen housing system.

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