Behavioural Processes 149 (2018) 43–51

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

An integrated analysis of social stress in laying hens: The interaction between physiology, behaviour, and hierarchy

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ARTICLE INFO

Keywords:
Corticosterone metabolites
Gallus gallus domesticus
Hierarchy
Social stress
Welfare

ABSTRACT

Livestock is the category of animals that suffers the most severe welfare problems. Among these, physical, physiological, and behavioural distress caused by artificial grouping are some of the challenges faced by these animals. Groups whose members are frequently changed have been reported as socially unstable, which could jeopardise the welfare of animals. Here, we assessed the effect of social instability on aggression, stress, and productivity in groups of laying hens (*Gallus gallus domesticus*). We studied 36 females, distributed into three stable groups (without group membership change) and three unstable groups (where the dominant member was rotated every week) over the course of 10 weeks. We evaluated the frequency of agonistic interactions, glucocorticoid metabolites (GCM) concentrations, and egg production. In both treatments, dominant hens produced more eggs compared to intermediate and subordinates, and intermediate hens had the highest GCM concentrations. Socially unstable groups had lower productivity and higher frequencies of agonistic interactions than stable groups. Social instability also affected GCM of the animals: in stable groups, subordinate hens had higher concentrations than dominants; in unstable groups, this pattern was reversed. Our results point to a social destabilisation in groups whose members were alternated, and suggest the welfare of individuals in unstable groups was compromised. Our results pointed to a complex relationship between hierarchy, productivity, physiological stress and aggression in laying hens, and have implications for their husbandry and management and, consequently, for their welfare levels.

1. Introduction

Livestock is the most numerous category of animals in direct contact with humans, and these are the animals that present the most severe welfare problems (Broom and Molento, 2004). They are often kept in artificial groups that can either foster positive social behaviour or lead to aggression, injury and chronic fear (Fraser et al., 2013). Behavioural distress caused by artificial confinement has been frequently reported in laying hens (*Gallus gallus domesticus;* Broom and Molento, 2004). Although not common worldwide, some poultry producers regroup or divide groups of hens at different stages of the laying cycle in order to keep the same number of individuals in the cages (Hester and Wilson, 1986). Laying hens kept in small groups (6–10 individuals) exhibit social behaviour similar to that of their wild ancestor, the red jungle fowl (*Gallus gallus*; Collias and Collias, 1996). Social organisation in chickens is typically stable and hierarchical (McBride et al., 1969). In stable hierarchies, social dominance tends to settle to avoid the costs and risks of increased and continual fighting (Creel, 2001; Enquist and Leimar, 1990). Therefore, allowing animals to form and maintain stable associations can create a positive social environment and improve their ability to cope with new stressors (Fraser et al., 2013). However, when the stability of the social group is disturbed, higher levels of aggression and, consequently, greater stress loads are expected (Rose and Croft, 2015).

Aggression in groups of domestic hens is targeted more to newcomers than towards older members (Cloutier and Newberry, 2002). Aggressive behaviour directed to unfamiliar birds was observed for up to eight weeks after the introduction of new members (Guhl and Allee, 1944). Therefore, regrouping, dividing or introducing new members may induce social stress due to the establishment of a new pecking order every time a new hen is introduced into the group, likely affecting the welfare of the birds (Cheng and Fahey, 2009). Previous studies

Abbreviations: AgI, agonistic interactions; AIC_c, Akaike's information criterion corrected for small samples; DVR, digital video recorder; GC, glucocorticoids; GCM, glucocorticoid metabolites; GLM, generalised linear model

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https://doi.org/10.1016/j.beproc.2018.01.016
Received 3 October 2016; Received in revised form 18 January 2018; Accepted 23 January 2018

considered either egg production (Hill, 1983), aggression (Cloutier and Newberry, 2002; Cordiner and Savory, 2001) or body condition and productivity (Guhl and Allee, 1944) as indicators of laying hens' health level. However, the measurement of only one or two stress parameters is often insufficient to evaluate the welfare of the animals because the interaction between these parameters may be complex (Scheiber et al., 2015), and lead to inconclusive results. In addition, differences in techniques and conditions used to measure stress parameters may prevent comparisons between studies. For instance, Guhl and Allee (1944) reported that unstable groups had higher levels of aggression and lower productivity than stable groups, and that hens that constantly switched groups had lower body weight and lower food consumption than individuals in stable groups. In contrast to these results, other studies comparing stability and egg productivity in laying hens found no correlation between these parameters (Feldkamp and Adams, 1973; Adams, 1974; Hester and Wilson, 1986). Studies evaluating the effects of stability in laying hens using different physiological parameters have also found different outcomes. For example, in two studies which were conducted by the same researchers, one study found no difference in stress levels between stable and unstable groups (Cheng and Fahey, 2009, based on the weight of adrenal glands and plasma concentrations of serotonin and tryptophan) while another study found lower concentrations of glucocorticoids (GC – used as a measure of stress levels) in the plasma of animals in unstable groups (Fahey and Cheng, 2008). Littin and Cockrem (2001) found no effect of mixing groups on plasma GC, and no correlation of GC responses to stressors with the birds' hierarchical positions.

For social species, the involvement in recurrent and/or prolonged agonistic interactions can lead to chronic stress (Sapolsky, 1992). In response to stressors, within seconds, adrenaline is released into the blood stream, promoting increases in blood pressure, heart and respiratory rate and, consequently, energy is mobilised and made available for immediate use. Within a few minutes, the adrenal cortex secrets GC that alter the metabolic pathway for the production of adenosine triphosphate, redirecting for immediate survival the energy that would otherwise be used for other physiological processes (such as digestion, growth and reproduction; Moberg and Mench, 2000). This response is beneficial to the animal in the short term, but it can become harmful if prolonged or repeated too often in conditions that preclude the animal from recovering from each stressful event. Animals that are constantly stressed exhibit higher basal GC concentrations, leading to inhibition of anabolic processes, which can result in reproductive problems, low disease resistance, neuronal loss and decreased life expectancy (Siegek, 1980; Sapolsky, 1992). Therefore, the evaluation of the stress-response system functioning may bring relevant information, and should be considered when evaluating welfare (Möstl and Palme, 2002).

Studies evaluating stress levels have used numerous methods, such as measuring glucocorticoid concentrations in blood, evaluating immune system functioning, etc. Non-invasive sampling methods are the best suited, since the negative effects of invasive collections – e.g. blood samples – impose limitations to this kind of analysis. Beuving and Vonder (1978) reported an increase in serum corticosterone only 45 s after restraining hens, which could influence the results and mask the effects of other variables on stress levels. The measurement of faecal glucocorticoid metabolites (GCM) is one of the non-invasive methods used more often (Möstl and Palme, 2002; Palme, 2012; Sheriff et al., 2011). Faeces are the matrix that offers the greatest advantages due to their ease of collection, and its collection being feedback free (Touma and Palme, 2005). Non-invasive techniques for GCM monitoring have been extensively used by researchers and conservationists with numerous species (Touma and Palme, 2005; Sheriff et al., 2011; Kersey and Dehnhard, 2014), and have been already validated for chickens to assist in assessing welfare levels (Rettenbacher et al., 2004). However, as stress mechanisms may interact with other physiological systems – introducing confounding variables to the results (Scheiber et al., 2015), the measurement of other variables in tandem with physiological parameters will bring accuracy to the evaluation of welfare. Stress measurements analysed together with hierarchy, productivity, and behaviour could provide a comprehensive evaluation of long-term stress levels in laying hens which are submitted to changes in their social environment.

The relationship between GC levels, aggression and social status has been studied since the 1950s (Creel et al., 2013). In the early years of study, it was believed that subordination represented a more significant stressor than dominance. However, it is not always so. In some societies, the actions required to maintain dominance can be, per se, stressors (Creel et al., 1992; Sands and Creel, 2004). The endocrine profiles of baboons (*Papio anubis*) in different social positions may change, depending on the type of hierarchy. During periods of unstable hierarchy, dominant baboons show higher GC levels than subordinates. However, in stable periods, they present lower GC concentrations than subordinates (Sapolsky, 1983). In bearded capuchins (*Sapajus libidinosus*), in both types of hierarchies – stable and unstable – the dominant male presented the highest stress loads (Mendonça-Furtado et al., 2014).

This study aimed at investigating the effects of social instability on the welfare of laying hens at a group level, and at distinct hierarchical levels, through the evaluation of: (1) egg productivity; (2) the frequency of agonistic interactions, and (3) stress levels, to perform a comprehensive analysis of the social stress experienced by these birds. Considering the well-established relationship between social stressors, physiological reactions and welfare levels (Hill, 1983; Creel, 2001; Broom and Molento, 2004), and based on studies carried out evaluating social stressors in livestock (Guhl, 1968; Cheng and Fahey, 2009; Matur et al., 2015), our hypotheses for this study were that a social instability would be connected to (a) higher GCM concentrations and (b) higher frequencies of conflicts, both leading to (c) a reduction in productivity. We also predicted (d) greater productivity for the most dominant hens compared to subordinates, since subordinates have been reported as being targets of aggression and having poor access to resources in poultry (Hill, 1983; Cunningham and von Tienhoven, 1984).

2. Material and methods

Procedures described herein were approved by the Committee of Ethics for Animal Research from the Pontifical Catholic University of Minas Gerais, Brazil (approval number 001.2015) and are in accordance with the ethical guidelines published by the International Society for Applied Ethology (ISAE, 2002).

2.1. Subjects

We studied 36 female hens (ISA Brown hybrids), acquired from a commercial breeder (Agropecuária Vila Verde, Betim-MG) at the age of 19 weeks. From acquisition until the start of the experiments (when the birds were 34 weeks old), the animals underwent 15 weeks of habituation (we waited until all hens were steadily laying) kept together in a 90 m^2 fenced space, containing eight feeders and eight water drinkers. During habituation, as well as the experimental period, every bird had water *ad libitum,* and received 300 g of commercial laying feed and around 70 g of corn grains, divided into two meals (at 08:00 h and at 16:00 h). Before the start of the experiment, all hens were clinically checked, and marked in different colours using non-toxic cloth dye (Acrilex®) for visual identification. Hens are less likely to identify a target when all of them are marked similarly, which is particularly important if they are in small groups (Marin et al., 2014), which is the case of the subjects of this study.

2.2. Facilities

The study was conducted in a property located in the municipality of Sete Lagoas, in the central region of Minas Gerais state, Brazil. After the habituation period described above, the birds were randomly