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INQUIRY INTO USE OF BATTERY CAGES FOR HENS IN THE EGG PRODUCTION INDUSTRY

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New South Wales Parliament Select Committee Inquiry into the use of battery cages for hens in the egg production industry

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Mass spectrometry measures of corticosterone in egg albumen show that this measure cannot be used as an indicator of hen welfare

Summary

We have measured the purported stress hormone corticosterone in egg albumen, using high accuracy liquid chromatography coupled with mass spectrometry. Our results show that levels vary from undetectable to very high in an unpredictable manner. This means that purported measures of corticosterone, including antibody-based measures, cannot be used as indicators of hen welfare.

Background

The science relating to the assessment of the welfare of layer hens has included attempts at measuring the levels of stress in different housing conditions. The level of an adrenocortical hormone, corticosterone, is claimed to reflect the level of stress in hens. There are serious doubts as to whether corticosterone can be used to indicate the level of stress, particularly in chronic situations, including different housing systems.[1] Notwithstanding this, there have been claims that corticosterone levels in hen egg white (albumen) are reflective of corticosterone in blood, so that the levels in albumen can be used as an indirect measure of stress in hens.[2] Industry-sponsored research has claimed to show that the corticosterone levels are indistinguishable in eggs from caged birds, birds kept in barns, and birds in free range systems, with the conclusion that stress levels are the same regardless of whether birds are free range or in cages.[3] This research uses a measure based on the claimed selectivity of an antibody for corticosterone, reporting levels of about 1-2 ng/g egg albumen. However, there is a serious possibility that this measure is not usable, as the antibody (and indeed any other antibodies used for these sorts of measures) measure molecules other than corticosterone, particularly female sex hormones (such as progesterone) and metabolites.[4] Recently, Engel et al have reported that egg albumen corticosterone levels did not vary regardless of the amount of space allocated to egg-laying hens.[5] This work was based on immunoassay measures of corticosterone, and was said to show corticosterone levels of about 0.3ng/g. However, earlier work from the same group, published in a PhD thesis and in an industry report, showed considerably

[1] See the analysis of this issue in MP Caulfield (2018) *Animals in Australia,* Vivid Press.
[2] Downing and Bryden (2008) *Physiology and Behavior* 95, 381.
[3] Downing JL (2012) Non-invasive assessment of stress in commercial housing systems. Australian Egg Corporation Limited. See https://www.australianeggs.org.au/what-we-do/leading-research/non-invasive-assessment-of-stress-in-commercial-housing-systems/
[4] Liquid chromatography coupled with mass spectrometry has shown that there are very small levels of corticosterone in egg albumen: De Baere et al (2015) *Analytical and Bioanalytical Chemistry* 407, 4351. Liquid chromatography followed by immunoassay has showed that progesterone levels in egg yolk are very high: Quillfeldt et al (2011) *General and Comparative Endocrinology* 173, 11.
[5] (2019) *Poultry Science* 98, 533.

higher corticosterone levels of about 20ng/g.[6] it is unclear why the authors chose not to report this significant discrepancy in levels in their recent paper.

Our work at the University of Technology Sydney confirms variable and inconsistent corticosterone levels in egg albumen

Over the past 18 months, we have been studying levels of corticosterone in egg albumen, comparing the extraction methods of Downing (footnote 2 – using diethyl ether) and De Baere et al (footnote 4, using acetonitrile). We have separated corticosterone using high performance liquid chromatography (hplc), followed by analysis with mass spectrometry (MS). The chromatographic gradient used initially was that used by De Baere et al (footnote 4; acetonitrile plus water / formic acid). We have used a Waters Vion mass spectrometer for our MS measures. Analysis of purified corticosterone reveals a good linear relationship between amount of corticosterone added and response, with a detection limit of around 0.2ng/g.

The eggs we used came from a variety of sources. Method development used eggs from what we would call 'genuine' free range hens aged from about 4 years to 10 years. These birds are not in any way restrained, and roam freely over a smallholding of some 6ha in Tasmania. What emerged from these studies was that on most occasions corticosterone was undetectable at the limit of detection of the MS instrument, which corresponded to about 0.2ng/g. Recovery of corticosterone using the Downing and De Baere methods was measured using an internal standard of deuterated corticosterone, and was found to be about 50%-55%. However, using a shortened version of the De Baere chromatographic gradient we were able to increase corticosterone recovery to about 70% to 75%. This means we can be confident that what we are measuring is in fact corticosterone which is being properly extracted. Also, our initial studies found that there were significant amounts of progesterone (about 30ng/g) in all our samples. This suggests that the antibody measures of Downing and Engel may have been confounded by false positive readings by the antibody detecting progesterone in the extracts.

The most important finding is that on some occasions we found very substantial amounts of corticosterone in our samples – up to about 30ng/g. In other words, it appears that a subtle change of conditions reveals what MS measurements say is genuine corticosterone. This appears to fit with the widely discrepant measures of the two reports by Engel and co-workers, referred to above.

Going on from our development work, we have also compared egg albumen obtained from commercial sources, where chickens were housed 'free range' or in cages.[7] Those measures revealed apparently high levels of corticosterone (ranging from about 5ng/g to about 30ng/g) in all samples, with no difference between free range and cage eggs.

We have no explanation for the hugely different levels in corticosterone we found in measures made on different experimental days on the same egg samples, with ostensibly the same extraction

[6] Engel (2016) The effects of floor space allowance and nest box access on the welfare of caged laying hens (*Gallus gallus domesticus*). PhD thesis, University of Melbourne; Engel (2009) Further development of non-invasive stress measures. Report 09-30, Australian Poultry CRC (attached)

[7] Eggs were kindly supplied by Danny Jones of Pure Foods Eggs.

methods. However, we think it is possible that differences in thawing procedures may have induced corticosterone formation in some experiments. Alternatively, the MS instrument may be detecting molecule(s) very similar to corticosterone which cannot be separated by our chromatography procedure. One such example is 11-deoxycortisol. These possibilities will be tested.

Our studies also indicate that, in spite of all our best efforts to reduce variability and to not introduce bias, the measurement of corticosterone in egg albumen is not currently able to be made reproducible and reliable. Nevertheless, it is possible our future work may resolve these issues. Regardless, our findings cast doubt on any claim that the 'stress hormone' can be measured accurately and strongly suggest that any purported measure of egg albumen corticosterone can not be regarded as a reliable indicator of stress levels in hens.

Conclusion

The overall conclusion from our work is that, as with Engel's two very disparate corticosterone levels (only one of which is reported in the most recent paper), we cannot obtain a consistent level of corticosterone in repeat experiments on the same egg samples. Free range and cage eggs show what seems to be indistinguishable corticosterone levels, but ranging widely.

This means that corticosterone measures in egg albumen cannot be used as a measure of hen welfare, simply because it is not consistent. Moreover, our 'genuine free range' birds are arguably in an optimal state of welfare. They are under continuous veterinary supervision, and have access to all parts of the large property where they stay. In fact, they are free to roam anywhere (there are no fences). Thus, when we find, in those same eggs, corticosterone levels ranging from undetectable to very high, depending on the day the experiment was done, the conclusion has to be that, even if the corticosterone is in fact high (and we do not believe that to be so), that level cannot indicate poor welfare.