

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Corticosterone responses, fear behaviour and sociality in laying hens

A thesis presented in partial fulfilment
of the requirements of the degree of

Master of Science
in Physiology at
Massey University

Lana Marie McLaughlin

2006

Abstract

The overall aim of this thesis was to compare behavioural measures of fear and sociality and corticosterone responses to a stressor in white Leghorn and brown Hyline hens and to examine the relationship between corticosterone and these behaviours. The first set of experiments involved taking behavioural measures of fear and sociality. Tonic immobility and open field tests were conducted to measure fear whilst a runway test was used to measure sociality. There was a distinct difference in underlying fear levels with white hens being more fearful than brown, principal components analysis further distinguished this difference. There was no difference in the levels of sociality between the two strains of hen. The second set of experiments investigated the hen's corticosterone response to a 15 min handling stressor and 15 min and 60 min restraint stressors. Corticosterone responses to these stressors were measured by the collection of blood samples at 0, 15, 30 and 60 min after the stressor had begun. Both strains of hen responded to the handling procedure with a greater corticosterone response than to either restraint procedure, with no difference between the strains of hen. There was no difference in corticosterone response to both the restraint procedures but the white hens had a greater corticosterone response than brown hens. The third part of this thesis investigated the repeatability of the tonic immobility test and examined the relationship between corticosterone and fear and sociality. No difference was found in the mean behavioural measures of the first and second tonic immobility test for either strain of hen, but correlations and statistical repeatability calculations indicated that the tonic immobility test was more repeatable for brown than white hens. Correlations were found between corticosterone and behavioural measures of fear and sociality in white hens only.

Principal components analysis supported these findings and indicated that there was a positive relationship between corticosterone and fear and a negative relationship between corticosterone and sociality. The findings of the present study have provided information about the behavioural and physiological responses of white Leghorn and brown Hyline hens and show that the use of derived measures such as principal components analysis can provide useful information about relationships between variables in laying hens.

Acknowledgements

Firstly I would like to thank my supervisor Associate Professor John Cockrem for his help and guidance throughout this study.

Many thanks to Martin Collin for allowing me to use his hens, and to Syliva Yalden, Michael Kelly and the other staff at Kairanga Poultry farm for their help during the time I spent at the farm.

I would like to thank Jane Candy, Cathy Davidson and Julian Wall for all their help out at the farm and back at the lab.

For suggestions and advice on the design of my behavioral apparatus I would like to thank Professor Bryan Jones and Professor Dan Satterlee.

To my friends, flatmates and fellow students Ange Harvey, Claire Mawson, Selina Meikle, George Newson, Jude Park, Renee Pedley, Lucy Phillips, Mandy Platt, Iain Thornton and Des Waters for supporting me and giving me encouragement through out this time.

The statistical analysis of the tonic immobility and open field corticosterone results in chapter two and the statistical analysis and results section of chapters three and four were completed by Associate Professor John Cockrem.

Finally I would like to thank my family for their support over the years.

Table of contents

Abstract.....	i
Acknowledgements	iii
Table of contents	iv
List of figures.....	viii
List of tables.....	ix
Chapter 1: General introduction	1
1.1 Stress, stressor and stress response	1
1.2 Hypothalamo-pituitary-adrenal (HPA) axis.....	3
1.2.1 Components	3
1.2.2 CRH and AVT	4
1.2.3 ACTH.....	5
1.2.4 Glucocorticoids	6
1.3 Corticosterone in birds.....	6
1.3.1 Synthesis and secretion	6
1.3.2 Corticosterone responses	8
1.3.3 Actions of corticosterone	8
1.3.3.1 Physiology.....	8
1.3.3.2 Behaviour	9
1.3.3.3 Other actions	11
1.4 Corticosterone in chickens.....	12
1.4.1 Stimuli for release	12
1.4.2 Corticosterone responses	12
1.4.3 Actions of corticosterone	12
1.4.3.1 Physiology.....	12
1.4.3.2 Behaviour.....	13
1.5 Fear	14
1.5.1 Basic emotions.....	14
1.5.2 What is fear?	14

1.5.3 Fear in chickens	15
1.5.3.1 How to measure fear	15
1.6 Outline of thesis	16
Chapter 2: Measurement of fear behaviour and sociality in white and brown hens	18
2.1 Abstract	18
2.2 Introduction	20
2.3 Materials and methods	22
2.3.1 Animals and husbandry	22
2.3.2 Experimental design	23
2.3.3 Behavioural observations and sample collection	23
2.3.3.1 Tonic immobility	23
2.3.3.2 Open field	24
2.3.3.3 Runway	25
2.3.4 Plasma sample preparation and corticosterone radioimmunoassay	25
2.3.4.1 Plasma sample preparation	25
2.3.4.2 Radioimmunoassay of corticosterone	25
2.3.4.3 Assay validation and characteristics	26
2.3.5 Statistical analysis	27
2.4 Results	29
2.4.1 Order of tests and comparisons between handlers	29
2.4.2 Tonic immobility, open field and runway tests	29
2.4.3 Principal components analyses	30
2.5 Discussion	49
2.5.1 Tonic immobility and open field tests	49
2.5.2 Runway test	52
2.5.3 Principal components analysis	52
Chapter 3: Effects of handling and restraint methods on corticosterone responses in white and brown hens	54
3.1 Abstract	54
3.2 Introduction	55

3.3 Materials and methods	56
3.3.1 Animals and husbandry	56
3.3.2 Experimental design	56
3.3.2.1 Corticosterone responses to handling in white and brown hens	56
3.3.2.2 Comparison of handling and restraint methods in white and brown hens	57
3.3.3 Plasma sample preparation and corticosterone radioimmunoassay	58
3.3.3.1 Plasma sample preparation	58
3.3.3.2 Radioimmunoassay of corticosterone	58
3.3.4 Statistical analysis	59
3.4 Results	61
3.4.1 Corticosterone responses to handling in white and brown hens	61
3.4.2 Comparison of handling and restraint methods in white and brown hens	61
3.5 Discussion	75
3.5.1 Corticosterone responses to handling in white and brown hens	75
3.5.2 Comparison of handling and restraint methods in white and brown hens	77
Chapter 4: Repeatability of behavioural tests of fear, and relationships between corticosterone and behaviour in white and brown hens	79
4.1 Abstract	79
4.2 Introduction	80
4.3 Materials and methods	82
4.3.1 Animals and husbandry	82
4.3.2 Experimental design and behavioural observations	83
4.3.2.1 Repeatability of tonic immobility behaviour test	83
4.3.2.1.1 Tonic immobility tests	83
4.3.2.2 Relationships between corticosterone and behaviour	84
4.3.2.3. Open field tests	85
4.3.2.4 Runway tests	85
4.3.3 Plasma sample preparation and corticosterone radioimmunoassay	86
4.3.3.1 Plasma sample preparation	86
4.3.3.2 Radioimmunoassay of corticosterone	86

4.3.4 Statistical analysis	87
4.3.4.1 Fear score ranks	87
4.3.4.2 Principal components analyses	89
4.3.4.3 Comparisons between the first and second tonic immobility tests	90
4.3.4.4 Statistical repeatability	91
4.3.4.5 Relationships between corticosterone and behaviour	91
4.4 Results	93
4.4.1 Repeatability of tonic immobility behaviour test	93
4.4.2 Relationships between corticosterone and behaviour	94
4.5 Discussion	102
4.5.1 Repeatability of tonic immobility behaviour test	102
4.5.2 Relationships between corticosterone and behaviour	103
Chapter 5: General discussion	106
5.1 General discussion	106
5.2 Major conclusions	106
5.3 Future directions	107
References	109
Appendix	125

List of figures

- Figure 2.1.** Plasma corticosterone concentrations in undisturbed white Leghorn and brown Hyline hens and in the hens after 10 min in an open field..... 34
- Figure 2.2.** Distributions of behavioural variables in relation to the first two principal components of principal components analyses of tonic immobility and open field variables for white and brown birds combined..... 35
- Figure 2.3.** Mean scores for white and brown birds for components identified in principal component analyses of variables in tonic immobility, open field and runway tests..... 37
- Figure 2.4.** Mean scores for white and brown birds for components identified in principal component analyses of all variables from tonic immobility, open field and runway tests combined..... 39
- Figure 3.1.** Corticosterone responses to a standard handling procedure in White Leghorn and brown Hyline hens. The handling procedure consisted of 15 min of repeated handling followed by 45 min of social isolation..... 64
- Figure 3.2.** Corticosterone responses to three handling and restraint methods in White Leghorn and brown Hyline hens. Hens were handled by a standard method or experienced 15 or 60 min of restraint..... 65
- Figure 3.3.** Corticosterone responses to three handling and restraint methods in White Leghorn and brown Hyline hens. Hens were handled by a standard method or experienced 15 or 60 min of restraint..... 66
- Figure 3.4.** Total and corrected integrated corticosterone responses to handling and restraint of White Leghorn and brown Hyline hens. The corrected response is the total response minus the integrated corticosterone secretion attributable to initial corticosterone concentrations at 0 min. Hens were handled by a standard method or experienced 15 or 60 min of restraint... 68

List of tables

Table 2.1.	Mean values and statistics for behavioural measures of tonic immobility open field and runway tests in white Leghorn and brown Hyline hens...	40
Table 2.2.	Two way repeat measures ANOVA for plasma corticosterone concentrations in white leghorn and brown Hyline hens in two situations. Hens were sampled when undisturbed and after 10 min in an open field test.....	41
Table 2.3.	Spearman rank correlations between four behaviour variables measured in tonic immobility tests for each strain and for all birds.....	42
Table 2.4.	Spearman rank correlations between five behaviour variables measured in open field tests for each strain and for all birds.....	43
Table 2.5.	Spearman rank correlations between four behaviour variables measured in runway tests for each strain and for all birds.....	44
Table 2.6.	Loadings from principal components analysis of variables in tonic immobility, open field and runway tests combined for white and brown birds together.....	45
Table 2.7.	Comparison of corticosterone responses in white and brown laying hens to different stressors.....	46
Table 3.1.	Two way repeat measures ANOVA for plasma corticosterone concentrations in white Leghorn and brown Hyline hens subjected to a standard handling procedure for 15 min.....	69
Table 3.2.	Statistical analysis for comparison of plasma concentrations of corticosterone between strains and handling and restraint methods.....	70
Table 3.3.	Statistical analysis for comparison of integrated corticosterone responses between handling and restraint methods.....	74
Table 4.1.	Mean values and statistics for behaviour variables from first and second tonic immobility tests.....	98
Table 4.2.	Pearson correlations between variables in first and second tonic immobility tests.....	99
Table 4.3.	Repeatabilities and statistics for behaviour variables from first and second tonic immobility tests.....	100

Table 4.4.	Spearman correlations between corticosterone variables and fear score ranks, and between corticosterone variables and PCA behaviour scores in white hens.....	101
-------------------	---	-----