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 throughout 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
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3 Materials and methods</td>
<td></td>
</tr>
<tr>
<td>3.3.1 Animals and husbandry</td>
<td>56</td>
</tr>
<tr>
<td>3.3.2 Experimental design</td>
<td>56</td>
</tr>
<tr>
<td>3.3.2.1 Corticosterone responses to handling in white and brown hens</td>
<td>56</td>
</tr>
<tr>
<td>3.3.2.2 Comparison of handling and restraint methods in white and brown hens</td>
<td>57</td>
</tr>
<tr>
<td>3.3.3 Plasma sample preparation and corticosterone radioimmunoassay</td>
<td>58</td>
</tr>
<tr>
<td>3.3.3.1 Plasma sample preparation</td>
<td>58</td>
</tr>
<tr>
<td>3.3.3.2 Radioimmunoassay of corticosterone</td>
<td>58</td>
</tr>
<tr>
<td>3.3.4 Statistical analysis</td>
<td>59</td>
</tr>
<tr>
<td>3.4 Results</td>
<td>61</td>
</tr>
<tr>
<td>3.4.1 Corticosterone responses to handling in white and brown hens</td>
<td>61</td>
</tr>
<tr>
<td>3.4.2 Comparison of handling and restraint methods in white and brown hens</td>
<td>61</td>
</tr>
<tr>
<td>3.5 Discussion</td>
<td>75</td>
</tr>
<tr>
<td>3.5.1 Corticosterone responses to handling in white and brown hens</td>
<td>75</td>
</tr>
<tr>
<td>3.5.2 Comparison of handling and restraint methods in white and brown hens</td>
<td>77</td>
</tr>
<tr>
<td>Chapter 4: Repeatability of behavioural tests of fear, and relationships between corticosterone and behaviour in white and brown hens</td>
<td>79</td>
</tr>
<tr>
<td>4.1 Abstract</td>
<td>79</td>
</tr>
<tr>
<td>4.2 Introduction</td>
<td>80</td>
</tr>
<tr>
<td>4.3 Materials and methods</td>
<td>82</td>
</tr>
<tr>
<td>4.3.1 Animals and husbandry</td>
<td>82</td>
</tr>
<tr>
<td>4.3.2 Experimental design and behavioural observations</td>
<td>83</td>
</tr>
<tr>
<td>4.3.2.1 Repeatability of tonic immobility behaviour test</td>
<td>83</td>
</tr>
<tr>
<td>4.3.2.1.1 Tonic immobility tests</td>
<td>83</td>
</tr>
<tr>
<td>4.3.2.2 Relationships between corticosterone and behaviour</td>
<td>84</td>
</tr>
<tr>
<td>4.2.3.3 Open field tests</td>
<td>85</td>
</tr>
<tr>
<td>4.2.3.4 Runway tests</td>
<td>85</td>
</tr>
<tr>
<td>4.3.3 Plasma sample preparation and corticosterone radioimmunoassay</td>
<td>86</td>
</tr>
<tr>
<td>4.3.3.1 Plasma sample preparation</td>
<td>86</td>
</tr>
<tr>
<td>4.3.3.2 Radioimmunoassay of corticosterone</td>
<td>86</td>
</tr>
</tbody>
</table>
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.