

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 to handling and  
effects of corticosterone injections  
in the Japanese quail  
(*Coturnix coturnix japonica*)

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

Master of Science  
in  
Physiology

at Massey University, Palmerston North,  
New Zealand

Raewyn Anne Boyd  
2000

## ABSTRACT

These studies examined the effects of corticosterone on the reproductive system, investigated the relationship between plasma and faecal corticosterone levels and defined corticosterones response to handling in the Japanese quail (*Coturnix coturnix japonica*). Six days of daily corticosterone injections decreased the area of the cloacal protuberance in both seven week old and six month old male quail. However, plasma testosterone levels 24 hours after an injection were only decreased in the six month old birds. There was a strong correlation between basal plasma and faecal corticosterone concentrations in the six month old birds.

The effects of corticosterone during the 24 hours after an injection were then examined before and after six days of corticosterone injections in male quail. Corticosterone injections decreased plasma testosterone levels three-fold for 6-12 hours both after a single corticosterone injection and after six days of treatment. However, there were no changes in plasma luteinising hormone levels during the 24 hours after an injection. This result is consistent with corticosterone acting directly on the testes to decrease testosterone release. The rate of corticosterone removal from the blood after an injection increased after six days of corticosterone injections.

Handling female Japanese quail for 15 minutes resulted in increased plasma corticosterone levels for less than 30 minutes. Mean corticosterone response curves were almost identical when the same birds were handled on three occasions. Although corticosterone response curves were similar during the early afternoon and during the night, basal corticosterone levels and the area under the corticosterone response curves were lower at night. Plasma corticosterone levels 0 and 15 minutes after the initiation of handling were more than twice as high in birds with large gonads than birds with small gonads.

This study provides the first information in birds of a decrease in plasma testosterone levels within three hours of a corticosterone injection, independent of changes in plasma LH levels. It is also the first study in a domestic species to show larger corticosterone responses in female birds with large gonads than in birds with small gonads.

## **ACKNOWLEDGMENTS**

I would first like to thank my Supervisor, Dr. John Cockrem for all his help, encouragement and direction over the past two years. Also profuse thanks to all the other members of the Conservation Endocrinology Research Group, who offered their time, energy and ideas and without whom my research would not have been possible. Thanks also to Jane Candy for teaching me the ins and outs of radioimmunoassay and carrying out the faecal corticosterone extractions.

Thanks to the Institute of Veterinary, Animal and Biomedical Sciences (IVABS), who provided the funding for my work. Many thanks to the staff, especially to Professor Keith Lapwood for his constructive criticism, and Allain Scott for all her help with all my administrative questions.

Finally, I'd like to thank my family and friends for their support and Peter for his patience, encouragement, and willingness to put up with me when it was all getting a bit much.

## TABLE OF CONTENTS

<b>ABSTRACT</b>	<b>ii</b>
<b>ACKNOWLEDGMENTS</b>	<b>iii</b>
<b>TABLE OF CONTENTS</b>	<b>iv</b>
<b>LIST OF FIGURES</b>	<b>ix</b>
<b>LIST OF TABLES</b>	<b>xiv</b>
<b>1 General Introduction</b>	<b>1</b>
1.1 Stressors, stress and the stress response	1
1.2 The hypothalamo-pituitary-adrenal axis	2
1.3 Circadian rhythms in hypothalamo-pituitary-adrenal axis activity	3
1.4 Measurement of the stress response	4
1.5 Actions of glucocorticoids in birds	5
1.6 Glucocorticoid metabolism	8
1.7 Relationship between faecal and plasma corticosterone	9
1.8 Outline of thesis	12
<b>2 Effects of daily corticosterone injections</b>	<b>13</b>
2.1 Abstract	13
2.2 Introduction	14
2.3 Materials and methods	17
2.3.1 Animals	17
2.3.2 Experimental design	17
2.3.2.1 Six month old birds	17
2.3.2.2 Seven week old birds	17
2.3.3 Hormone administration	18
2.3.3.1 Six month old birds	18
2.3.3.2 Seven week old birds	19

2.3.4 Data collection	19
2.3.4.1 Six month old birds	19
2.3.4.1.1 Cloacal gland area and foam production	19
2.3.4.1.2 Condition index	20
2.3.4.1.3 Blood, faecal and tissue samples	20
2.3.4.2 Seven week old birds	22
2.3.4.2.1 Weight and cloacal gland area	22
2.3.4.2.2 Blood and tissue samples	22
2.3.5 Hormone Assays	22
2.3.5.1 Hormone extraction from faeces	22
2.3.5.2 Extraction from plasma	24
2.3.5.3 Radioimmunoassay of LH	26
2.3.5.4 Radioimmunoassay of testosterone	26
2.3.5.5 Radioimmunoassay of corticosterone	27
2.3.6 Calculation of the area under the curve	29
2.3.7 Statistics	29
2.4 Results	30
2.4.1 Six month old birds	30
2.4.1.1 Plasma corticosterone	30
2.4.1.2 Faecal corticosterone	30
2.4.1.3 Relationship between plasma and faecal corticosterone	36
2.4.1.4 Plasma LH and testosterone	36
2.4.1.5 Faecal composition	41
2.4.1.6 Body measurements	41
2.4.1.6.1 Body weight and condition index	41
2.4.1.6.2 Muscle to fat ratio	41
2.4.1.6.3 Organ weights	45
2.4.1.6.4 Secondary sexual characteristics	45
2.4.2 Seven week old birds	49
2.4.2.1 Plasma corticosterone	49
2.4.2.2 Plasma LH and testosterone	49
2.4.2.3 Body measurements	57

2.4.2.3.1 Body weight	57
2.4.2.3.2 Testes weight	57
2.4.2.3.3 Secondary sexual characteristics	57
2.5 Discussion	61
2.5.1 Plasma corticosterone	61
2.5.2 Faecal corticosterone	63
2.5.3 Relationship between plasma and faecal corticosterone	65
2.5.4 Plasma LH and testosterone	65
2.5.5 Faecal parameters	67
2.5.6 Body measurements	68
2.5.6.1 Body weight and condition index	68
2.5.6.2 Muscle to fat ratio	69
2.5.6.3 Organ weights	70
2.5.6.3.1 Liver weight	70
2.5.6.3.2 Testes weight	70
2.5.6.4 Secondary sexual characteristics	71
2.5.6.4.1 Cloacal gland area	71
2.5.6.4.2 Foam production	72
2.6 Conclusions	73
<b>Chapter 3: Acute effects of a corticosterone injection</b>	<b>74</b>
3.1 Abstract	74
3.2 Introduction	75
3.3 Materials and methods	79
3.3.1 Animals	79
3.3.2 Experimental design	79
3.3.3 Hormone administration	80
3.3.4 Data collection	80
3.3.4.1 Body weight and cloacal gland area	80
3.3.4.2 Blood and faecal samples	80
3.3.5 Hormone assays	81

3.3.6 Calculation of the area under the curve	81
3.3.7 Statistics	82
3.4 Results:	83
3.4.1 Plasma corticosterone:	83
3.4.2 Faecal corticosterone	87
3.4.4 Plasma LH	92
3.4.3 Plasma testosterone	92
3.5 Discussion	106
3.5.1 Plasma corticosterone	106
3.5.2 Faecal corticosterone	107
3.5.3 Plasma LH	109
3.5.4 Plasma testosterone	112
3.6 Conclusions	114
<b>4 Corticosterone responses to handling</b>	<b>115</b>
4.1 Abstract	115
4.2 Introduction	116
4.3 Materials and methods	120
4.3.1 Animals	120
4.3.2 Experimental Design	120
4.3.2.1 Duration of the corticosterone response	120
4.3.2.2 Individual variation	121
4.3.2.3 Day versus night	121
4.3.2.4 Effect of reproductive status	122
4.3.3 Data Collection	124
4.3.3.1 Cloacal diameter, gonad weight and egg laying	124
4.3.3.2 Blood samples	124
4.3.4 Hormone assays	125
4.3.5 Calculation of the area under the curve	125
4.3.6 Statistics	125
4.4 Results	126

4.4.1 Duration of the corticosterone response	126
4.1.2 Effect of sampling speed	126
4.4.3 Individual variation	127
4.4.4 Day versus night	137
4.4.5 Effect of reproductive status	140
4.4.5.1 Cloacal diameter and gonad weights	140
4.4.5.2 Plasma corticosterone	140
4.5 Discussion	146
4.5.1 Duration of the corticosterone response	146
4.5.2 Effect of sampling speed	147
4.5.3 Individual variation	148
4.5.4 Day versus night	149
4.5.5 Effect of reproductive status	151
4.6 Conclusions	154
<b>5 General Discussion</b>	<b>155</b>
5.1 Conclusions	155
5.2 Future work	156
5.2.1 Corticosterone and reproduction	156
5.2.2 Non-invasive measures of corticosterone	157
5.2.3 Corticosterone responses in the Japanese quail	159
<b>6 References</b>	<b>161</b>

## LIST OF FIGURES

Fig. 2.1: Individual faecal corticosterone levels on days 0 - 6 .....	32
Fig. 2.2: Mean faecal corticosterone levels on days 0 - 6 .....	33
Fig. 2.3: Areas under faecal corticosterone versus time curves over the treatment period .....	34
Fig. 2.4: Correlation between faecal and plasma corticosterone levels in oil injected birds during the treatment period. ....	37
Fig. 2.5: Mean plasma LH levels on days 0, 2, 4 and 6.....	38
Fig. 2.6: Mean plasma testosterone levels on days 0, 2, 4 and 6 .....	38
Fig. 2.7: Individual areas under plasma testosterone versus time curves over the treatment period .....	39
Fig. 2.8: Correlation between plasma testosterone and faecal corticosterone levels during the treatment period.....	40
Fig. 2.9: Composition of faecal collections on days 0 - 6 .....	42
Fig. 2.10: Relative body weights on days 0, 2, 4, and 6 and condition index on days 0 and 6. ....	43
Fig. 2.11 Pectoral muscle weight, neck skin weight and ratio of pectoral muscle to neck skin weight on day 6.....	44
Fig. 2.12: Liver weight as a percentage of body weight on day 6.....	46

Fig. 2.13: Paired testes weights on day 6.....	46
Fig. 2.14: Cloacal gland area on days 0, 2 and 4 relative to area on day 0 .....	47
Fig. 2.15: Cloacal foam production over 24 hours on days 0, 2, 4 and 6 .....	48
Fig. 2.16: Plasma testosterone levels of quail producing different amounts of cloacal foam over the treatment period .....	48
Fig. 2.17: Mean plasma corticosterone levels on days 0, 2, 4 and 6.....	50
Fig. 2.18: Areas under the plasma corticosterone versus time curves over the treatment period .....	52
Fig. 2.19: Mean plasma LH levels on days 0 and 6 for birds treated with daily injections of oil or 0.6 or 1.2 mg corticosterone.....	52
Fig. 2.20: Mean plasma testosterone levels on days 0, 2, 4 and 6 .....	54
Fig. 2.21: Areas under the testosterone versus time curves over the treatment period .....	56
Fig. 2.22: Body weights on day 6 relative to weight on day 0.....	58
Fig. 2.23: Paired testes weights on day 6.....	58

Fig. 2.24: Cloacal gland areas on days 0, 2, 4 and 6 relative to area on day 0.....	59
Fig. 3.1: Individual plasma corticosterone levels on day 6 .....	85
Fig. 3.2: Mean plasma corticosterone levels on day 6 .....	86
Fig. 3.3: Areas under the plasma corticosterone versus time curves for 0-12 hours and 12-24 hours on day 6. ....	86
Fig. 3.4: Mean faecal corticosterone levels on day 0. ....	89
Fig. 3.5: Individual plasma LH levels on day 6 .....	93
Fig. 3.6: Mean plasma LH levels on day 6. ....	94
Fig. 3.7: Areas under the plasma LH versus time curves on day 6. ....	96
Fig. 3.8: Individual plasma testosterone levels on day 0.....	97
Fig. 3.9: Mean plasma testosterone levels on day 0. ....	99
Fig. 3.10: Individual plasma testosterone levels on day 6. ....	101
Fig. 3.11: Mean plasma testosterone levels on days 0 and 6 for each treatment group .....	102
Fig. 3.12: Areas under the plasma testosterone versus time curves for 0-12 hours and 12-24 hours. ....	104

Fig. 3.13: Correlation between areas under the plasma corticosterone versus time curves 0-12 hours after a corticosterone injection and areas under the plasma testosterone versus time curves 0-12 hours on day 6.....	105
Fig. 4.1: Corticosterone response to handling curve for birds held on long days (LD 16:8) and sampled at time 0 and one subsequent time.....	128
Fig. 4.2: Plasma corticosterone levels in samples collected in $\leq 2$ minutes or $> 2$ minutes at 0, 15, 30 and 60 minutes .....	130
Fig. 4.3: Individual corticosterone response to handling curves for birds bled on three occasions.....	131
Fig. 4.4: Mean plasma corticosterone response to handling curves for birds bled on three occasions.....	133
Fig. 4.5: Areas under corticosterone response to handling curves on three bleeding sessions.....	135
Fig. 4.6: Individual corticosterone response to handling curves for birds sampled during the day and at night.....	138
Fig. 4.7: Mean corticosterone response to handling curves for birds bled on two occasions .....	139
Fig. 4.8: Areas under corticosterone response to handling curves during the day and at night.....	139

Fig. 4.9: Cloacal diameters from three to six weeks of age for birds with small gonads (from the studies of individual variation and day versus night) and large gonads .....	142
Fig. 4.10: Ovary and oviduct weights for birds with small gonads (from the studies of individual variation and day versus night) and large gonads.....	143
Fig. 4.11: Individual corticosterone response to handling curves for birds with large gonads.....	144
Fig. 4.12: Corticosterone response to handling curves for birds with small gonads (from the studies of individual variation and day versus night) and large gonads.....	145
Fig. 4.13: Areas under corticosterone response to handling curves for birds with small gonads (from the studies of individual variation and day versus night) and large gonads .....	145

## LIST OF TABLES

Table 2.1: Extraction efficiency table for testosterone and corticosterone extracted from quail plasma. ....	25
Table 2.2: Percentage of injected corticosterone that was excreted into the faeces of corticosterone treated birds during each 24 hour period over the six days of treatment .....	35
Table 2.3: Summary of two-way repeat measures ANOVA for plasma corticosterone levels on days 0, 2, 4 and 6 .....	51
Table 2.4: Summary of two-way repeat measures ANOVA for plasma LH levels on days 0 and 6 .....	53
Table 2.5: Summary of one-way repeat measures ANOVA for plasma testosterone levels on days 0, 2, 4 and 6 .....	55
Table 2.6: Summary of two-way repeat measures ANOVA for relative cloacal gland areas on days 2, 4 and 6 .....	60
Table 3.1: Summary of Mann-Whitney U tests for faecal corticosterone concentration (ng/g) and rate of excretion (ng/h) on day 0 .....	90
Table 3.2: Percentage of corticosterone excreted in the faeces in six-month-old and seven-week-old male quail during the 24 hours after an injection of corticosterone on day 0. ....	91
Table 3.3: Summary of two-way repeat measures ANOVA for plasma LH levels on day 6 .....	95

Table 3.4: Summary of two-way repeat measures ANOVA for plasma testosterone levels on day 0 .....	98
Table 3.5: Summary of two-way repeat measures ANOVAs comparing plasma testosterone levels on days 0 and 6 .....	103
Table 4.1: Sampling times for birds from the studies examining individual variation in corticosterone responses and responses during the day and at night. ....	125
Table 4.2: Correlations between plasma corticosterone levels and the length of time taken to collect each blood sample at 0, 15, 30 and 60 minutes. ....	129
Table 4.3: Summary of two-way repeat measures ANOVA for plasma corticosterone response curves on three bleeding sessions.....	134
Table 4.4: Variation in plasma corticosterone levels within and between birds at 0, 15, 30 and 60 minutes after handling. ....	136