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The effect of introduction to training on some haematological and immunological parameters in the thoroughbred horse

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The Effect of Introduction to Training on Some
Haematological and Immunological Parameters in the
Thoroughbred Horse



UNIVERSITY *of* LIMERICK

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Submitted in fulfilment of the requirements for the award of the Master of Science
(Equine Science) at the University of Limerick, 2010

Abstract

Good health of the racing horse is essential to optimal training and, thus, high levels of performance. However, epidemiological research has shown that respiratory disease, especially in young horses, is an important contributor to wastage in the industry, and, as such causes a significant economic loss to the thoroughbred industry. Both, acute and chronic exercise, have been associated with changes in immunological, haematological and biochemical parameters. A connection between exercise, disease and *in vitro* measurements has been suggested by research studies published to date. Mechanisms underlying these changes are complex and multifactorial, but have been linked to changes in the endocrine system as a response to the stress imposed on the body by exercise. The present study sought to investigate the effects of introduction to training on some immunological parameters in the thoroughbred horse. Blood samples from 20 thoroughbreds in National Hunt training in two local yards were taken prior to and after two, eight and ten weeks of training. Lymphoproliferative response against stimulation with Concanavalin A was examined by a nonradioactive assay immediately upon collection of the blood samples. The functioning of the innate immune system was evaluated by establishing phagocytosis and oxidative burst capacity of granulocytes and monocytes. Results showed that oxidative burst capacity of granulocytes and monocytes significantly increased during training (Yard One: $p < 0.05$; Yard Two: $p < 0.001$). Granulocyte and monocyte phagocytosis fluctuated during training ($p < 0.01$). Changes were associated with other external factors such as training intensity and duration. Lymphocyte proliferation was not significantly affected by chronic exercise training ($p > 0.05$). A second aim of this study was to assess the effect of introduction to training on some plasma haematological and biochemical parameters. Analysis of blood samples was undertaken by the Irish Equine Centre. Both, plasma haematology and biochemistry remained largely uninfluenced by chronic exercise training ($p > 0.05$). However, significant changes that resembled a 'stress leukogram' in neutrophil and lymphocyte percentage were observed in one yard during training ($p < 0.001$) which may indicate that factors such as training intensity moderate the effect of exercise on leukocytes. Elevated levels of GGT, total bilirubin and globulins were observed in horses during the training period examined. A trend towards decreasing levels of eosinophils, total bilirubin, total protein, albumin and globulins was observed in this study ($p < 0.05$). Also, a trend towards increasing levels of total erythrocytes, PCV and monocytes was detected in the present study, however, changes were non-significant ($p > 0.05$) except in the case of haemoglobin ($p < 0.05$). In summary, several components of the immune system appeared to change with training. However, components of the innate system seemed to be more susceptible to change than components of the adaptive immune system. Although the clinical consequences of these training-induced alterations of the immune response are not yet known, managers of horses should take into account that the immune system of a horse may be affected by chronic exercise training. This appears particularly important considering the importance of wastage in racehorses caused by infectious disease.

Declaration

The Effect of Introduction to Training on Some Haematological and Immunological Parameters in the Thoroughbred Horse

Supervisor: Prof Sean Arkins

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This dissertation is presented in fulfilment of the requirements for Master of Science in Equine Science. It is entirely my own work and has not been submitted to any other university or higher education institution, or for any other academic award in this university. Where use has been made of the work of other people it has been fully acknowledged and fully referenced.

Signed

Julia Luehr

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Table of Contents

Abstract	i
Declaration	ii
Acknowledgements	iii
Table of Contents	iv
List of Figures	viii
List of Tables	xi

CHAPTER ONE

LITERATURE REVIEW

Introduction	1
Wastage in Training	3
Cost of Wastage	4
Effects of Exercise and Training on the Immune System	4
Brief Introduction to the Immune System	5
Effects of Acute Exercise versus Chronic Exercise on Leukocyte Function	7
Effects of Acute Exercise on Lymphocyte Function	8
Human Studies	8
Animal Studies	8
Summary of the Effect of Acute Exercise on Lymphocyte Function	11
Effect of Chronic Exercise on Lymphocyte Function	12
Human Studies	12
Animal Studies	13
Summary of the Effect of Chronic Exercise on Lymphocyte Function	14
Effects of Acute Exercise on Peripheral Blood Granulocyte and Monocyte Function – Phagocytosis	15
Human Studies	15
Animal Studies	16
Summary of the Effect of Acute Exercise on Peripheral Blood Granulocyte and Monocyte Function - Phagocytosis	17
Effects of Chronic Exercise on Peripheral Blood Granulocyte and Monocyte Function – Phagocytosis	18
Human Studies	18
Animal Studies	19
Summary of the Effect of Chronic Exercise on Peripheral Blood Granulocyte and Monocyte Function - Phagocytosis	21
Effects of Acute Exercise on Peripheral Blood Granulocyte and Monocyte Function – Oxidative Burst Capacity	22
Human Studies	22
Animal Studies	23
Summary of the Effect of Acute Exercise on Peripheral Blood Granulocyte and Monocyte Function – Oxidative Burst Capacity	24
Effects of Chronic Exercise on Peripheral Blood Granulocyte and Monocyte Function – Oxidative Burst Capacity	25
Human Studies	25
Animal Studies	26

Summary of the Effect of Chronic Exercise on Peripheral Blood Granulocyte and Monocyte Function – Oxidative Burst Capacity	28
Effects of Acute and Chronic Exercise on Plasma Haematology	29
Total Erythrocytes	29
Packed Cell Volume (PCV)	30
Haemoglobin	30
Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH)	31
Platelets	31
Summary of the Effect of Exercise on Plasma Haematology	32
Total Leukocytes	32
Neutrophils	34
Lymphocytes	35
Monocytes	37
Eosinophils	37
Summary of the Effect of Exercise on Leukocyte Parameters	38
Effects of Acute and Chronic Exercise on Plasma Biochemistry	38
Total Protein	38
Albumin	39
Globulin	40
Summary of the Effect of Exercise on plasma protein parameters	40
Muscle Enzymes – Aspartate Transaminase (AST)	41
Muscle Enzymes – Creatine Kinase (CK)	42
Gamma-Glutamyl Transferase (GGT)	42
Total Bilirubin	43
Summary of the Effect of Exercise on Serum Enzymes	43
Electrolytes – Sodium (Na), Potassium (K), Chloride (Cl)	44
Summary of the Effect of Exercise on Plasma Electrolytes	44
Summary	46
CHAPTER TWO	
RESEARCH METHODOLOGY	
Research Objectives	48
Animals	48
Experimental Design and Blood Sampling	49
Training Schedule	49
Isolation of Lymphocytes / Peripheral Blood Cell Preparation	50
Lymphocyte Proliferation Assay	51
Flow Cytometric Analysis	51
Phagocytosis Assay	52
Oxidative Burst Capacity Assay	55
Haematology Analysis	59
Biochemistry Analysis	59
Data Analysis	60

CHAPTER THREE

RESEARCH FINDINGS AND ANALYSIS

Effect of Training on Plasma Haematology, Biochemistry and Immunological Parameters in the Horse	62
Effect of Training on T-Cell Proliferation	62
Effect of Training on Leukocyte Function – Phagocytosis	72
Granulocytes	72
Monocytes	74
Effect of Training on Leukocyte Function – Oxidative Burst Capacity	77
Oxidative Burst Capacity of Granulocytes – Yard One	77
Oxidative Burst Capacity of Monocytes – Yard One	81
Oxidative Burst Capacity of Granulocytes – Yard Two	87
Oxidative Burst Capacity of Monocytes – Yard Two	90
Effect of Training on Plasma Haematology	93
Red Blood Cells	93
Packed Cell Volume (PCV)	94
Haemoglobin	96
Mean Corpuscular Volume (MCV)	98
Mean Corpuscular Haemoglobin Concentration (MCHC)	99
Mean Corpuscular Haemoglobin (MCH)	102
Platelets	104
Total leukocytes	106
Neutrophils	107
Lymphocytes	111
Monocytes	114
Eosinophils	117
Effect of Training on Plasma Biochemistry	121
Total Protein	121
Albumin	123
Globulin	125
Aspartate Transaminase (AST)	127
Creatine Kinase (CK)	129
Gamma-Glutamyl Transferase (GGT)	131
Total Bilirubin	132
Sodium (Na)	134
Potassium (K)	136
Chloride (Cl)	138

CHAPTER FOUR

DISCUSSION

Introduction	141
Effect of Training on Lymphocyte Function	142
Effect of Training on Leukocyte Function – Phagocytosis	144
Granulocytes	144
Monocytes	146

Effect of Training on Leukocyte Function – Oxidative Burst Capacity	148
Granulocytes	148
Monocytes	149
Effect of Training on Plasma Haematology	151
Total Erythrocytes	151
Packed Cell Volume (PCV)	152
Haemoglobin	152
Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and	
Mean Corpuscular Haemoglobin (MCH)	153
Platelets	154
Total Leukocytes	154
Neutrophils	155
Lymphocytes	156
Monocytes	157
Eosinophils	158
Effect of Training on Plasma Biochemistry	159
Total Protein	159
Albumin	159
Globulin	160
Muscle Enzymes – Aspartate Transaminase (AST)	161
Muscle Enzymes – Creatine Kinase (CK)	161
Gamma-Glutamyl Transferase (GGT)	162
Total Bilirubin	162
Electrolytes – Sodium (Na), Potassium (K), Chloride (Cl)	163
CHAPTER FIVE	
CONCLUSIONS AND RECOMMENDATIONS	
Conclusions and Recommendations	165
REFERENCES	
Bibliography	168
APPENDICES	
Haematology Reference Values	177
Biochemistry Reference Values	178

List of Figures

CHAPTER TWO

RESEARCH METHODOLOGY

Figure 2.1 Gating on leukocytes	53
Figure 2.2 Gated on granulocytes (R6) and monocytes (R5)	53
Figure 2.3 Phagocytosis – Control Sample	54
Figure 2.4 Phagocytosis – Activated	54
Figure 2.5 Gated granulocytes	55
Figure 2.6 Gated monocytes	55
Figure 2.7 Gating on leukocytes	56
Figure 2.8 Oxidative Burst Capacity – Control Sample	57
Figure 2.9 Oxidative Burst Capacity – Positive Control	57
Figure 2.10 Oxidative Burst Capacity – Test Sample	57
Figure 2.11 Gated granulocytes (Positive Control)	58
Figure 2.12 Gated monocytes (Positive Control)	58
Figure 2.13 Gated granulocytes (Test Sample)	59
Figure 2.14 Gated monocytes (Test Sample)	59

CHAPTER THREE

RESEARCH FINDINGS AND ANALYSIS

Figure 3.1 Average T-cell proliferation (mean) in response to stimulation with Con A throughout training period	63
Figure 3.2 Mean T-cell proliferation in response to stimulation with 10µg/ml Con A throughout training period	65
Figure 3.3 Mean T-cell proliferation in response to stimulation with 5 µg/ml Con A throughout training period	66
Figure 3.4 Mean T-cell proliferation in response to stimulation with 2.5 µg/ml Con A throughout training period	68
Figure 3.5 Mean T-cell proliferation in response to stimulation with 1.25 µg/ml Con A throughout training period	70
Figure 3.6 Mean T-cell proliferation in response to stimulation with 0.625 µg/ml Con A throughout training period	71

Figure 3.7 Mean percentage of phagocytosing granulocytes throughout training period	74
Figure 3.8 Mean percentage of phagocytosing monocytes throughout training period	76
Figure 3.9 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria in week zero and week two of training – Yard One	78
Figure 3.10 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria in week zero and week eight of training – Yard One	78
Figure 3.11 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria in week zero and week ten of training – Yard One	79
Figure 3.12 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria throughout training period – Yard One	80
Figure 3.13 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with PMA in week zero and week two of training – Yard One	81
Figure 3.14 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria in week zero and week two of training – Yard One	82
Figure 3.15 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria in week zero and week eight of training – Yard One	83
Figure 3.16 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria in week zero and week ten of training – Yard One	83
Figure 3.17 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria throughout training period – Yard One	84
Figure 3.18 Mean oxidative burst capacity (%) of monocytes in response to stimulation with PMA in week zero and week two of training – Yard One	86
Figure 3.19 Mean oxidative burst capacity (%) of monocytes in response to stimulation with PMA in week zero and week ten of training – Yard One	86
Figure 3.20 Mean oxidative burst capacity (%) of monocytes in response to stimulation with PMA in week two and week ten of training – Yard One	87
Figure 3.21 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria throughout training period – Yard Two	88
Figure 3.22 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with PMA throughout training period – Yard Two	90
Figure 3.23 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria throughout training period – Yard Two	91
Figure 3.24 Mean oxidative burst capacity (%) of monocytes in response to stimulation with PMA throughout training period – Yard Two	92
Figure 3.25 Mean total erythrocyte levels throughout training period	94
Figure 3.26 Mean packed cell volume levels throughout training period	96
Figure 3.27 Mean haemoglobin levels throughout training period	98
Figure 3.28 Mean MCV levels throughout training period	99

Figure 3.29 Mean MCHC levels throughout training period	101
Figure 3.30 Mean MCH levels throughout training period	104
Figure 3.31 Mean platelet levels throughout training period	106
Figure 3.32 Mean total leukocyte levels throughout training period	107
Figure 3.33 Mean neutrophil levels throughout training period	109
Figure 3.34 Mean neutrophil percentage levels throughout training period	110
Figure 3.35 Mean lymphocyte levels throughout training period	112
Figure 3.36 Mean lymphocyte percentage levels throughout training period	114
Figure 3.37 Monocyte levels throughout training period	115
Figure 3.38 Mean monocyte percentage levels throughout training period	117
Figure 3.39 Mean eosinophil levels throughout training period	119
Figure 3.40 Mean eosinophil percentage levels throughout training period	120
Figure 3.41 Mean total protein levels throughout training period	123
Figure 3.40 Mean albumin levels throughout training period	125
Figure 3.43 Mean globulin levels throughout training period	127
Figure 3.44 Mean AST levels throughout training period	129
Figure 3.45 Mean CK levels throughout training period	131
Figure 3.46 Mean GGT levels throughout training period	132
Figure 3.47 Mean total bilirubin levels throughout training	134
Figure 3.48 Mean sodium levels throughout training period	136
Figure 3.49 Mean potassium levels throughout training period	138
Figure 3.48 Mean chloride levels throughout training	140

List of Tables

CHAPTER ONE

LITERATURE REVIEW

Table 1.1 Effects of acute exercise on equine lymphocyte proliferative response	11
Table 1.2 Effect of chronic exercise training on equine lymphocyte proliferative response	13
Table 1.3 Effect of chronic exercise training on equine granulocyte phagocytosis	21
Table 1.4 Effect of chronic exercise training on equine granulocyte oxidative burst capacity	28
Table 1.5 Effects of exercise on plasma haematology	32
Table 1.6 Effects of exercise on leukocyte parameters	38
Table 1.7 Effects of exercise on plasma protein parameters	40
Table 1.8 Effects of exercise on serum enzymes	44
Table 1.9 Effects of exercise on plasma electrolytes	45

CHAPTER TWO

RESEARCH METHODOLOGY

Table 2.1 Training schedule in yard one	50
Table 2.2 Training schedule in yard two	50
Table 2.3 Post hoc comparisons	61

CHAPTER THREE

RESEARCH FINDINGS AND ANALYSIS

Table 3.1 Descriptive Statistics – Average T-cell proliferation	62
Table 3.2 Average T-cell proliferation – Change throughout training period and difference between yard one and yard two	63
Table 3.3 Descriptive Statistics – T-cell proliferation– 10 µg/ml Con A	64
Table 3.4 T-cell proliferation (10 µg/ml Con A) – Change throughout training period and difference between yard one and yard two	64
Table 3.5 Descriptive Statistics – T-cell proliferation – 5 µg/ml Con A	65
Table 3.6 T-cell proliferation (5 µg/ml Con A) – Change throughout training period and difference between yard one and yard two	66

Table 3.7 T-cell proliferation (5 µg/ml Con A) change throughout training period by weeks	66
Table 3.8 Descriptive Statistics – T-cell proliferation – 2.5 µg/ml Con A	67
Table 3.9 T-cell proliferation (2.5 µg/ml Con A) – Change throughout training period and difference between yard one and yard two	67
Table 3.10 T-cell proliferation (2.5 µg/ml Con A) change throughout training period by weeks	68
Table 3.11 Descriptive Statistics – T-cell proliferation – 1.25 µg/ml Con A	69
Table 3.12 T-cell proliferation (1.25 µg/ml Con A) – Change throughout training period and difference between yard one and yard two	69
Table 3.13 Descriptive Statistics – T-cell proliferation – 0.625 µg/ml Con A	70
Table 3.14 T-cell proliferation (0.625 µg/ml Con A) – Change throughout training period and difference between yard one and yard two	71
Table 3.15 Descriptive Statistics – Granulocyte phagocytosis	72
Table 3.16 Granulocyte Phagocytosis – Change throughout training period and difference between yard one and yard two	73
Table 3.17 Granulocyte phagocytosis change throughout training period by weeks	73
Table 3.18 Descriptive Statistics – Monocyte phagocytosis	74
Table 3.19 Monocyte Phagocytosis – Change throughout training period and difference between yard one and yard two	75
Table 3.20 Monocyte phagocytosis change throughout training period by weeks	75
Table 3.21 Descriptive Statistics – Granulocyte oxidative burst capacity	77
Table 3.22 Granulocyte oxidative burst capacity change throughout training period by weeks	77
Table 3.23 Granulocyte oxidative burst capacity– Change throughout training period (n=4)	79
Table 3.24 Descriptive Statistics – Granulocyte oxidative burst capacity	80
Table 3.25 Granulocyte oxidative burst capacity change throughout training period by weeks	81
Table 3.26 Descriptive Statistics – Monocyte oxidative burst capacity	81
Table 3.27 Monocyte oxidative burst capacity change throughout training period by weeks	82
Table 3.28 Monocyte oxidative burst capacity – Change throughout training period (n = 4)	84
Table 3.29 Descriptive Statistics – Monocyte oxidative burst capacity	85
Table 3.30 Monocyte oxidative burst capacity change throughout training period by weeks	85
Table 3.31 Descriptive Statistics – Granulocyte oxidative burst capacity	87
Table 3.32 Granulocyte oxidative burst capacity – Change throughout training period	88
Table 3.33 Granulocyte oxidative burst capacity change throughout training period by weeks	88

Table 3.34 Descriptive Statistics – Granulocyte oxidative burst capacity	89
Table 3.35 Granulocyte oxidative burst capacity – Change throughout training period and difference between yard one and yard two	89
Table 3.36 Granulocyte oxidative burst capacity change throughout training period by weeks	89
Table 3.37 Descriptive Statistics – Monocyte oxidative burst capacity	90
Table 3.38 Monocyte oxidative burst capacity – Change throughout training period	91
Table 3.39 Monocyte oxidative burst capacity change throughout training period by weeks	91
Table 3.40 Descriptive Statistics – Monocyte oxidative burst capacity	92
Table 3.41 Monocyte oxidative burst capacity – Change throughout training period and difference between yard one and yard two	92
Table 3.42 Descriptive Statistics – Total erythrocytes	93
Table 3.43 Total Erythrocytes – Change throughout training period and difference between yard one and yard two	94
Table 3.44 Descriptive Statistics – Packed cell volume	95
Table 3.45 Packed Cell Volume – Change throughout training period and difference between yard one and yard two	95
Table 3.46 Descriptive Statistics – Haemoglobin concentration	96
Table 3.47 Haemoglobin – Change throughout training period and difference between yard one and yard two	97
Table 3.48 Haemoglobin change throughout training period by weeks	97
Table 3.49 Descriptive Statistics – MCV	98
Table 3.50 MCV – Change throughout training period and difference between yard one and yard two	99
Table 3.51 Descriptive Statistics – MCHC	100
Table 3.52 MCHC – Change throughout training period and difference between yard one and yard two	100
Table 3.53 MCHC change throughout training period by weeks	101
Table 3.54 Descriptive Statistics – Mean corpuscular haemoglobin	102
Table 3.55 MCH – Change throughout training period and difference between yard one and yard two	103
Table 3.56 MCH change throughout training period by weeks	103
Table 3.57 Descriptive Statistics – Platelet counts	104
Table 3.58 Platelets – Change throughout training period and difference between yard one and yard two	105

Table 3.59 Platelets change throughout training period by weeks	105
Table 3.60 Descriptive Statistics – Total leukocytes	106
Table 3.61 Total Leukocytes – Change throughout training period and difference between yard one and yard two	107
Table 3.62 Descriptive Statistics – Neutrophils	108
Table 3.63 Neutrophils – Change throughout training period and difference between yard one and yard two	108
Table 3.64 Descriptive Statistics – Neutrophils	109
Table 3.65 Neutrophil % – Change throughout training period and difference between yard one and yard two	110
Table 3.66 Neutrophil % change throughout training period by weeks	110
Table 3.67 Descriptive Statistics – Lymphocytes	111
Table 3.68 Lymphocytes – Change throughout training period and difference between yard one and yard two	111
Table 3.69 Descriptive Statistics – Lymphocytes	112
Table 3.70 Lymphocyte % – Change throughout training period and difference between yard one and yard two	113
Table 3.71 Lymphocyte % change throughout training period by weeks	113
Table 3.72 Descriptive Statistics – Monocytes	114
Table 3.73 Monocytes – Change throughout training period and difference between yard one and yard two	115
Table 3.74 Descriptive Statistics – Monocytes	116
Table 3.75 Monocyte % – Change throughout training period and difference between yard one and yard two	116
Table 3.76 Descriptive Statistics – Eosinophils	117
Table 3.77 Eosinophils – Change throughout training period and difference between yard one and yard two	118
Table 3.78 Eosinophils change throughout training period by weeks	118
Table 3.79 Descriptive Statistics – Eosinophils	119
Table 3.80 Eosionophil % – Change throughout training period and difference between yard one and yard two	120
Table 3.81 Eosinophil % change throughout training period by weeks	120
Table 3.82 Descriptive Statistics – Total Protein	121
Table 3.83 Total Protein – Change throughout training period and difference between yard one and yard two	122

Table 3.84 Total protein change throughout training period by weeks	122
Table 3.85 Descriptive Statistics – Albumin	123
Table 3.86 Albumin – Change throughout training period and difference between yard one and yard two	124
Table 3.87 Albumin change throughout training period by weeks	124
Table 3.88 Descriptive Statistics – Globulin	125
Table 3.89 Globulin – Change throughout training period and difference between yard one and yard two	126
Table 3.90 Globulin change throughout training period by weeks	126
Table 3.91 Descriptive Statistics – AST	127
Table 3.92 AST – Change throughout training period and difference between yard one and yard two	128
Table 3.93 AST change throughout training period by weeks	128
Table 3.94 Descriptive Statistics – CK	129
Table 3.95 CK – Change throughout training period and difference between yard one and yard two	130
Table 3.96 CK change throughout training period by weeks	130
Table 3.97 Descriptive Statistics – GGT	131
Table 3.98 GGT – Change throughout training period and difference between yard one and yard two	132
Table 3.99 Descriptive Statistics – Total Bilirubin	133
Table 3.100 Total Bilirubin – Change throughout training period and difference between yard one and yard two	133
Table 3.101 Total Bilirubin change throughout training period by weeks	133
Table 3.102 Descriptive Statistics – Sodium	134
Table 3.103 Sodium – Change throughout training period and difference between yard one and yard two	135
Table 3.104 Sodium change throughout training period by weeks	135
Table 3.105 Descriptive Statistics – Potassium	136
Table 3.106 Potassium – Change throughout training period and difference between yard one and yard two	137
Table 3.107 Potassium change throughout training period by weeks	137
Table 3.108 Descriptive Statistics – Chloride	138

Table 3.109 Chloride – Change throughout training period and difference between yard one and yard two 139

Table 3.110 Chloride change throughout training period by weeks 139

APPENDICES

Table A Reference Values for the Resting Haemogram in Normal Adult Thoroughbred Horses 177

Table B Normal Ranges for Plasma Biochemical Measurements in Mature Performance Horses 178

Chapter One

Literature Review

Introduction

Good health of the racing horse is essential to optimal training and, thus, high levels of performance (Vigre *et al.*, 2002). However, epidemiological studies have established that respiratory infections of racehorses during training are an important contributor to wastage in the thoroughbred industry (Jeffcott *et al.*, 1982; Bailey *et al.*, 1999; Hernandez and Hawkins, 2001; Perkins *et al.*, 2005a; Perkins *et al.*, 2005b; Wilsher *et al.*, 2006). Young horses, i.e. those being introduced to training, were reported to be more susceptible to contracting respiratory disease than older ones (Rossdale *et al.*, 1985; Bailey *et al.*, 1999; Morley *et al.*, 2000b; Wilsher *et al.*, 2006). Incidence of respiratory disease during training was associated with substantial costs connected to veterinary care, failure to race or perform as well as premature retirement and, hence, the loss of the potential to earn prize money (Bailey *et al.*, 1999; Perkins *et al.*, 2005a). Investigating the link between exercise and the immune system and improving understanding of the mechanisms underlying this relationship, is, therefore, warranted.

Exercise has been widely recognised as a stress, which may affect the immune system and, thus, an individual's susceptibility to disease (Klarlund Pedersen and Hoffman-Goetz, 2000; Raidal *et al.*, 2000a; Hines, *et al.*, 2008). Whereas acute exercise has been found to cause transient suppression of immunological, haematological and biochemical parameters, much less is known regarding the effects of chronic exercise training on these parameters (Pedersen and Hoffman-Goetz, 2000). It appears that chronic exercise has fewer effects on immunological, haematological and biochemical (Rose and Hodgson, 1982; Tyler-McGowan *et al.*, 1999) parameters. However, moderate training is generally considered to have beneficial effects (Hines *et al.*, 2008), while prolonged high-intensity training may cause slight impairment of immunological parameters (Mackinnon, 2000; Gleeson, 2007). A similar effect has been described in studies investigating the effects of acute exercise on immunity (Nieman, 1997; Pedersen and Hoffman-Goetz, 2000; Nieman, 2000; Gleeson, 2007; Hines *et al.*, 2008). The scientific literature therefore suggests that the effects of exercise and training on the immune system can be rather contradictory and, as previously indicated, are complex and dependent upon multiple factors, including (i) the effects of acute exercise versus chronic exercise; (ii) the immunological parameter

analysed; (iii) the intensity, duration and type of the exercise; (iv) the timing of the measurement of immune function in relation to completion of the exercise bout; and (v) the age of the individuals studied (Nieman, 2000; Horohov, 2004; Malm, 2004). The mechanisms behind changes in immunological parameters are multifactorial and complex, though changes in many parameters are mainly associated with changes in the endocrine system as a result of exercise stress (MacKinnon, 2000, Pedersen and Hoffman-Goetz, 2000; Hines *et al.*, 2008).

This study aims to investigate the hypothesis that introduction to training in the horse is associated with immunosuppression. The previous, brief introduction of the background of this study indicates some of the changes in the horse's immune system thought to take place with acute and chronic exercise. Based on literature, this study sought to examine the effects of chronic exercise training on components of, both, the innate immune system (phagocytosis and oxidative burst capacity of granulocytes and monocytes) and the adaptive immune system (mitogen-induced T-lymphocyte proliferation). Additionally, this study investigated the effects of chronic exercise training on several biochemical and haematological variables.

Wastage in Training

Increased susceptibility of racehorses in training to respiratory infections has been widely recognised within the industry (Morley *et al.*, 2000a; Wood *et al.*, 2005) with young horses and those with low antibody concentrations appearing to be especially at risk to viral upper respiratory tract infection (Morley *et al.*, 2000b).

Epidemiological studies have confirmed that respiratory infections of racehorses during training are an important contributor to wastage in the thoroughbred industry. Several studies conducted in the UK, the United States, New Zealand and Australia found that respiratory infections are the second most prevalent cause of wastage and lost racing and training performance in the thoroughbred industry after musculoskeletal injuries (Jeffcott *et al.*, 1982; Bailey *et al.*, 1999; Hernandez and Hawkins, 2001; Perkins *et al.*, 2004a; Perkins *et al.*, 2004b; Wilsher *et al.*, 2006). Also, it is important to note that respiratory infections were more common in two year olds than in older horses, i.e. after introduction to training (Rossdale *et al.*, 1985; Bailey *et al.*, 1999; Wilsher *et al.*, 2006). Respiratory viruses have been found to be the primary cause of disease in Australian racehorses (Christley *et al.*, 2000). An Australian survey further established that racehorse trainers perceived respiratory infection to be the most common cause of wastage in the industry followed by general lameness and shin soreness (Bailey *et al.*, 2008). Rossdale *et al.* (1985) reported that 8.5 % out of 114,919 available training days of British racehorses were lost due to injury or disease in 1982 and 1983. While lameness accounted for 67.6 % of days lost, respiratory problems accounted for 20.5 %. In an Australian study, Bailey *et al.* (1999) found that the total number of modified training days of a cohort of two and three year old racehorses was 1075. This figure amounted to 2.7 % of available training days. Of the total days modified, 15.8 % were due to cough and nasal discharge. Also, 24.4 % of the total training days lost was due to respiratory conditions, while 5.4 % of total reduced training days were accounted for by coughing and nasal discharge. If the horses had to rest at pasture due to cough and nasal discharge, the median duration of this spell was 6.5 weeks. The perception of Australian trainers that respiratory conditions were the major cause of wastage might be explained by the finding that shin soreness rarely resulted in the prevention of training, whereas coughing and nasal discharge established their major effect by

preventing training from occurring at all. Respiratory disease of pinhooked yearlings in the U.S. resulted in a total loss of 126 training days (Hernandez and Hawkins, 2001). In New Zealand, Perkins *et al.* (2005a) determined that involuntary spells resulting from respiratory conditions accounted for 7,984 out of 67,853 horse days. Furthermore, 22.4 % of all exits (deaths or retirement) happened as a result of respiratory conditions (Perkins *et al.*, 2005b). Poor racing performance was also linked to respiratory conditions (Morris and Seeherman, 1991; Bourke, 1995; Knight and Evans, 2008).

Cost of Wastage

Lost training days, poor performance and the end of the racing career incur costs associated with veterinary care, failure to race or perform as well as premature retirement and, hence, the loss of the potential to earn prize money (Bailey *et al.*, 1999; Perkins *et al.*, 2005b). Jeffcott *et al.* (1982) further stated that the economic loss of wastage in the industry amounted to a total of at least £15.2 million for the 1974 season and its progeny. Keep and training fees of horses that did not run amounted to £3.7 million of the financial cost.

The economic implementations of lost training days and wastage associated with disease as well as the welfare issue arising from disease as a result of exercise warrant further research into this area. Hence, a multitude of research projects have examined the effects of exercise on the immune system in humans and horses to date.

Effects of Exercise and Training on the Immune System

As epidemiological studies have linked exercise to an increased risk of respiratory infections, it has been suggested that parameters measuring immune function in vitro should, thus, be adversely affected by intensive exercise, whereas moderate exercise should show a beneficial effect (Nieman, 1995). A study by Folsom *et al.* (2001) found vaccinated ponies were susceptible to equine influenza virus infection after having been subjected to a five day strenuous exercise programme, whereas non-exercise control ponies remained protected. The susceptibility to infection of exercised ponies was also marked by a significant suppression of the T-cell mediated

immune response to equine influenza virus in vitro, thus indicating the connection between exercise, disease and in vitro measures of immune function.

Brief Introduction to the Immune System

Exercise is considered a physical stressor and as such has been shown to have profound effects on the immune response of many species (Woods *et al.*, 2000; MacKinnon, 2000; Pedersen and Hoffman-Goetz, 2000; Folsom *et al.*, 2001; Cappelli *et al.*, 2007). The immune response is regulated by the immune system, which consists of a complex network of cells and tissues that, both peripherally and autonomously, interact with each other to elicit an appropriate host response to infection or cancer (Woods *et al.*, 2000). Different cell types in the immune system are specialised to perform a particular function and work together to create the integrated immune response (Kadowaki *et al.*, 2000). Mechanisms that determine the functioning of the immune system can either be innate or adaptive (Medzhitov and Janeway, 1997; Horohov, 2004). The innate immune response is non-specific, thus, the induction of the response does not require previous exposure to the invading organism (Horohov, 2004). Innate immunity plays an important role in directly eliminating pathogens and in determining the initiation and type of adaptive immune responses (Kadowaki *et al.*, 2000; Horohov, 2004). Hence, the innate immune response proceeds, and is necessary for, the adaptive immune response (Medzhitov and Janeway, 1997). The principal cells that mediate innate or non-specific immunity are neutrophils, macrophages, and natural killer (NK) cells. Therefore, these cells constitute the first line of defence against invading pathogens (Robson *et al.*, 2003; da Costa and Carvalho, 2003; Escribano *et al.*, 2005a; Ferreira-Dias *et al.*, 2005; Yamamoto *et al.*, 2008), migrating to the affected area in response to chemotactic factors released during the inflammatory response (Raidal *et al.*, 1998; da Costa and Carvalho, 2003).

A key aspect of neutrophil and macrophage function is the phagocytic process – chemotaxis, adherence, phagocytosis, degranulation and the respiratory burst (Pyne *et al.*, 2000), which are essential for the eradication of invading organisms (Raidal *et al.*, 1998; Woods *et al.*, 2000; da Costa and Carvalho, 2003; Kampen *et al.*, 2004; Yamamoto *et al.*, 2008). Within the co-ordinated sequence of steps of the phagocytic

process, the activity of the neutrophil respiratory burst can be described as the limiting factor in the cytotoxic response (Pyne *et al.*, 2000). Monocytes constitute an immature form of macrophage in peripheral blood, which upon entering tissues alter their function and differentiate into macrophages (Woods *et al.*, 2000). Natural killer (NK) cells are a type of lymphocytes capable of killing cells without previous antigenic stimulation and are, therefore, part of the innate immune system, however, as other parts of the innate immune system, NK cells interact with the adaptive immune system (Raulet, 2004). The adaptive immune response is highly specific and remembers invading organisms that it has previously encountered. The immune response to a previously encountered pathogen is augmented due to the immunological memory of the adaptive immune system, which is the basis for vaccination (Gourley *et al.*, 2004). The adaptive or specific immune response is mediated by humoral and cellular components. The humoral immune response consists of B-lymphocytes, which on contact with antigen, proliferate and differentiate into plasma cells that produce antigen-specific antibodies (immunoglobulins). Immunoglobulins are specific to the antigen and are, thus, able to neutralise and/or eliminate it (Horohov, 2004). Pre-existing antibodies represent the humoral part of immunological memory (Gourley *et al.*, 2004). The cell-mediated immune response is composed of cytotoxic T-lymphocytes, which are antigen specific and exhibit immunological memory, and T-helper lymphocytes. T-helper lymphocytes produce various cytokines, which control growth, differentiation, and activation state of various cells of the immune system (Horohov, 2004).

The functioning of the innate immune response can be assessed *in vitro* by measuring phagocytic ability or oxidative burst capacity of neutrophils and macrophages (Wong *et al.*, 1990; Woods *et al.*, 2000; Robson *et al.*, 2003; da Costa and Carvalho, 2003; Escribano *et al.*, 2005a; Yamamoto *et al.*, 2008). Furthermore, the cytotoxic ability of NK cells can be assessed to provide information on NK cell lytic activity (Hines *et al.*, 2008). To provide information about the functioning of the adaptive immune response, the ability of lymphocytes to proliferate in response to stimulation with a mitogen can be assessed *in vitro* (Ansar Ahmed *et al.*, 1994; Gogal *et al.*, 1997). Mitogens (Saker *et al.*, 2001) or antigens (Hines *et al.*, 2008) stimulate lymphocyte proliferation in cell culture. Mitogens used to stimulate lymphoproliferative response include, for example, concanavalin A (Con A), pokeweed mitogen (PWM) or

phytohaemagglutinin (PHA) (Nesse *et al.*, 2002). The ability of lymphocytes to produce cytokines in response to various mitogens and specific antigens can also be assessed in vitro (Hines *et al.*, 2008).

Effects of Acute Exercise versus Chronic Exercise on Leukocyte Function

It has been suggested that acute exercise leads to changes in leukocyte function for an 'open window' of several hours during recovery from high-intensity exercise (Nieman, 1997; Nieman, 2000; Pedersen and Hoffman-Goetz, 2000; Gleeson, 2007; Hines *et al.*, 2008). Mechanisms responsible for changes in leukocyte numbers and function following acute exercise are believed to be multifactorial and complex; although, alterations in neuroendocrine hormones, in particular, catecholamines and corticosteroids, in response to an exercise challenge have been associated with transient immunosuppression (Mackinnon, 2000, Pedersen and Hoffman-Goetz, 2000; Hines *et al.*, 2008). Furthermore, changes in levels of β -endorphins, cytokines, and sex steroids have been linked to altered leukocyte numbers and function after acute exercise (Mackinnon, 2000, Pedersen and Hoffman-Goetz, 2000; Hines *et al.*, 2008).

In general, and as previously indicated, moderate training is considered to have beneficial effects on host defence mechanisms (Hines *et al.*, 2008), whereas prolonged periods of high-intensity training may lead to slight impairment of several immune parameters (Mackinnon, 2000). However, compared to the large amounts of accrued data relating to the effects of acute exercise on the immune response, much less is known regarding the effect of training on the immune function (Pedersen and Hoffman-Goetz, 2000). Alterations in cellular function as a response to chronic exercise challenge are also probably mediated by endocrine response to exercise (Raidal *et al.*, 2000a). As previously described, immunosuppression, as indicated by measurements of immune function in vitro, could present the link between increased risk of respiratory infection and exercise.

Effects of Acute Exercise on Lymphocyte Function

Human Studies

Several recent research studies on the immune function in sports and exercise described a decrease in mitogen-induced lymphocyte proliferation following an acute bout of high-intensity exercise (Nielsen and Pedersen, 1997; Nieman and Pedersen, 1999; Mackinnon, 2000; Pedersen and Hoffman-Goetz, 2000; Nieman, 2000, Gleeson, 2007).

Other research studies also reported a transient reduction in lymphocyte mitogenic response following acute exercise (Hinton *et al.*, 1997; Ceddia *et al.*, 1999; Green *et al.*, 2002). However, Ceddia *et al.* (1999) also observed that findings depended on the mitogen used, mitogen concentration and age.

Espersen *et al.* (2007) observed that mitogen-induced lymphocyte proliferation in response to stimulation with PHA, Con A and PWM had increased two hours after intense exercise, but returned to resting levels 24 hours post-exercise. The subjects tested were eleven elite middle- and long-distance runners taking part in a 5 km race on a running track.

Animal Studies

Similar to the effect of acute exercise on mitogen-induced lymphocyte proliferation in the human, scientific evidence suggested that lymphocyte proliferation in the horse decreases post-exercise and, as such, could be responsible for the previously described increased risk of respiratory infection in equine athletes.

Kurcz *et al.* (1988) examined the effect of acute exercise on cell-mediated immune response in four Quarter Horse mares aged five to nine years. Prior to the high-intensity exercise challenge, test horses were conditioned on a treadmill on an 11 % incline for four weeks. Lymphoproliferative response to Con A was reduced significantly 30 minutes post-exercise, but not 15 minutes post-exercise, and had returned to pre-exercise values 24 hours after the exercise challenge. Lymphoproliferative response to PHA did not show any significant changes at a 95 % confidence interval when comparing pre- and post-exercise values. Transient

reduction in lymphoproliferative response after exercise coincided with elevated cortisol levels. Interactions with glucocorticoids and specific receptors on lymphoid cells leading to possible inhibitory effects on IL-1 synthesis as well as on IL-2 and its receptor had been suggested as the cause for suppression of lymphoid activity.

Keadle *et al.* (1993) investigated the effect of acute exercise on lymphoproliferative response to PWM and equine influenza virus in six unconditioned Thoroughbreds. The exercise challenge was conducted on a high-speed treadmill with peak heart rate values exceeding 200 bpm. Blood samples were collected immediately after exercise, 20 minutes and 120 minutes post-exercise. A significant decrease in lymphoproliferative response to both PWM and equine influenza virus was recorded immediately post-exercise relative to pre-exercise values, but not at 20 or 120 minutes post-exercise. Increases in plasma cortisol were linked to a decreased lymphoproliferative response after exercise.

Horohov *et al.* (1999) examined the effect of exercise on the immune response of six young and six aged horses at different maximum heart rates (160, 180, and 200 beats/min (bpm)) on a high-speed treadmill. Horses in both groups were female Standardbreds or Thoroughbreds. In the younger horses, levels of mitogen-induced lymphocyte proliferation were found to be significantly decreased immediately after exercise when compared to pre-exercise levels. The lymphoproliferative response to PHA and PWM was significantly decreased post-exercise at a maximum heart rate of 200 bpm, but not at 160 and 180 bpm, whereas the lymphoproliferative response to Con A was found to have decreased after exercise at maximum heart rates of 180 and 200 bpm. Although older horses had significantly lower proliferative responses prior to exercise than younger horses, lymphoproliferative response of older horses remained unchanged after exercise. As indicated by Keadle *et al.* (1993), corticosteroids were suggested to have caused transient exercise-induced immunosuppression by suppressing production of IL-2 and other cytokines.

Lunn *et al.* (2001), who examined the lymphoproliferative response of five ponies to PWM, found that these responses were significantly depressed after exercise during a five day strenuous exercise programme on a high-speed treadmill indicating that exercise caused immunosuppression. It was interesting to note that vaccination

(modified-live virus) against equine influenza virus during the period of transient immunosuppression was concluded to be safe according to their research findings.

Nesse *et al.* (2002) examined the effects of racing on lymphocyte proliferation in eight trained Thoroughbreds. Each horse competed in one to three races. Lymphoproliferative response to the mitogens PHA, PWM and Con A were significantly reduced 12 to 16 hours after racing when compared to pre-race values, however, lymphocyte proliferation response 40 to 60 minutes after the race remained unchanged. These results were probably connected to increased plasma cortisol and other neuroendocrine hormone levels after exercise. As previously indicated, other studies by Kurcz *et al.* (1988), Keadle *et al.* (1993), Horohov *et al.* (1999) and Lunn *et al.* (2001) differed from these findings in that a reduction in lymphoproliferative response became apparent as early as 0 to 30 minutes after exercise was completed.

As described in an earlier section, when comparing the equine influenza virus-specific lymphoproliferative response of four immunised, mixed-breed ponies after a five day strenuous exercise programme on a high-performance treadmill to both their own pre-exercise response and those of four rested ponies, the proliferative response was significantly reduced. Blood samples for the measurement of antigen-specific lymphoproliferative response were collected on day 1 and day 5 prior to exercise (Folsom *et al.*, 2001). In contrast, a study by Wong *et al.* (1992) using eight three to seven year old Thoroughbreds that were subjected to a single bout of high-intensity exercise observed that mitogen-induced (PHA, PWM and Con A) lymphocyte proliferation response remained unchanged in response to exercise (30 min, 6, 24, 48, 72 and 120 hours post-exercise). Various exercise-induced mediators, other than cortisol alone, were thought to have affected results. Table 1.0 summarises the effects of acute exercise on equine lymphocyte proliferative response taking breed, age, gender and exercise regime into account, which were found to vary across all research studies.

Table 1.1 Effects of acute exercise on equine lymphocyte proliferative response**(n number of subjects, Stim. Stimulant, time time of blood sampling after exercise challenge)**

Reference	Exercise	N	Subject	Stim.	Time	Effect	Notes
Kurcz <i>et al.</i> (1988)	High speed treadmill (11%) incline until fatigue at 4.5 m/s	4	Quarter Horse mares, 5 - 9 years; conditioned for 4 wks	Con A, PHA	15,30,1440 min	- ↓ - - - -	carried 27kg lead
Wong <i>et al.</i> (1992)	High intensity exercise bout on high speed treadmill	8	Thoroughbreds, 3 - 7years	PHA, PWM, Con A	30 min to 120 hours	- - -	
Keadle <i>et al.</i> (1993)	High speed treadmill; peak heart rate values exceeded 200bpm	6	Thoroughbreds, 2 mares (5-6yrs) and 4 geldings (6-10yrs); unconditioned	PWM, EIV	0,20,120 min	↓ - - ↓ - -	
Horohov <i>et al.</i> (1999)	High speed treadmill until target heart rate of 160, 180 or 200bpm was achieved	6 (young) 6 (aged)	Female Standarbreds or Thoroughbreds	PHA, PWM, Con A	0-30 min	↓ ↓ ↓	200bpm 200bpm 180bpm, 200bpm
Lunn <i>et al.</i> (2001)	5 day strenuous exercise programme on high speed treadmill	5	Mixed breed ponies, 9-15 mths, either sex	PWM	Day 1,2,4; 0 min	↓ ↓ ↓	
Nesse <i>et al.</i> (2002)	1 to 3 races/horse	8	Thoroughbreds, 4 mares & 4 geldings, 2-9yrs; conditioned	PHA, PWM, Con A	40-60 min, 12-16 h	- ↓ - ↓ - ↓	
Folsom <i>et al.</i> (2001)	5 day strenuous exercise programme on high speed treadmill	8	Mixed breed ponies, 4 exercised and 4 non-exercised	EIV	at rest (Day 1&5)	↓ ↓	

Summary of the Effect of Acute Exercise on Lymphocyte Function

It has been shown that in, both, humans and horses, acute exercise was mostly associated with a transient decrease (Kurcz *et al.*, 1988; Keadle *et al.*, 1993; Hinton *et al.*, 1997; Nielsen and Pedersen, 1997; Ceddia *et al.*, 1999; Horohov *et al.*, 1999; Nieman and Pedersen, 1999; Mackinnon, 1999; Klarlund Pedersen and Hoffman-Goetz, 2000; Nieman, 2000; Lunn *et al.*, 2001; Folsom *et al.*, 2001; Nesse *et al.*, 2002; Green *et al.*, 2002; Gleeson, 2007) in lymphocyte function as measured by lymphocyte proliferation. Only two studies examined reported an increase in lymphocyte proliferation following exercise (Wong *et al.*, 1992; Espersen *et al.*, 2007). Thus, research findings indicate a possible association between acute exercise and impairment of lymphocyte function following acute exercise. This effect was

largely attributed to increases in circulating corticosteroids following acute exercise stress (Kurcz *et al.*, 1988; Wong *et al.*, 1992; Keadle *et al.*, 1993; Horohov *et al.*, 1999). As lymphocyte function is essential to initiate effective immune responses to pathogens, a potential decrease in this capacity might expose the individual to an increased risk of respiratory infection for a period following acute exercise.

Effect of Chronic Exercise on Lymphocyte Function

Human Studies

Reviews suggest that the response of the adaptive immune system to chronic exercise training is largely non-consistent such that lymphocyte proliferative response has been found to decrease, increase or remain unchanged with training (Pedersen and Hoffman-Goetz, 2000; Nieman, 2000). In humans, both a cross-sectional and a longitudinal approach to examining the effect of training on immune function have been adopted. Cross-sectional study designs compare resting levels of immune parameters in conditioned and unconditioned (control) individuals, whereas in longitudinal models, immune parameters are measured repeatedly over a designated time period such as a training season.

Using a cross-sectional approach, Papa *et al.* (1989) observed that lymphocyte proliferation response to PHA and particularly to PWM at rest was significantly lower in seven water-polo players than in seven non-athlete control individuals. In contrast, Nieman *et al.* (2000) reported that 20 elite female rowers exhibited a significantly higher lymphocyte proliferation response to PHA at rest than 19 non-athletic female controls. In this study whole blood data was adjusted as counts per minute for each CD3⁺ or T cell.

When employing a longitudinal approach, Verde *et al.* (1992) observed that lymphocyte proliferation response to stimulation with PHA and Con A of ten elite male runners significantly increased after a three week period of increased training. It was also reported that lymphoproliferative response to acute exercise remained initially unchanged, however, after the three week period of intense exercise training an acute exercise challenge resulted in decreased lymphocyte proliferation response to PHA.

Also, using a longitudinal approach, Nehlsen-Cannarella *et al.* (1991) found that spontaneous blastogenesis remained unchanged in a group of 36 sedentary, mildly obese women during and following a 15 week moderate exercise training programme.

Animal Studies

Studies relating to the effect of training on lymphocyte function in animals mainly employed longitudinal research design models. A study by Buschmann and Baumann (1991) that investigated the effect of training on cellular immune response in three-day event horses. The training programme for the four subjects was divided in three stages of work with blood samples taken at weekly intervals. The three stages of work included a basic training phase (January until April), an intensive training phase (April until June) and a detraining phase. Lymphocyte proliferation in response to stimulation with PHA remained unchanged throughout the period examined. As only phagocytic capacity in the granulocytes of tested horses decreased during this study, it was suggested that an impairment of the first line of defence against invading pathogens, rather than an impairment of specific immunity, might increase a horse's susceptibility to disease during high intensity training. Malinowski *et al.* (2004) evaluated the effect of exercise training (and chronic clenbuterol administration) on immune function in horses. In this study five mares given exercise alone were trained aerobically three days per week for a total period of eight weeks, while six mares remained sedentary. It was reported that lymphoproliferative response to PHA, PWM and Con A was not significantly affected by exercise treatment. This finding was attributed to the relatively short duration, but high intensity of the acute exercise bouts.

Table 1.2 Effect of chronic exercise training on equine lymphocyte proliferative response
***n* number of subjects, *Stim.* stimulant**

Reference	Training Schedule	N	Subject	Stim.	Effect (at rest)
Buschmann and Baumann (1991)	Basic: Jan-April Intensive: April-June Detraining: June onwards	4	3-day event horses	PHA	unchanged
Malinowski <i>et al.</i> (2004)	Exercise group: Exercised aerobically 3 days per week for 8 weeks	11	Standardbreds mares, 5 exercised and 6 non-exercised	PHA, PWM, Con A	unchanged

A further study (cross sectional design) examined the effect of chronic exercise on splenic T lymphocyte response to Con A (Hoffman-Goetz *et al.*, 1986). Sixty-two male C57BL/6J mice were randomly assigned to one of four treatment groups. Treatment one consisted of regular exercise training for six weeks on a treadmill. Treatment two included similar exercise training followed by a high-intensity exercise challenge; treatment three consisted of a non-exercise control group, while treatment four consisted of a non-exercise control group followed by a high-intensity exercise challenge. It was established that mice undergoing regular training exercise exhibited reduced splenic lymphoproliferative response to Con A compared with control animals. Splenic T lymphocyte response to Con A was most highly reduced in mice undergoing regular training followed by an acute exercise trial.

Summary of the Effect of Chronic Exercise on Lymphocyte Function

In summary, the effect of chronic exercise on lymphocyte function is not well established as research findings appeared to vary with study design in, both, humans and horses. Whereas cross-sectional research studies reported lower (Papa *et al.*, 1989) and higher (Nieman *et al.*, 2000) lymphocyte proliferation in athletes, longitudinal research studies reported increases (Verde *et al.*, 1992), decreases (Hoffman-Goetz *et al.*, 1986) or unchanged (Buschmann and Baumann, 1991; Nehlsen-Cannarella *et al.*, 1991; Malinowski *et al.*, 2004) lymphocyte proliferation throughout training programmes. Also, in one study, a negative effect of training on the lymphoproliferative response to acute exercise was observed (Verde *et al.*, 1992). It became apparent that acute exercise had a more coherent effect on lymphocyte proliferation and, as such, might go some way towards explaining the increased incidence of infectious disease in athletes as described in epidemiological studies. This observation has been previously described in the section on the effect of acute exercise on lymphocyte function.

Effects of Acute Exercise on Peripheral Blood Granulocyte and Monocyte Function – Phagocytosis

Human Studies

Research studies examining granulocyte and monocyte function reported varying effects of acute exercise on phagocytic activity. Yamamoto *et al.* (2008) stated that phagocytosis of neutrophils was found to either decrease, increase or remain unchanged according to previous studies. Other research studies reviewing the effect of exercise on granulocyte or monocyte function found that it was either suppressed (Pedersen and Hoffman-Goetz, 2000), remained unchanged in response to acute exercise (Pedersen and Hoffman-Goetz, 2000), or was enhanced following exercise (Nieman, 1997; Nieman, 2000; Woods *et al.*, 2000).

These observations are supported by individual research studies. Hack *et al.* (1992) examined the effect of maximal exercise on the activity of neutrophil granulocytes in highly endurance-trained athletes during a moderate training period and compared results with those of untrained individuals. After intensive exercise on a treadmill neutrophil phagocytosis was significantly increased 24 hours post-exercise compared to resting values in both the control and the trained athletes group. Another study by Hack *et al.* (1994) further demonstrated that the phagocytic index increased significantly until 24 hours after intensive exercise on a treadmill in highly trained athletes during moderate and intensive training periods as well as in a control group of untrained individuals. Research findings in a study by Wolach *et al.* (2000) on the response of neutrophil function to aerobic and anaerobic exercise in female judoka and untrained subjects differed from these observations. No significant changes in bactericidal activity of neutrophils with both autologous and homologous serum after exercise were reported irrespective of exercise type or training status of the individuals.

Yamamoto *et al.* (2008) investigated the effect of unified exercise regime on neutrophil function in male university judoists at different stages during long-term training. It was found that phagocytic activity significantly decreased after acute exercise prior to the beginning of training and at two months into training.

Phagocytic activity remained unchanged post exercise at four months and six months into training. However, it was observed that at four and six months into training post-exercise values of phagocytic activity showed a slight increase, rather than the decrease that was observed as a response to exercise in the earlier stages of training. These findings were suggested to comprise part of the long-term training effect; as the subject's physical condition improved through training, the body was better able to cope with the exercise stresses.

Animal Studies

Research findings relating to the equine and other animals appeared to be broadly similar to those relating to the human in that the reported effect of acute exercise on granulocyte and monocyte function seemed to vary depending on the study design and method. Woods *et al.* (2000) reviewed exercise-induced modulation of macrophage function and reported that phagocytosis of murine peritoneal macrophages increased following moderate and exhaustive exercise.

Several research studies conducted on the horse observed varying effects of acute exercise on granulocyte function. Raidal *et al.* (2000b) investigated the effect of high-intensity exercise on the functional capacity of equine pulmonary alveolar macrophages (PAM) in eight Standardbred geldings prior to training and seven weeks into training. Prior to the beginning of training, no significant difference between pre- and post-exercise values was observed, however, after seven weeks of training post-exercise values of phagocytosis assays performed on PAM showed a significant decrease compared to pre-exercise values. Raidal *et al.* (2000a) further examined the effect of single bouts of moderate and high-intensity exercise and training on equine peripheral blood neutrophil function in eight Standardbred geldings. Prior to the beginning of training, moderate-intensity exercise and high-intensity exercise appeared to enhance phagocytic capacity (six hours post-exercise). A non-significant decrease immediately following high-intensity exercise was noted. After ten weeks of training no changes in phagocytic capacity following exercise were shown. However, after 17 weeks of training, phagocytic capacity was found to be significantly reduced at two and 22 hours following high-intensity exercise. Decreased phagocytosis of bacteria and oxidative cell functions were attributed to increased levels of circulating cortisol, released in response to increased adrenocorticotrophic hormone (ACTH)

following acute exercise. Enhanced neutrophil function during moderate exercise had been contributed to increases in growth hormone, and, amongst others, possibly prolactin (Raidal *et al.*, 2000a).

When comparing the non-specific immune response of seven colts in training for national show-jumping events to exercise at the aerobic-anaerobic threshold with the response of five untrained horses, it was shown that the phagocytic capacity of neutrophils in the trained animals was enhanced with respect to the untrained ones immediately after exercise (Escribano *et al.*, 2002). Escribano *et al.* (2005b) also evaluated the effect of exercise at the aerobic-anaerobic threshold on the non-specific immune response of peripheral blood neutrophils in eleven untrained horses of two different breeds. The percentage of phagocytosing neutrophils remained unchanged following exercise in both breeds. However, a difference in the nature of the response between the two breeds (Spanish Arabian and Anglo-Arabian) was observed. In the Anglo-Arabian breed a post-exercise reduction of phagocytic activity was shown, whereas in the Spanish Arabian breed a general trend towards increasing phagocytic activity following exercise was noted.

A different study by Escribano *et al.* (2005a) observed that, following exercise of eleven trained and eight untrained horses in the aerobic – anaerobic transition area, significantly higher mean values for the internalization of particles were seen in trained horses during the recovery phase. Also, the phagocytosis percentage was significantly higher in trained horses immediately after exercise. No apparent comparison was made between pre- and post-exercise values, hence, it could not be determined whether trained, untrained or both horse groups displayed these changes following exercise. Increased phagocytosis percentage was linked to increased epinephrine levels following exercise in trained horses.

Summary of the Effect of Acute Exercise on Peripheral Blood Granulocyte and Monocyte Function - Phagocytosis

It has been shown that acute exercise had varied effects on granulocyte and monocyte phagocytosis depending on exercise intensity as well as training status of the subject. Phagocytosis was found to have transiently increased (Hack *et al.*, 1992; Hack *et al.*, 1994; Raidal *et al.*, 2000a; Woods *et al.*, 2000; Escribano *et al.*, 2002; Yamamoto *et*

al., 2008), decreased (Raidal *et al.*, 2000a/b; Yamamoto *et al.*, 2008) or remained unchanged (Raidal *et al.*, 2000a/b; Wolach *et al.*, 2000; Escribano *et al.*, 2005b; Yamamoto *et al.*, 2008) following acute exercise. It should be noted that, due to different research designs, a direct comparison between these studies is not advisable. Furthermore, variation in results between studies suggests, that it cannot be concluded whether or not acute exercise causes transient suppression of the immune system. It has become apparent that each case should be considered individually taking exercise intensity, duration and training status into account. Suppression of phagocytic activity was linked to increases in circulating cortisol (Raidal *et al.*, 2000a/b), while enhancement of phagocytic activity was attributed to increases in growth hormone, prolactin and substance P (Raidal *et al.*, 2000a) or increases in epinephrine levels following exercise (Escribano *et al.*, 2005a).

Effects of Chronic Exercise on Peripheral Blood Granulocyte and Monocyte Function – Phagocytosis

Human Studies

As with the effects of acute exercise on granulocyte and monocyte function, the effects of chronic exercise on these immunological parameters appear to vary widely. A review on exercise and the immune system by Pedersen and Hoffman-Goetz (2000) stated that neutrophil function was unchanged in athletes during periods of low-intensity training, but decreased during high-intensity training. Mackinnon (2000) summarized that resting values in athletes appeared to be lower than in non-athletes. Furthermore, normal or moderate training had no effect on neutrophil function, whereas intensive training lead to decreased neutrophil function. This was found to be compatible with anecdotal evidence suggesting that moderate training either has no effect, or may stimulate various immune parameters, while intensive training might lead to slight impairment in immune parameters.

Hack *et al.* (1992) observed that the ingestion capacity of neutrophil granulocytes did not differ between 20 highly trained athletes in a moderate training period and ten untrained control subjects at rest and after exercise. Another research study by Hack *et al.* (1994) suggested that polymorphonuclear leukocyte (PMN) cell counts and phagocytic activity of highly trained athletes depended on the training period. There

was no significant difference in phagocytic activity of neutrophil granulocytes at rest and after exercise between athletes during moderate training and untrained control subjects. However, a significant decrease in the phagocytic activity of neutrophil granulocytes at rest and after exercise during intensive training was shown, when compared to the phagocytic activity of athletes in moderate training or the control subjects. Furthermore, plasma epinephrine and norepinephrine were found to be increased at rest in the intensive training group compared to controls and the moderate training group. Contrary to findings by Escribano *et al.* (2005a), marked increases in epinephrine were thought to have been responsible for the impairment of phagocytic activity observed in this study. This previously indicated weakness in the first line of defence, again, might present the link between exercise and increased risk of disease.

Nieman *et al.* (2000) compared the immune function in female elite rowers and non-athletes over a period of two months. It was established that neither granulocyte nor monocyte phagocytosis significantly differed between the groups. A similar finding was made by Wolach *et al.* (2000) in a cross-sectional study design, who observed no significant differences in the basal values of bactericidal activity of polymorphonuclear leukocytes (PMNs) in female judoka and untrained control subjects. When investigating the effects of long-term training on neutrophil function in male university judoists, it was found that phagocytic activity per cell was significantly reduced two months into training when compared to pre-training values (Yamamoto *et al.*, 2008). However, after four and six months of training phagocytic activity had returned to pre-training levels, which was suggested to have indicated an adaptation of the body to physical exercise.

Animal Studies

Research findings of studies examining the effects of chronic exercise on granulocyte and monocyte phagocytosis in horses were mainly similar to those of studies relating to humans.

During periods of intensive training (April to June), the *in vitro* killing rate of phagocytosed yeast cells by the blood granulocytes of four three-day event horses was found to be significantly reduced when compared to resting phase, basic training and detraining levels (Buschmann and Baumann, 1991). Research findings by Raidal *et*

al. (2000a) support this evaluation, as phagocytosis of peripheral blood neutrophils was reduced pre- and post-exercise in week 17 of training, which marked the end of the high-intensity training phase, when compared to results of week 10. It was stated that, due to laboratory procedures, no comparison of high-intensity versus moderate intensity and pre-training levels of phagocytosis in the eight Standardbred geldings was undertaken. Altered functions were associated with the duration and intensity of training. Also, as resting cortisol levels were only measured prior to the beginning of training and after eight and after thirty-two weeks of training, no correlation with functional changes could be made (resting levels had not significantly changed at sampling times). It was, however, suggested that more frequent sampling might have explained the mechanism behind the changes in phagocytic activity of neutrophils and PAMs.

When examining the effects of training on resting peripheral blood and BAL-derived leukocyte function in eight Standardbred geldings, Raidal *et al.* (2001) found that peripheral blood neutrophil phagocytosis (internalisation) significantly increased throughout the initial high-intensity training period (week 10 to 14 including performance testing in week 15 and 16) when compared to results from week seven of the training schedule. A subsequent overtraining phase was associated with a decline of blood neutrophil phagocytosis. Phagocytic capacity of PAMs did not significantly fluctuate throughout the 32 week training programme. Basal cortisol or β -endorphin levels were not changed after 32 weeks of training compared to pre-training values.

Escibano *et al.* (2002) determined that there were no differences between the neutrophil function of trained and untrained horses at rest and after a post-exercise recovery period, when comparing the non-specific immune response of seven colts in training for national show-jumping events with that of five untrained male horses. As previously indicated, immediately following exercise, significantly lower values were observed in trained colts. However, phagocytic ability ('digestive stage') was increased in trained colts, when compared to untrained ones. This finding indicated that training could improve immune function. A further study by Escibano *et al.* (2005a) supported this research finding. At rest, no difference in phagocytic capacity between the eleven untrained and the eight horses in training for national jumping events was apparent. Table 1.3 summarises these findings.

Table 1.3 Effect of chronic exercise training on equine granulocyte phagocytosis
(n number of subjects)

Reference	Training Schedule	N	Subject	Effect (at rest)
Buschmann and Baumann (1991)	Basic: Jan-April Intensive: April-June Detraining: June onwards	4	3-day event horses	Basic: unchanged Intensive: reduced Detraining: unchanged
Raidal et al. (2000)	week 1 - 10: moderate intensity week 10 - 17: high intensity	8	Standardbred geldings	week 17: lower than week 10 no comparison pre-training vs. post-training
Raidal et al. (2001)	week 1 - 7: moderate intensity week 7 - 16: high intensity after week 16: overtraining	8	Standardbred geldings	week 14 - 16: increased compared to wk 7 overtraining: reduced
Escribano et al. (2002)	Trained vs. untrained horses	12	7 trained colts (show jumping) 5 untrained colts	no difference / unchanged
Escribano et al. (2005a)	Trained vs. untrained horses	19	8 trained horses (show jumping) 11 untrained horses	no difference / unchanged

Summary of the Effect of Chronic Exercise on Peripheral Blood Granulocyte and Monocyte Function - Phagocytosis

Training and exercise systems, age, gender, group size and discipline examined varied in research studies, both, on human and equine subjects making direct comparisons of results difficult. This might explain some of the variation in the results observed. Nonetheless, phagocytic activity of neutrophils appeared to have been reduced during periods of intensive training (Buschmann and Baumann, 1991; Hack *et al.*, 1994; Raidal *et al.*, 2000) or overtraining (Raidal *et al.*, 2001) whereas it appeared unchanged (Hack *et al.*, 1992; Hack *et al.*, 1994) during periods of moderate-intensity training. Also, in cross-sectional study designs no difference between phagocytic activity of athletes and non-athletes was observed at rest (Nieman *et al.*, 2000; Wolach *et al.*, 2000, Escribano *et al.*, 2002; Escribano *et al.*, 2005). One study examined reported increased phagocytic activity of granulocytes during a period of high-intensity training. Furthermore, another study reported initial reduction in phagocytosis, though; pre-training values were regained as training progressed (Yamamoto *et al.*, 2008). However, overall results indicate an impairment of the first line of defence against invading pathogens during high intensity training periods,

which could increase the risk of contracting infectious disease. Changes in cortisol levels were suggested as a mechanism causing changes in phagocytic activity of granulocytes during training, however, this was not confirmed (Raidal *et al.*, 2000). Increases in plasma epinephrine and norepinephrine during intensive training were linked to suppressed phagocytic activity (Hack *et al.*, 1994).

Effects of Acute Exercise on Peripheral Blood Granulocyte and Monocyte Function – Oxidative Burst Capacity

Human Studies

Most researchers contend that moderate exercise (typically $\leq 60\%$ $\text{VO}_2 \text{max}$) had a tendency to enhance oxidative burst activity of neutrophils and monocytes (Nieman, 1997; Klarlund Pedersen and Hoffman-Goetz, 2000), whereas sustained, high-intensity exercise results in suppressed activity (Nieman, 1997; Klarlund Pedersen and Hoffman-Goetz, 2000; Nieman, 2000).

Individual research studies generally appear to agree with these observations. However, different exercise conditions seem to have led to slight differences in the exact nature of the response. Hack *et al.* (1992) found that the ability of neutrophils to produce microbicidal reactive oxygen species was reduced immediately after maximal intensity exercise in, both, highly trained athletes in a moderate training period and untrained control subjects. Following this initial decrease, the production of reactive oxygen species then increased significantly in all subjects (long-distance runners, triathletes and controls) up to 24 hours post-exercise. Research findings from a different study by Hack *et al.* (1994) taking the effect of training period into account supported the earlier observation. Superoxide anion (O_2^-) production of PMNs in male long-distance runners during moderate and intensive training periods, as well as in untrained subjects, was suppressed immediately following heavy exertion for up to 30 minutes post-exercise and then increased compared with pre-exercise values up to 24 hours after exercise. Increased epinephrine and norepinephrine levels following the acute exercise bout were associated with suppressed superoxide anion production following exercise. Epinephrine, in particular, which was considered a more potent β_2 -adrenergic agonist than norepinephrine was found to be markedly increased after acute exercise.

Pyne *et al.* (2000) established that neutrophil oxidative activity was differentially affected by exercise intensity and type. Chemiluminescence (CL) activity was consistently suppressed after an intense downhill run, whereas downhill and a moderate-intensity near-level run lead to an initial marginal decrease in CL activity, which was followed by a significant enhancement of this response at six hours post-exercise. The capacity of neutrophils to release the superoxide anion was reduced following both uphill and near-level running in response to stimulation with Opsonized Zymosan (OZ) but not with PMA. Mechanisms that could have caused these findings were either catecholamine-mediated inhibition or a post-activation refractory period. Wolach *et al.* (2000) found that superoxide anion release by neutrophils in response to stimulation with PMA and FMLP was not affected by aerobic or anaerobic exercise in untrained subjects or female trained judoka.

Yamamoto *et al.* (2008) observed that training status might affect neutrophil functional response to exercise. The ability of neutrophils to produce reactive oxygen species in response to stimulation with OZ following exercise did not change in male judoists before training begin, however, at two months, four months and six months after training values were significantly elevated following exercise.

Animal Studies

A research study using eight Standardbred geldings by Raidal *et al.* (2000a) showed that prior to the beginning of training, oxidative burst capacity of PMNs stimulated with PMA was not significantly affected by moderate-intensity exercise. However, high-intensity exercise was associated with a significant reduction of oxidative burst activity for up to six hours post-exercise. Following training, the oxidative burst capacity tended to be lower post-exercise. This effect was significant after 17 weeks of training, but not after ten weeks of training.

The oxidative burst capacity of PAMs in eight Standardbred geldings was found to be significantly reduced following high-intensity exercise both before training and after seven weeks of endurance training (Raidal *et al.*, 2000b). Research findings by Escribano *et al.* (2002) indicated that sub-maximal exercise at the aerobic-anaerobic threshold had no effect on the digestion and microbicidal capacity of neutrophils in either trained or untrained horses.

Robson *et al.* (2003) observed a prolonged suppression of the innate immune system in the horse following an 80 kilometres endurance race. Neutrophil and monocyte oxidative burst activity decreased after the endurance race and had not returned to pre-race values after three days of rest. Possible mechanisms underlying this decrease in functional activity of neutrophils and monocytes might have been changes in stress hormones, cytokines and haematological factors. Haematological factors relate to more immature neutrophils, which are reported to have a lower phagocytic capacity than mature cells, entering circulation under the influence of cortisol. Another haematological factor relates to neutrophils entering a refractory state after initial activation reducing their capacity for subsequent stimulation.

A further study examining the effect of exercise at the aerobic-anaerobic threshold in untrained horses of two different breeds found that moderate exercise did not affect the oxygen-dependent microbicide capacity of neutrophils in either breed (Escribano *et al.*, 2005b). These findings are similar to those of another by Escribano *et al.* (2005a), which also established that moderate exercise at the aerobic-anaerobic threshold had no effect on oxidative metabolism of peripheral neutrophils in trained and untrained horses.

Summary of the Effect of Acute Exercise on Peripheral Blood Granulocyte and Monocyte Function – Oxidative Burst Capacity

In summary, oxidative burst capacity immediately following, especially high intensity, exercise was mostly found to have been transiently suppressed in both humans and horses (Hack *et al.*, 1992; Hack *et al.*, 1994; Pyne *et al.*, 2000; Raidal *et al.*, 2000a, Robson *et al.*, 2003). Moderate exercise was found to either have no effect on granulocyte or monocyte oxidative burst capacity (Wolach *et al.*, 2000; Escribano *et al.*, 2002; Escribano *et al.*, 2005a/b) or to increase this parameter (Pyne *et al.*, 2000; Raidal *et al.*, 2000a). One study established that oxidative burst capacity increased following acute exercise as training progressed (Yamamoto *et al.*, 2008). Again, findings indicate impairment of the first line of defence of the immune system following intensive acute exercise, which athletes and trainers should be aware of, as it could increase susceptibility of the human or equine athlete to infectious disease immediately after exercise. Also, as previously indicated, transient immunosuppression was thought to be linked to elevated cortisol levels (Robson *et*

al., 2003), elevated catecholamine levels (Hack *et al.*, 1994; Pyne *et al.*, 2000) or a post-activation refractory period (Pyne *et al.*, 2000) as a response to acute exercise.

Effects of Chronic Exercise on Peripheral Blood Granulocyte and Monocyte Function – Oxidative Burst Capacity

Human Studies

As with the examination of effects of chronic exercise or training on any immune parameter, a cross-sectional study design comparing resting levels of oxidative burst capacity of neutrophils or monocytes in trained athletes and untrained controls is often used as an indication of the nature of the immune response to training. A research study by Pedersen and Hoffman-Goetz (2000) reviewing several cross-sectional studies indicated that neutrophil function was either suppressed or not significantly influenced by exercise training. Mackinnon (2000) found that resting values relating to neutrophil function in athletes were often lower than in unconditioned individuals. It was also observed that resting values either changed or decreased after a period of moderate training or decreased after a period of high-intensity training.

A cross-sectional research study by Hack *et al.* (1992) investigating whether superoxide anion production levels in highly trained endurance long-distance runners and triathletes in a moderate training period differed from those of untrained subjects found no significant differences between these groups at rest and after exercise. Hack *et al.* (1994) also found that when comparing a group of male long-distance runners during a moderate training period to untrained subjects, no significant differences in superoxide anion production at rest became apparent. However, when comparing superoxide anion production at rest of the same long-distance runners during a high-intensity training period, shortly before the competition event, to the results obtained during the moderate training period, a significant decrease in superoxide anion production was shown. It was important to note that no significant differences between resting values of athletes during the high-intensity training period and untrained subjects could be seen. Also, a strong negative correlation between plasma epinephrine and O_2^- production at rest and after exercise was observed.

Another cross-sectional study by Nieman *et al.* (2000) found that oxidative burst capacity of granulocytes and monocytes did not differ between 20 elite female rowers and 19 non-athletic female control subjects. Similar research findings were established by Wolach *et al.* (2000). Neither resting levels of superoxide anion release by neutrophils nor levels after aerobic or anaerobic exercise differed between female trained judoka and untrained subjects. On the other hand, a longitudinal research study by Yamamoto *et al.* (2008) showed that oxidative burst activity of neutrophils in male judoists first declined after two months of training, but then increased significantly after both four and six months of training compared with pre-training values.

Animal Studies

Research studies investigating the effect of training on oxidative burst activity in horses have shown varying results. Buschmann and Baumann (1991) found that PMA induced chemiluminescence in granulocytes appeared higher during the high-intensity training period of four three-day event horses than during moderate training or detraining periods, although no significant differences were shown.

Raidal *et al.* (2000a) observed that oxidative burst activity of neutrophils at rest and post-exercise in eight Standardbred geldings appeared to be reduced after endurance training in week 17, when comparing results to those of week 10, however, no significant differences were determined. Changes were likely to have been mediated by training induced alterations in the endocrine response to exercise.

Pre- and post-exercise values of oxidative burst activity of PAMs in eight Standardbred geldings acquired prior to the commencement of training were not significantly different to those obtained after seven weeks of endurance training. This indicates that moderate training does not affect oxidative burst activity (Raidal *et al.*, 2000b). As discussed in the section on phagocytosis, resting cortisol levels after thirty-two weeks of training did not differ significantly from those after eight weeks of training and pre-training. A study by Freestone *et al.* (1991) observed an increase in cortisol during the first five weeks of training, followed by a subsequent decrease between weeks six to week ten. Although, in this study, reduced phagocytic function

has been mainly associated with an increase in cortisol during the latter stages of training.

When examining the effects of training on resting peripheral blood and BAL-derived leukocyte function in eight Standardbred geldings, Raidal *et al.* (2001) found that oxidative burst activity of peripheral blood neutrophils first increased, then decreased slightly and peaked at the end of the high-intensity training period (at 16 weeks) to be followed by a progressive decrease in activity during the overtraining phase. Whereas PAM oxidative burst activity was found to increase until the early stages of the overtraining period, but then decreased at week 28 and increased again at week 32 (end of the study).

Contrary to research findings in most cross-sectional human studies, Escribano *et al.* (2002) indicated a higher microbicidal activity of neutrophils in horses trained for national show-jumping events than in untrained horses. A further study by Escribano *et al.* (2005a) confirmed these results. Oxidative metabolism by neutrophils was found to be significantly higher in horses trained for national jumping events than in untrained horses both before and immediately after exercise, but not in the recovery stage. However, during the recovery stage, norepinephrine was found to have significantly decreased in trained horses; this could have led to a reduction in oxidative metabolism from post-exercise values.

Table 1.4 summarises the effect of chronic exercise on oxidative burst capacity in horses depending on intensity, duration and breed of horses.

Table 1.4 Effect of chronic exercise training on equine granulocyte oxidative burst capacity
(n number of subjects)

Reference	Training Schedule	N	Subject	Effect (at rest)
Buschmann and Baumann (1991)	Basic: Jan-April Intensive: April-June Detraining: June onwards	4	3-day event horses	Chemiluminescence higher during high-intensity phase, however: change not significant
Raidal et al. (2000a)	week 1 - 10: moderate intensity week 10 - 17: high intensity	8	Standardbred geldings	Oxidative burst activity reduced after endurance training in wk17, however: change not significant
Raidal et al. (2001)	week 1 - 7: moderate intensity week 7 - 16: high intensity after week 16: overtraining	8	Standardbred geldings	BAL-derived neutrophils: wk 7-16: increase =>decrease => peak after wk 16: decrease
Escribano et al. (2002)	Trained vs. untrained horses	12	7 trained colts (show jumping) 5 untrained colts	Higher in trained horses
Escribano et al. (2005a)	Trained vs. untrained horses	19	8 trained horses (show jumping) 11 untrained horses	Higher in trained horses

Summary of the Effect of Chronic Exercise on Peripheral Blood Granulocyte and Monocyte Function – Oxidative Burst Capacity

Varied effects of chronic exercise on oxidative metabolism were observed. While values remained unchanged throughout training and cross-sectional research studies reported no differences between athletes and non-athletes in many cases (Buschmann and Baumann, 1991; Hack *et al.*, 1992; Hack *et al.*, 1994; Nieman *et al.*, 2000; Raidal *et al.*, 2000a/b; Wolach *et al.*, 2000; Raidal *et al.*, 2001), a reduction of oxidative burst capacity during high intensity training periods was indicated in one instance (Hack *et al.*, 1994). Increases in oxidative metabolism were also noted with increasing duration of training (Raidal *et al.*, 2001; Escribano *et al.*, 2002; Escribano *et al.*, 2005a; Yamamoto *et al.*, 2008). Training induced changes in the endocrine system were associated with changes in oxidative burst capacity in, both, humans (Hack *et al.*, 1994) and horses (Raidal *et al.*, 2000a; Raidal *et al.*, 2001). Increases in circulating catecholamines during training were thought to have occurred due to repeated exercise bouts during a training programme and there from resulting increases in plasma epinephrine and norepinephrine. An implication of reduced oxidative burst capacity would be an increased risk to infectious diseases. Overall

however, evidence points to the conclusion that exercise has only little effects on granulocyte or monocyte oxidative burst capacity.

Effects of Acute and Chronic Exercise on Plasma Haematology

Whereas acute exercise is thought to affect haematological variables in both humans and horses, chronic exercise appears to have little effect on haematological variables. However, due to differences in exercise intensity and duration and the varying sports disciplines examined as well as differences in gender, age and weight of individuals, comparison across studies is not always possible or advisable. Unless otherwise stated, research studies reviewed in the following referred to the effect of acute and/or chronic exercise on plasma haematology in horses.

Total Erythrocytes

According to Hodgson (1994), the erythrocyte pool in the horse is directly influenced by catecholamine concentrations, which show significant alterations with exercise. Therefore, acute exercise is understood to have variable effects on the erythrocyte population. Krumrych (2006) reported increases in circulating erythrocyte counts of jumping horses immediately after the exercise challenge, but values returned to pre-exercise level within 30 minutes after the exercise bout. Erythrocytes are released from the spleen in response to exercise stress with the size of the final response depending on inherent splenic capacity and ability for subsequent release during exercise (Hodgson and Rose, 1994; Krumrych, 2006). Piccione *et al.* (2007) also reported increased red blood cell counts after exercise on a sandy track, while exercise on a horse-walker lead to a reduction. However, these changes were not significant.

No significant changes in erythrocyte counts of endurance horses were observed during a 12 week training programme in a study by Rose and Hodgson (1982). On the other hand, erythrocyte counts of Standardbred horses increased throughout a 34 week progressive training programme with the highest levels being reported following the moderate-intensity training phase in week 18 (Tyler-McGowan *et al.*, 1999). In the same study, hypothesized overtraining appeared to have no effect on total erythrocyte numbers in horses (Tyler-McGowan *et al.*, 1999). In a study by Padalino

et al. (2007), hypothetically overtrained horses displayed higher erythrocyte counts. Also, it should be noted that separate studies have described normal haematological values in different populations of thoroughbreds as high as $11.6 \times 10^{12}/l$ as described by Hodgson and Rose (1994) depending on age and training status of the horse (Rose and Allen, 1985); whereby mainly younger horses upon introduction to training displayed higher normal haematological values at rest than older thoroughbreds.

Packed Cell Volume (PCV)

Changes in haematocrit are mainly attributed to fluid movement during exercise (Hodgson, 1994; Krumrych, 2006). Therefore, research studies evaluating the effect of acute exercise on PCV in horses of varying breeds, gender, age and discipline reported increased PCV levels following the exercise bout that returned to pre-exercise levels shortly after (Robson *et al.*, 2003; Krumrych, 2006; Piccione *et al.*, 2007).

In humans, a research study employing a cross-sectional design showed no significant differences in PCV at rest between female elite rowers and untrained control subjects (Nieman *et al.*, 2000). Similarly in horses, a research study examining haematological parameters of endurance horses during 12 weeks of training observed no significant changes in resting PCV (Rose and Hodgson, 1982). Conversely, a research study investigating haematological parameters in Standardbred horses during a 34 week progressive training programme found that resting PCV increased in line with resting erythrocyte counts (Tyler-McGowan *et al.*, 1999). Highest levels were seen at the end of the moderate-intensity training phase. No differences were observed between overtrained and normally trained horses and, also, no increase in erythrocyte indices was shown while signs of overtraining were apparent. A research study by Padalino *et al.* (2007) supports this finding.

Haemoglobin

Depending on exercise intensity, haemoglobin levels in horses were found to increase immediately following acute exercise, but returned to resting levels 30 minutes post-exercise (Krumrych, 2006; Piccione *et al.*, 2007). In humans, Nieman *et al.* (2000) found no significant difference between resting haemoglobin levels of female elite rowers and untrained control subjects.

Haemoglobin concentration in endurance horses did not significantly change during a 12 week training programme as described by Rose and Hodgson (1982). In a research study by Tyler-McGowan *et al.* (1999), haemoglobin in Standardbred horses was shown to increase throughout a 34 week training period in line with other red blood indices. With respect to training intensity, neither Tyler-McGowan *et al.* (1999) nor Padalino *et al.* (2007) reported significant differences between regularly trained and hypothetically overtrained horses.

Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH)

Hodgson and Rose (1994) reported small changes in the erythrocytes themselves following acute exercise, manifesting in decreases in mean corpuscular volume and increases in mean corpuscular haemoglobin concentration. Also, erythrocytes appeared to be more resistant to osmotic stress and, hence, cell shape and degree of deformity remain unaffected by exercise.

Few changes appear to take place in the erythrocytes themselves as a result of chronic exercise. Nevertheless, MCV and MCH have been suggested as markers for overtraining in the horse by Padalino *et al.* (2007) as both variables were lower in horses subjected to hypothesized overtraining than in those subjected to less intensive training. In conjunction with a higher average red cell distribution weight, this was thought to be a sign of anisocytosis.

Platelets

As above, information regarding the effect of exercise on platelet counts could only be obtained with respect to training intensity. Padalino *et al.* (2007) found no significant differences in hypothetically overtrained and less intensively trained horses.

Summary of the Effect of Exercise on Plasma Haematology

Table 1.5 summarises the nature of observed effects of acute and chronic exercise on total erythrocytes, PCV, Haemoglobin, MCV, MCHC, MCH and platelets. An *x* denotes that research studies as included in this literature review have observed the marked effect of acute (or chronic) exercise on the according haematological parameter. Different research studies as included in this study, for example, have observed that acute exercise had either no effect on total erythrocyte levels or lead to increased erythrocyte levels. In the case of this example, the effects were observed in research studies by Krumrych (2006) ('increased') and Piccione *et al.* (2007) ('no effect'). A blank space illustrates that no research study, which was found during the course of this secondary research observed the unmarked effect of exercise on the according variable.

Table 1.5 Effects of exercise on plasma haematology

<i>Parameter</i>	<i>Acute Exercise</i>			<i>Chronic Exercise/Training</i>		
	<i>↑</i>	<i>↓</i>	<i>No effect</i>	<i>↑</i>	<i>↓</i>	<i>No effect</i>
<i>Total Erythrocytes</i>	<i>x</i>		<i>x</i>	<i>x</i>		<i>x</i>
<i>PCV</i>	<i>x</i>			<i>x</i>		<i>x</i>
<i>Haemoglobin</i>	<i>x</i>			<i>x</i>		<i>x</i>
<i>MCV</i>		<i>x</i>			<i>x</i>	
<i>MCHC</i>	<i>x</i>					
<i>MCH</i>					<i>x</i>	
<i>Platelets</i>						<i>x</i>

Total Leukocytes

Studies are broadly in agreement that, the numbers of leukocytes changes depend on exercise intensity and duration with prolonged endurance exercise and high-intensity exercise leading to leukocytosis resulting from changes in leukocyte subsets. This increase in circulating leukocytes following acute exercise has been linked to increases in endogenous cortisol (Rose, 1984; in Hodgson, 1994; Nieman, 2000; Miyazaki *et al.*, 2001; Padalino *et al.*, 2007).

Hack *et al.* (1994) found that the total number of leukocytes increased significantly after high-intensity exercise in human long-distance runners during a moderate and intensive training period as well as in untrained control subjects.

In a study examining the response of untrained horses of two breeds to exercise, Escribano *et al.* (2005b) did not observe any changes in circulating total leukocytes following exercise at the aerobic-anaerobic threshold in either breed. On the other hand, Krumrych (2006) observed a transient increase in white blood cell count in trained jumping horses immediately following acute exercise. Piccione *et al.* (2007) found that total leukocyte counts did not change significantly after exercise on a horse-walker or on a sandy track. However, a trend towards increasing white blood cell levels was seen following exercise on the sandy track and a trend towards decreasing leukocyte numbers was shown after exercise on a walker. Pre-exercise levels were reached again 30 minutes post-exercise. Despite some contrary results, there seems to be few changes in total leukocytes during training.

Consistent with this observation, Nehlsen-Cannarella *et al.* (1991) found that total leukocyte numbers did not change in human subjects conducting a 15 week training programme or a non-exercise control group. Hack *et al.* (1994) did not report any differences between total leukocyte count at rest in highly trained long-distance runners during a moderate or an intensive training period and untrained control subjects or between athletes during training periods of varying intensity. Furthermore, Gleeson *et al.* (1995) showed that total numbers of leukocytes of elite swimmers did not change significantly throughout a seven months training period. No significant changes were observed in untrained control subjects; however, at the beginning of the training season, a marginally lower total leukocyte count was reported in athletes than in the control group. In a cross-sectional research study, Nieman *et al.* (2000) found that the total number of leukocytes at rest did not differ between elite female rowers in training and untrained control subjects. Yamamoto *et al.* (2008) observed that total leukocyte count in male judoists did not vary throughout a six months training period.

Rose and Hodgson (1982) reported a slight but significant increase in total leukocyte count in endurance horses in week 10 of a 12 week training schedule. However, it

was concluded that this increase was of no clinical importance as values fell within normal ranges. Tyler-McGowan *et al.* (1999) found that total leukocyte count at rest did not change with training or hypothesized overtraining in Standardbred geldings throughout the 34 week period examined. Contrary to this finding, Padalino *et al.* (2007) detected that total leukocyte count in hypothetically overtrained Standardbreds was significantly higher than horses undergoing a less intense training programme.

Neutrophils

Changes in total leukocyte count following exercise can partially be explained by transient neutrophilia occurring after acute exercise in both humans and horses. Research studies reviewing the effect of acute exercise on leukocytes in humans have described an increase in neutrophil counts occurring after acute exercise induced by high plasma cortisol levels (Nieman *et al.*, 1997; Nieman, 2000). Hack *et al.* (1992) found that neutrophil counts increased in long-distance runners and triathletes during a moderate training period as well as in untrained individuals after a high-intensity exercise test on a treadmill. In agreement with finding from this study, Hack *et al.* (1994) also reported increased levels of PMNs after exercise compared with pre-exercise values in long-distance runners during a moderate or intensive training period and untrained subjects. PMN counts returned to pre-exercise levels within 30 minutes after exercise. Pyne *et al.* (2000) observed increased blood granulocyte counts after an intense uphill run, an eccentrically-based downhill run and a moderate-intensity near-level run in aerobically trained male runners. The transient increase in neutrophil counts after uphill running was linked to endocrine responses such as an increase of epinephrine secretion associated with intense exercise. On the other hand, the transient increase of neutrophils following downhill running was thought to be associated with a mild localised inflammatory response to tissue damage, in the absence of increased heart rate or epinephrine concentrations.

Robson *et al.* (2003) observed that, in horses, following an 80 km endurance race, a significant neutrophilia was apparent until one day past the exercise challenge, but a return to pre-exercise levels was evident three days post-exercise. This study also reported an increase in serum cortisol one hour after the exercise challenge, which could explain the occurrence of neutrophilia as cortisol stimulates the release of

neutrophils from the bone marrow into circulation (Yang and Hill, 1991). Research studies examining the effect of exercise on neutrophil counts in untrained horses (Escribano *et al.*, 2005b) and trained jumping horses (Krumrych, 2006) reported that acute exercise of different intensity did not affect neutrophil counts.

Chronic exercise was reported to have slightly more variable effects on neutrophil counts. In humans, Hack *et al.* (1992) found no significant differences in the absolute number of neutrophils of endurance-trained athletes during a moderate training period compared to untrained subjects. In accordance with this research finding, a different study by Hack *et al.* (1994) reported no difference in resting PMN cell counts of untrained subjects and long-distance runners during a moderate training period. However, PMN cell counts of athletes during an intensive training phase were reduced significantly at rest and after exercise compared to athletes during a moderate training phase and controls. Nieman *et al.* (2000) observed no significant difference between neutrophil counts of elite female rowers and untrained control subjects. Interestingly, Yamamoto *et al.* (2008) showed an increase in blood neutrophil counts in male judoists two months after training began, which decreased again in line with decreasing levels of myogenic enzymes from the two-month point onwards. This was attributed to a decrease in muscle damage and inflammation after the initial training period.

Training of endurance horses was found to have no effect on neutrophil counts throughout the 12 week period examined in a research study by Rose and Hodgson (1982). Conversely, a research study by Tyler-McGowan *et al.* (1999) on the haematological and biochemical responses of Standardbred geldings to training and overtraining found that neutrophil numbers increased throughout a 34 week training programme. Padalino *et al.* (2007) observed no differences between horses in normal training and horses in hypothesized overtraining.

Lymphocytes

In humans, whereas neutrophil counts are thought to increase as a result of acute exercise, a tendency towards transient lymphopenia after acute exercise has been reported in research studies related to changes in stress hormone and cytokine

concentrations, body temperature changes, increases in blood flow, lymphocyte apoptosis and dehydration (Nieman, 1997; Nieman *et al.*, 2000).

Contrary to these observations, Hack *et al.* (1992) observed an increase in lymphocyte counts in highly trained athletes in a moderate training period and in untrained controls after intensive exercise. Hack *et al.* (1994) further reported an increase in lymphocyte counts immediately after intensive exercise in untrained subjects as well as in highly trained long-distance runners during moderate and intensive training periods. However, 30 minutes post-exercise a modest lymphopenia was evident in all subjects.

In horses, Robson *et al.* (2003) found that numbers of circulating lymphocytes were significantly reduced after an 80 km endurance race for up to three days with the largest reduction visible one hour post-exercise. This transient lymphopenia was linked to increased levels of cortisol (Shinkai *et al.*, 1996), epinephrine (Tonnesen *et al.*, 2008) and growth hormone (Kappel *et al.*, 1993) in the blood following acute exercise. In accordance with findings by Hack *et al.* (1992; 1994), a research study by Krumrych (2006) showed that lymphocyte counts in jumping horses increased immediately after exercise, but returned to resting levels 30 minutes later.

Chronic exercise appeared to have varying effects on lymphocyte counts in horses and humans. In humans, Nehlsen-Cannarella *et al.* (1991) observed a decrease in lymphocyte counts and percentages in subjects of the exercise group six weeks after training began, while no change was detected in subjects of the non-exercise group at the same time point. In week 15, only the percentage of lymphocytes remained reduced relative to pre-training values. With respect to lymphocyte counts at rest and after intensive exercise, no differences were observed between highly trained athletes in moderate and intensive training periods and untrained subjects in a study by Hack *et al.* (1994). No differences regarding lymphocyte counts and percentages following long-term training in untrained subjects and, either elite swimmers (Gleeson *et al.*, 1995) or elite rowers (Nieman *et al.*, 2000) were evident.

During a 12 week training programme for endurance horses, no changes in lymphocyte percentages were observed in the animals (Rose and Hodgson, 1982).

However, a reduction in lymphocyte number during a 34 week training programme was apparent in a study by Tyler-McGowan *et al.* (1999). Padalino *et al.* (2007) did not report differences in lymphocyte percentages between normally trained and hypothetically overtrained horses.

Monocytes

Research studies describe an increase in monocyte counts in humans (Nieman, 1997; Nieman, 2000) following acute exercise and in horses after a prolonged endurance exercise challenge (Robson *et al.*, 2003). In horses, the numbers of circulating monocytes had regained pre-exercise levels one hour after exercise.

In humans, Nieman *et al.* (2000) found no differences in monocyte counts between highly trained female rowers and non-athletes in a cross-sectional study design indicating training might have few effects on resting monocyte levels.

In a study investigating haematological responses to training and hypothesized overtraining in horses, Tyler-McGowan *et al.* (1999) reported elevated numbers of monocytes throughout the entire training period. No differences were shown between regularly trained and overtrained horses. With further respect to training intensity, Padalino *et al.* (2007) did not observe differences in monocyte percentages between horses undergoing regular training or hypothesized overtraining.

Eosinophils

Rose and Hodgson (1982) observed significant decreases in eosinophil counts during week 6, 8 and 12 of a 12 week training programme for endurance horses. This was attributed to elevated levels of eosinophils during the initial training period due to suspected parasite burden as all horses had been out on pasture three to five months prior to training begin. Eosinophil counts decreased following anthelmintic treatment two to four weeks into training. Tyler-McGowan *et al.* (1999) also observed a decrease in eosinophil counts of Standardbred horses throughout a 34 week progressive training programme that were not linked to a potential parasite burden. It was suggested that eosinopenia was a more sensitive indicator of training stress than other members of the leukocyte series as only eosinophil levels fell outside the normal

range during training. Also, absolute eosinopenia was associated with clinical signs of illness. With respect to training intensity, no differences were observed between hypothetically overtrained and normally trained horses (Tyler-McGowan *et al.*, 1999; Padalino *et al.*, 2007).

Summary of the Effect of Exercise on Leukocyte Parameters

Table 1.6 summarises the nature of observed effects of acute and chronic exercise on total leukocytes, PCV, neutrophils, lymphocytes, monocytes and eosinophils. An *x* denotes that research studies as included in this literature review have observed the marked effect of acute (or chronic) exercise on the according haematological parameter. A blank space illustrates that no research study, which was found during the course of this secondary research observed the unmarked effect of exercise on the according variable.

Table 1.6 Effects of exercise on leukocyte parameters

<i>Parameter</i>	<i>Acute Exercise</i>			<i>Chronic Exercise/Training</i>		
	<i>↑</i>	<i>↓</i>	<i>No effect</i>	<i>↑</i>	<i>↓</i>	<i>No effect</i>
<i>Total Leukocytes</i>	<i>x</i>		<i>x</i>	<i>x</i>		<i>x</i>
<i>Neutrophils</i>	<i>x</i>		<i>x</i>	<i>x</i>	<i>x</i>	<i>x</i>
<i>Lymphocytes</i>	<i>x</i>	<i>x</i>			<i>x</i>	<i>x</i>
<i>Monocytes</i>	<i>x</i>					<i>x</i>
<i>Eosinophils</i>					<i>x</i>	

Effects of Acute and Chronic Exercise on Plasma Biochemistry

Acute exercise has been reported to have varying effects on biochemical parameters, whereas chronic exercise is thought to have few effects on biochemical values (Hodgson, 1994). Unless otherwise stated, research studies reviewed in the following referred to the effect of exercise on plasma haematology in horses.

Total Protein

Alterations in total protein as a result of exercise are mainly attributed to fluid shift out of the plasma and fluid loss (Hodgson, 1994; Poracova *et al.*, 1998). Rose *et al.*

(1983) examined total protein of fourteen horses at different points during and up to three days after a 160 km endurance ride. Total protein was significantly increased at 85 km and then remained unchanged with respect to pre-race values at 160 km and 30 minutes after the ride. One day after the ride total protein was significantly reduced compared to pre-exercise values. Fluid loss was thought to be highest at mid-point of the ride, resulting in the highest total protein values at this point. Robson *et al.* (2003) found that immediately following an 80 km endurance race, serum total protein concentration was significantly elevated, but had regained pre-race concentrations one-day after the race. It was suggested that this finding in conjunction with an increased haematocrit provided a qualitative, if not precise, indication of the extent of dehydration following the endurance race.

Rose and Hodgson (1982) observed no change in resting total protein concentration in endurance horses during a 12 week training programme. Training intensity appeared to have no effect on total protein in horses, as no differences between horses that were undergoing hypothesized overtraining and those undergoing less intensive training were reported (Tyler-McGowan *et al.*, 1999; Padalino *et al.*, 2007).

Albumin

Measurements of albumin, like all other protein measurements, provide information on hydration status of the individual as well as indices of infection, inflammation, or a variety of pathologic states such as increased protein loss, or reduced protein synthesis (Hodgson and Rose, 1994; Poracova *et al.*, 1998). Rose *et al.* (1983) found that plasma albumin was increased in horses during a 160 km endurance race at 85 km and 160 km as well as 30 min after the ride. Pre-race values of albumin were regained one day after the race. These changes in plasma albumin were linked to fluid loss associated with prolonged, strenuous endurance exercise.

No changes in plasma albumin of endurance horses were observed throughout the duration of a 12 week training programme in a study by Rose and Hodgson (1982). However, a research study by Tyler-McGowan *et al.* (1999) examining biochemical responses to training in Standardbred horses during a 34 week progressive training programme demonstrated increases in albumin throughout the entire programme. Increases were observed during moderate and intensive training phases as well as

during a hypothesized overtraining phase. However, no differences were found between horses in the overtraining and regular training groups at any stage. Padalino *et al.* (2007) also reported no differences in the albumin level of hypothetically overtrained horses and those undergoing less intensive training.

Globulin

Globulins include α -globulins, β -globulins, γ -globulins and immunoglobulins (Kingston, 2004). Padalino *et al.* (2007) suggested that increases in the percentage of α_1 and α_2 -globulins in horses might indicate a condition of overtraining in combination with alterations in other haematological and biochemical parameters. This parameter was higher in a group of horses subjected to hypothesized overtraining than in horses subjected to less intensive training. No differences between β_1 and β_2 -globulins or γ -globulins in either group were observed. Coyne *et al.* (1990) reported increases in plasma globulin group concentrations after acute exercise, which could not solely be attributed to changes in fluid concentration due to a heterogenous nature of the increase in plasma protein concentration. However, mechanisms behind this observation are unclear, but could include compartmental redistribution, accelerated biosynthesis, increased degradation and bolus release.

Summary of the Effect of Exercise on plasma protein parameters

Table 1.7 summarises the nature of observed effects of acute and chronic exercise on total protein, albumin and globulin. An *x* denotes that research studies as included in this literature review have observed the marked effect of acute (or chronic) exercise on the according biochemical parameter. A blank space illustrates that no research study, which was found during the course of this secondary research observed the unmarked effect of exercise on the according variable.

Table 1.7 Effects of exercise on plasma protein parameters

<i>Parameter</i>	<i>Acute Exercise</i>			<i>Chronic Exercise/Training</i>		
	<i>↑</i>	<i>↓</i>	<i>No effect</i>	<i>↑</i>	<i>↓</i>	<i>No effect</i>
<i>Total Protein</i>	<i>x</i>	<i>x</i>	<i>x</i>			<i>x</i>
<i>Albumin</i>	<i>x</i>			<i>x</i>		<i>x</i>
<i>Globulin</i>				<i>x (OT)</i>		

Muscle Enzymes – Aspartate Transaminase (AST)

Aspartate Transaminase (AST) is a muscle-derived enzyme and as such is commonly used as an indicator of muscle damage in the human athlete or athletic horse. AST was reported to increase during or following acute exercise in horses during and immediately after a 160 km endurance race (Rose *et al.*, 1983). Pre-race values were regained one day after the event. Also, pre-race AST values were significantly higher in the horses examined than in eventing and endurance horses from previous studies, where the same measurement technique was used. This was explained by an extensive training programme horses had undergone four to six weeks prior to the 160 km endurance race and might as such have indicated earlier muscle damage. AST had been reported to have a prolonged half life compared to other muscle enzymes such as CK (Cardinet *et al.*, 1967; in Harris *et al.*, 1998). Therefore, increased AST levels could potentially be detected for longer periods following acute and chronic exercise.

Yamamoto *et al.* (2008) detected increased AST levels in human athletes after two months of training. After four and six months of training no differences to pre-training values were observed. Similarly increasing neutrophil counts indicated muscle damage and inflammation occurring as a result of initial training. Values also showed that from the two-month point onwards, muscle damage and inflammation declined.

Whereas Rose and Hodgson (1982) observed no change in AST in endurance horses throughout a 12 week training regime, Harris *et al.* (1998) showed that AST in Thoroughbred race horses increased during the initial training phase in three year old colts. Gender and age were also found to significantly affect AST in horses. Tyler-McGowan *et al.* (1999) detected increasing AST levels in Standardbred horses throughout a 34 week progressive training regime. Furthermore, horses subjected to overtraining showed higher mean AST activity than those subjected to less intensive training. Horses with increased AST levels also displayed signs of clinical muscle damage including gluteal muscle injury during the high-intensity training phase. These findings are similar to those of Padalino *et al.* (2007), who also found elevated AST in overtrained horses.

Muscle Enzymes – Creatine Kinase (CK)

Creatine kinase (CK), another muscle-derived enzyme, was found to increase significantly as a response to acute exercise in endurance horses (Rose *et al.*, 1983). Following a 160 km endurance race, CK was reported to have returned to pre-exercise values one day after the event.

Similar to AST levels, CK levels were reported to peak two months after training began in male human athletes in a study by Yamamoto *et al.* (2008). CK levels decreased thereafter, although remaining significantly elevated compared to pre-training values. It was suggested that as muscle tissue and function were strengthened by chronic exercise, muscle damage and inflammation decreased accordingly.

No changes in CK values were observed in endurance horses during 12 weeks of training (Rose and Hodgson, 1982) or in Standardbred horses during 34 weeks of training (Tyler-McGowan *et al.*, 1999). Also, hypothesized overtraining had no effect on CK. However, a study by Padalino *et al.* (2007) found elevations in CK in a group of horses subjected to overtraining when compared to horses undergoing less intensive training.

Gamma-Glutamyl Transferase (GGT)

Gamma-Glutamyltransferase (GGT) is an indicator of liver function (Poracova *et al.*, 1998; Tyler-McGowan *et al.*, 1999). It has been found to increase during and immediately following prolonged, intensive endurance exercise in horses, however pre-exercise values were regained one-day after the exercise challenge (Rose *et al.*, 1983). In conjunction with increased plasma bilirubin concentrations, some degree of hepatic dysfunction was thought to have contributed to this result.

As with other biochemical parameters examined, Rose and Hodgson (1982) detected no significant changes in GGT levels of endurance horses during a 12 week training scheme. Tyler-McGowan *et al.* (1999), on the other hand, found that GGT underwent a significant increase with training throughout the 34 week period examined. No difference in GGT between overtrained and regularly trained horses was found by Tyler-McGowan *et al.* (1999) or Padalino *et al.* (2007).

Total Bilirubin

Plasma bilirubin concentration, an indicator of hepatic function, was found to be increased in horses after 85 km and 160 km of an endurance race as well as 30 minutes and one day after the event (Rose *et al.*, 1983). Intravascular hemolysis following acute exercise or some degree of hepatic dysfunction independent of exercise taking increased GGT into account was suggested as possible reasons.

Training of endurance horses appeared to have no effect on total bilirubin (Rose and Hodgson, 1982), while long-term training with increasing exercise-intensity of Standardbred horses was seen to lead to increases in total bilirubin (Tyler-McGowan *et al.*, 1999). The latter study showed no difference in total bilirubin of overtrained and regularly trained horses. Padalino *et al.* (2007) reported higher levels of total bilirubin in hypothetically overtrained horses than in horses that were less intensively trained.

Also, increased total bilirubin values have been reported in fasting horses by Gronwall and Mia (1972), although no significant increase in bilirubin production was reported.

Summary of the Effect of Exercise on Serum Enzymes

Table 1.8 summarises the nature of observed effects of acute and chronic exercise on AST, CK, GGT and total bilirubin. An *x* denotes that research studies as included in this literature review have observed the marked effect of acute (or chronic) exercise on the according biochemical parameter. A blank space illustrates that no research study, which was found during the course of this secondary research observed the unmarked effect of exercise on the according variable.

Table 1.8 Effects of exercise on serum enzymes

Parameter	Acute Exercise			Chronic Exercise/Training		
	↑	↓	No effect	↑	↓	No effect
AST	x			x		x
CK	x			x		x
GGT	x			x		x
Total Bilirubin	x			x		x

Electrolytes – Sodium (Na), Potassium (K), Chloride (Cl)

Changes in plasma electrolytes following exercise are associated with changes in extracellular fluid balance (sodium) or accumulation of hydrogen ions in active muscle, leading to a reduced re-uptake by the fibres and an impairment of the Na⁺, K⁺-ATPase pump (potassium) occurring during high-intensity exercise. Changes in plasma electrolytes (sodium, potassium, chloride) occurring during low and moderate endurance exercise can be explained by fluid loss such as perspiration (Rose *et al.*, 1983; Hodgson, 1994).

Several changes in plasma electrolytes of horses were reported during and following a 160 km endurance race in a study by Rose *et al.* (1983). Sodium was increased after 85 km, but had returned to pre-race levels after 160 km. Potassium was increased at 85 km during the ride, which was associated with fluid movement between intra- and extracellular compartments. Decreased levels of potassium 30 minutes after the race could possibly have been explained by potassium leaving muscle fibres bound to phosphate during exercise. Chloride levels remained unchanged during and after the race. Unusually cold conditions leading to low fluid loss by perspiration were likely to have contributed to the minimal changes in plasma electrolytes seen during and after this endurance race. Plasma electrolyte levels in endurance horses did not fluctuate during a 12 week training regime as reported by Rose and Hodgson (1982) indicating that changes in plasma electrolytes are linked to acute exercise.

Summary of the Effect of Exercise on Plasma Electrolytes

Table 1.9 summarises the nature of observed effects of acute and chronic exercise on sodium, potassium and chloride. An x denotes that research studies as included in this

literature review have observed the marked effect of acute (or chronic) exercise on the according biochemical parameter. A blank space illustrates that no research study, which was found during the course of this secondary research observed the unmarked effect of exercise on the according variable.

Table 1.9 Effects of exercise on plasma electrolytes

<i>Parameter</i>	<i>Acute Exercise</i>			<i>Chronic Exercise/Training</i>		
	↑	↓	<i>No effect</i>	↑	↓	<i>No effect</i>
<i>Sodium</i>	<i>x</i>		<i>x</i>			<i>x</i>
<i>Potassium</i>	<i>x</i>	<i>x</i>	<i>x</i>			<i>x</i>
<i>Chloride</i>			<i>x</i>			<i>x</i>

Summary

Epidemiological research has shown that respiratory disease, especially in young horses, is an important contributor to wastage in the industry, and, as such causes a significant economic loss to the thoroughbred industry. Both, acute and chronic exercise, have been associated with changes in immunological, haematological and biochemical parameters. A connection between exercise, disease and *in vitro* measurements has been suggested by research studies published to date. The response of the body to acute exercise and training is complex depending on several factors such as parameter measured, exercise intensity and duration, timing of blood sampling as well as the age of the individual human or equine athlete.

Lymphocyte proliferation, as a measure of the adaptive immune response to exercise and training, is generally reduced following acute exercise, whereas varied results have been observed during training though research findings pointed towards training not causing any significant changes. The response of parameters of the innate immune system to exercise and training appears even more diverse. Acute exercise further has varied effects on phagocytic activity of granulocytes and monocytes. Granulocyte and monocyte phagocytosis are generally suppressed during intensive training periods, but appear to remain unchanged throughout more moderate periods of exercise. On the other hand, oxidative burst capacity is more commonly found to be reduced after high-intensity acute exercise, while no effect is observed following moderate acute exercise bouts. Although oxidative burst capacity remains unchanged during training in a number of research studies, results, in general, are varied.

Haematological variables exhibit a more prominent reaction to acute exercise, often displayed in form of increases in values observed, even though some variety is observed, for example, relating to stress leukograms. The effect of chronic exercise on haematological parameters is much more varied, while it has been indicated that values often remained unchanged throughout the training periods examined.

Similar to the effect of acute exercise on haematological parameters, biochemical parameters generally increase following acute exercise, but this varies in some instances. The effect of chronic exercise on biochemical variables is more

inconsistent leading to increase, decreases and no effects being detected depending on study design.

As previously indicated, physiological mechanisms underlying reported changes are multifactorial and complex; however, changes associated with exercise and training have been shown to be dependent on changes in the endocrine system.

Although several studies examining the effect of exercise and training on immunity in the horse have been published to date, no coherent response of immunological, biochemical and haematological parameters could be reported due to differences in study design, type of horses used as well as the parameters measured. To the knowledge of the researcher no study published to date has taken the effect of racehorse training on immunological parameters of the innate and specific immune system as well as haematological and biochemical parameters into consideration. Thus, the current study aimed at presenting new results on the effects of introduction to training on immunity in the racehorse, which is considered of particular importance in furthering the understanding of the possible occurrence of exercise-induced immunomodulation in the racehorse population. The confirmed incidence of respiratory infections in racehorses in training and its associated economic costs warrants further research into this area.

Chapter Two

Research Methodology

Research Objectives

As described in the previous chapter, research suggests that a link between exercise or training and the immune system as well as plasma biochemistry and haematology exists. However, effects of exercise or training on these parameters appear to be complex and dependent on multiple factors, for example, exercise-intensity or duration and type of exercise. The effects of exercise or training on immunity can also make athletes more susceptible to disease. Increased incidences of upper respiratory tract infection in horses following introduction to training have been reported according to clinical and anecdotal evidence. It has not been established if this is as a result of immunosuppression.

The objective of this research project was to determine whether introduction to training of has an effect on components of the innate and specific immune system as well as on plasma haematology and biochemistry in racehorses. And to further establish the nature of any effects observed throughout the duration of this study to investigate the hypothesis that chronic exercise training is associated with immunosuppression. Phagocytosis and oxidative burst capacity of granulocytes and monocytes were examined as components of the innate immune system, while mitogen-induced T-cell proliferation was examined as a component of the specific immune system. The effect of training on several haematological as well as biochemical variables as described further on in this chapter were examined in this study.

Animals

Twenty thoroughbred horses from two local training yards were used in this study. All horses were moved to the training yards prior to commencement of training. The horses in each training yard were housed in the same environment, respectively, and were of a similar age (4 years old ($n=5$), 5 years old ($n=7$), six years old ($n=6$) with the exception of two horses aged 8 years ($n=1$) and ten years ($n=1$)). Horses included in this study were mares ($n=3$) and geldings ($n=17$). All horses were regularly wormed and vaccinated. Each horse was trained for National Hunt racing. Two

horses from training yard one had to discontinue training between two and eight weeks of training due to lameness or personal circumstances of the owner. These two horses were geldings aged eight and six years. Due to the nature of the statistical analysis these horses were excluded from all analysis resulting in total sample size being reduced to $N=18$ ($n = 8$ (yard one), 10 (yard two)) unless otherwise stated.

Experimental Design and Blood Sampling

The animals were blood sampled upon introduction and upon commencement of training. Blood sampling took place on week 0 (prior to first training session), week 2 as well as in week 8 and week 10. All sampling was performed prior to exercise. Blood samples were obtained by jugular venipuncture into heparinised and EDTA blood tubes (Labstocks Microservices Ltd., Clonee, Ireland). Samples were analysed immediately upon arrival to the laboratory. Samples kept for the purpose of haematological and biochemical analyses were posted to the Irish Equine Centre immediately upon arrival to the laboratory.

Training Schedule

The training programmes were non-unified as trainers adapted the training schedule according to the individual horse and the individual training programme developed by each yard. Training, however, was naturally progressive in each yard as can be observed in table 2.1 and 2.2. Each bout of acute exercise in either yard was followed by a cooling down period on the walker and/or turnout depending on weather conditions.

Table 2.1 Training schedule in yard one

<i>Week</i>	<i>Activity</i>
<i>1</i>	60 min. Walker
<i>2</i>	45 min. workout (walk & trot)
<i>3</i>	10 min. workout (walk & trot) followed by 1 ½ mile canter
<i>4</i>	10 min. workout (walk & trot) followed by 1 ½ mile canter
<i>5</i>	10 min. workout (walk & trot) followed by 3 mile canter
<i>6-8</i>	10 min. workout (walk & trot) followed by 3 mile canter building up to 4 miles canter
<i>>8</i>	45 min. workout including walk, trot, canter and gallop Schooling over hurdles and fences up to 8 jumps

Each acute exercise bout in yard two included a 40 minute warm up period as well as a 30 min cool down period on the walker. Workout in some horses alternatively included 5 minutes of swimming during week one to six due to individual differences in health and response to exercise.

Table 2.2 Training schedule in yard two

<i>Week</i>	<i>Activity</i>
<i>1-2</i>	20 min. workout (trot & slow canter)
<i>3-4</i>	20 min. 'half-speed' workout (trot, slow and strong canter)
<i>>5</i>	20 min. 'full-speed' workout (trot, canter and gallop)

Isolation of Lymphocytes / Peripheral Blood Cell Preparation

Peripheral blood leukocytes were separated from blood by means of density gradient centrifugation using Histopaque-1077 (Sigma-Aldrich, Schnelldorf, Germany) immediately upon arrival in the laboratory to ensure viability of lymphocytes. Diluted blood was layered on top of an equal amount of Histopaque-1077 in a conical centrifuge tube. All reagents and blood samples were allowed to warm to room temperature. Blood was then centrifuged at 400 x g for 30 minutes at room temperature. The buffy coat layer was collected and washed 3x with Phosphate Buffered Saline (PBS) (250 x g for 10 minutes at 21°C). Following the third wash the cell pellet was re-suspended in DMEM (Sigma-Aldrich, Schnelldorf, Germany) at

10% heat-inactivated foetal bovine serum (FBS) (Sigma-Aldrich, Schnellendorf, Germany).

Lymphocyte Proliferation Assay

A nonradioactive lymphocyte proliferation assay was used, as previously described by Witonsky *et al.* (2003), Saker *et al.* (2001), Gogal *et al.* (1997), and Ahmed *et al.* (1994). After isolation of lymphocytes, 100 μ l aliquots of the cells (3.5×10^6 cells/ml) were plated in duplicate wells in 96-well flat-bottom tissue culture plates (Sarstedt, Wexford, Ireland). The wells into which the cell suspension was aliquoted contained 100 μ l of either medium alone (DMEM at 10% FBS), concanavalin A (Sigma-Aldrich, Schnellendorf, Germany) (Con A, 0.625 μ g/ml to 10 μ g/ml). Subsequently, after stimulation with a mitogen, cells were incubated in a CO₂ incubator (5% at 37°C) for approximately 44 hours. Following incubation, 20 μ l of the reagent dye Alamar Blue (Europa Bioproducts Ltd., Cambridge, UK) was added to each well and the cells were incubated for a further 6 to 8 hours. Cell proliferation was measured by use of a microplate reader. Absorbance was measured at 570 nm and 600 nm. Subtraction of the optical density at 600 nm from the optical density at 570 nm accurately determines the true absorbance, which reflects proliferation. The specific absorbance of unstimulated cells in media alone was subtracted from the specific absorbance of cells incubated with a mitogen to determine the Δ -specific absorbance (Saker *et al.*, 2001). The mean specific absorbance of duplicate wells was then calculated. Results were reported as the Δ -specific absorbance.

Flow Cytometric Analysis

The flow cytometer used for analysis of phagocytosis and oxidative burst capacity in this study was the BD LSRI (BD Biosciences, Shannon, Ireland). The sheath fluid used for all experiments was FacsflowTM (BD Biosciences, Shannon, Ireland). The flow cytometer was calibrated on a daily basis using CalibriteTM three colour calibration beads (BD Biosciences, Shannon, Ireland) and SpheroTM Ultra Rainbow Fluorescent Particles (Spherotech Inc., Libertyville, IL, USA). Cleaning procedures as recommended by the manufacturer were carried out on a daily basis. Instrument

settings (forward scatter (FSC) and side scatter (SSC)) were adjusted so that lymphocytes, monocytes and granulocytes could be distinguished when cells were run through the cytometer. The software that was used for data acquisition and analysis was CellQuest™ (BD Biosciences, Shannon, Ireland).

Phagocytosis Assay

The quantitative determination of phagocytic activity in whole blood was performed using the Phagotest® Kit (ORPEGEN Pharma, Heidelberg, Germany) according to the manufacturer's protocol, except that the temperature of the water bath was kept at 39°C, as previously described (Kampen *et al.*, 2004) in order to adjust for different requirements arising from the cell type used (equine) . Briefly, for each sample 100 µl of pre-cooled heparinised whole blood was incubated with 20 µl opsonised FITC-labelled *E.coli* suspension for ten minutes at 39°C to activate cells. A second tube, prepared in the same way, served as a control sample and as such remained on ice for ten minutes at 0°C. Subsequently, 100 µl of quenching solution was added to each tube at 0°C in order to stop phagocytosis. Afterwards, the samples were washed twice by adding 3 ml of washing solution to each tube at 0°C and centrifugation at 300 x g for five minutes at 4°C, and discarding the supernatant. The whole blood was next lysed by the addition 2 ml of lysing solution, which had been pre-warmed to room temperature, to each tube. Following addition of the lysing solution, samples were kept at room temperature for 20 minutes and were then centrifuged at 300 x g for five minutes at 4°C. The supernatant was discarded prior to washing the samples once, as described previously. Finally, 100 µl of DNA staining solution was added to each tube at 0°C. Samples were incubated for at least 10 minutes protected from light at 0°C and, subsequently, each tube was analysed by flow cytometry within 60 minutes.

Data acquisition and analysis

The results of the analysis of 10,000 leukocytes per sample were recorded as the percentage of phagocytosing cells. Figure 2.1 displays gating on leukocytes to discriminate between bacteria and leukocytes.

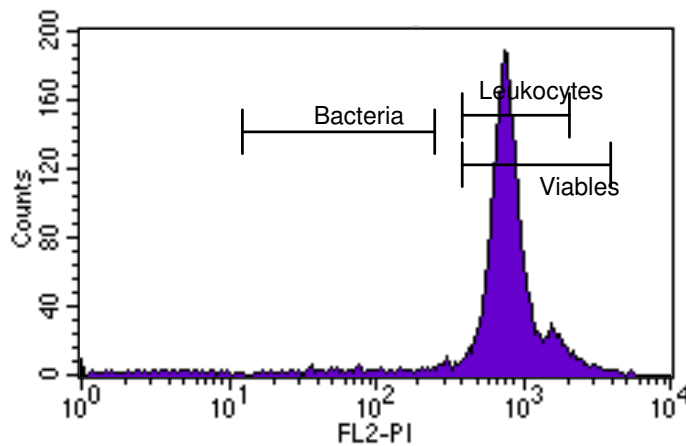


Figure 2.1 Gating on leukocytes

The relevant leukocyte cluster was gated on granulocytes and monocytes for the purpose of data evaluation as can be seen from figure 2.2.

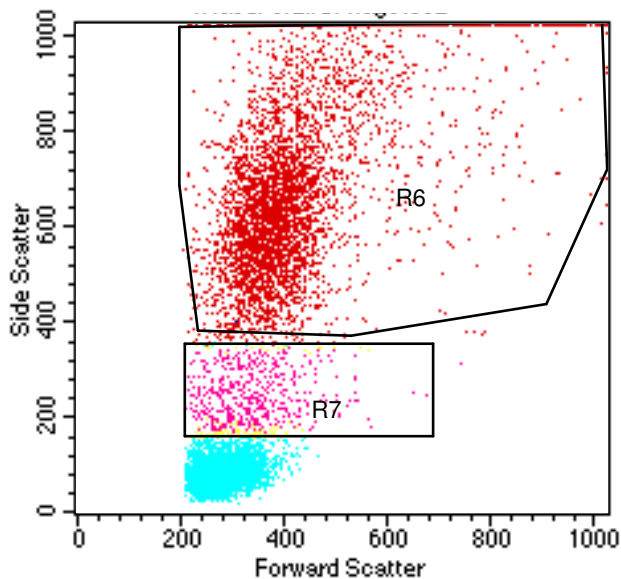


Figure 2.2 Gated on granulocytes (R6) and monocytes (R5)

Figure 2.3 and 2.4 respectively illustrate example dot plot displays during data acquisition of the control sample, which was incubated at 0°C, and the activated sample, which was incubated at 39°C.

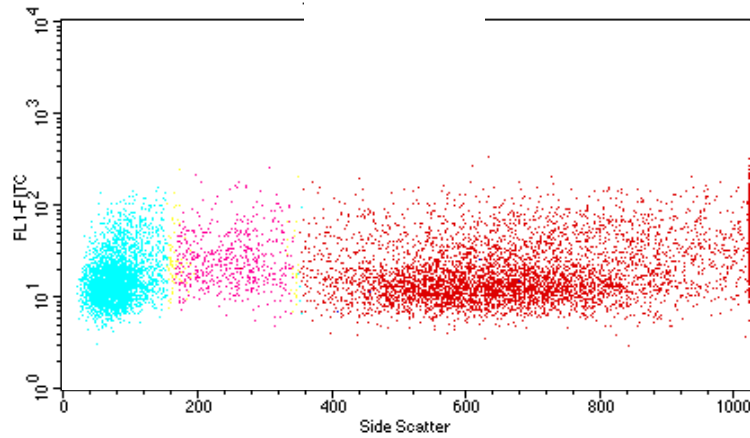


Figure 2.3 Phagocytosis – Control Sample

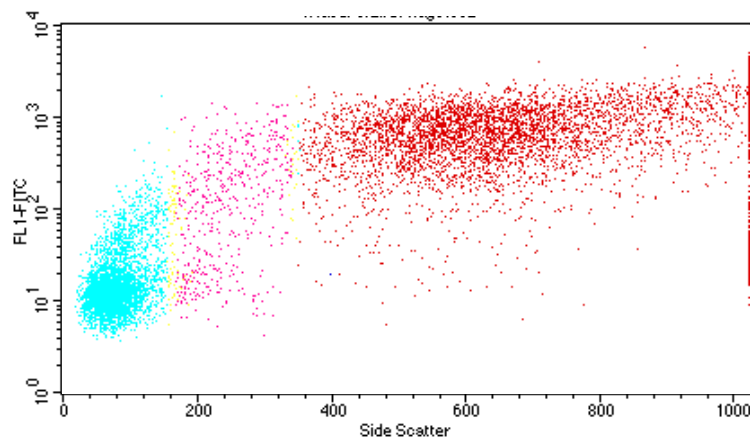


Figure 2.4 Phagocytosis – Activated

To determine the percentage of cells having performed phagocytosis the according green fluorescence histogram (FL1-FITC) was analysed. Figures 2.5 and 2.6 respectively represent sample histograms gated on granulocytes and monocytes. The sample displayed in figure 2.5 included 94.47% of positive (i.e. of phagocytosing cells). The sample histogram in figure 2.6 showed 37.69 % of positive cells.

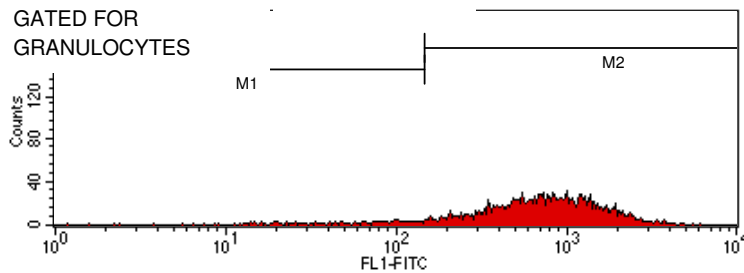


Figure 2.5 Gated granulocytes

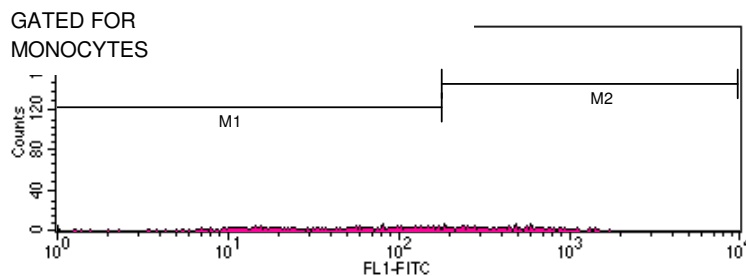


Figure 2.6 Gated monocytes

Oxidative Burst Capacity Assay

The determination of oxidative burst function of PMNs in whole blood was performed using the Bursttest® Kit (ORPEGEN Pharma, Heidelberg, Germany) according to the manufacturer's protocol, except that the temperature of the water bath was kept at 39°C, as previously described (Kampen *et al.*, 2004) in order to adjust for different requirements arising from the cell type used (equine). Tests in the laboratory showed that the use of fMLP (Chemotactic peptide f-formyl-MetLeuPhe) (intended as a 'negative or low control') could not be adapted to analyse equine blood as results obtained were inconsistent and therefore this reagent was omitted from use in the experiment. Briefly, for each sample 100 µl of pre-cooled heparinised whole blood was pipetted into three tubes at 0°C. Afterwards, 20 µl of opsonised, non-labelled *E.coli* suspension was added to one tube, 20 µl of washing solution was added to another tube as a 'positive or high control' and 20 µl of PMA (Phorbol 12-myristate 13-acetate) working solution was added to the third tube as a 'positive control'. The samples were then incubated for ten minutes at 39°C. Subsequently, 20 µl of substrate solution was added to each tube and tubes were incubated for ten minutes at 39°C. The whole blood was next lysed by adding 2 ml of lysing solution, which had

been pre-warmed to room temperature, to each tube. Following the addition of the lysing solution, samples were kept at room temperature for 20 minutes and were then centrifuged at 300 x g for five minutes at 4°C. The supernatant was discarded and samples were washed once by the addition of 3 ml washing solution to each tube followed by centrifugation at 300 x g for five minutes at 4°C. After discarding the supernatant, 100 µl of DNA staining solution was added to each tube at 0°C. Samples were incubated for at least 10 minutes protected from light at 0°C and, subsequently, each tube was analysed by flow cytometry within 30 minutes.

Data acquisition and analysis

The results of the analysis of 10,000 leukocytes per sample were recorded as the percentage of oxidising cells. Figure 2.7 displays gating on leukocytes to discriminate between bacteria and leukocytes. The relevant leukocyte cluster was then gated on granulocytes and monocytes for the purpose of data evaluation.

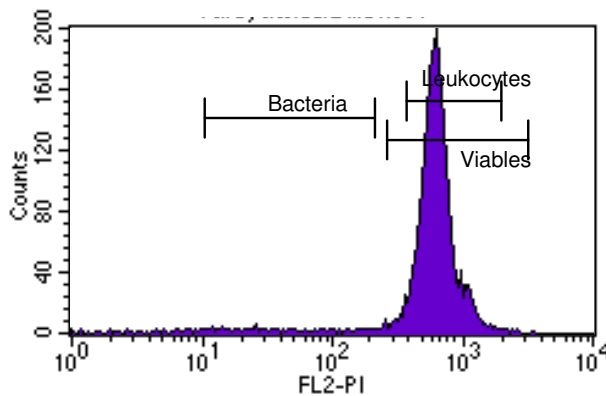


Figure 2.7 Gating on leukocytes

Figure 2.8, 2.9 and 2.10 respectively illustrate example dot plot displays during data acquisition of the control sample (no stimulation), the positive control (stimulation with PMA) and the test sample (stimulation with E.coli).

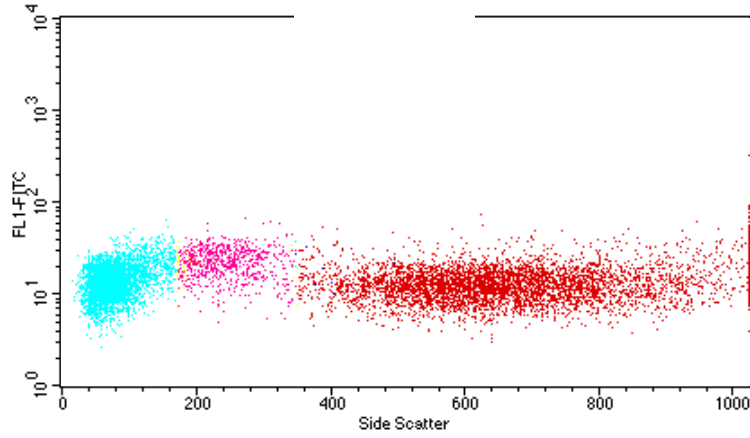


Figure 2.8 Oxidative Burst Capacity – Control Sample

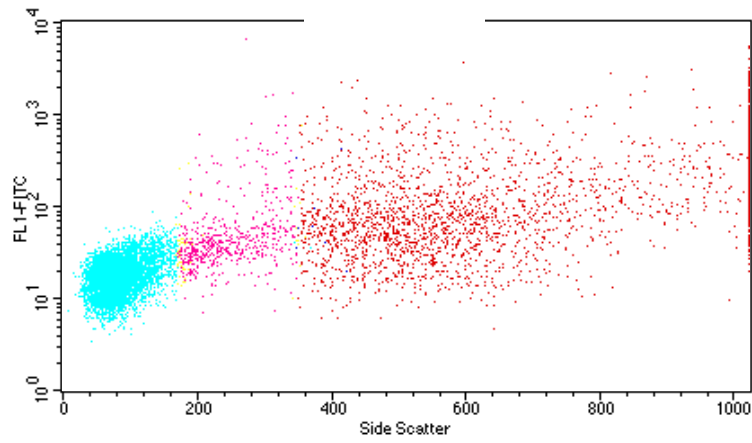


Figure 2.9 Oxidative Burst Capacity – Positive Control

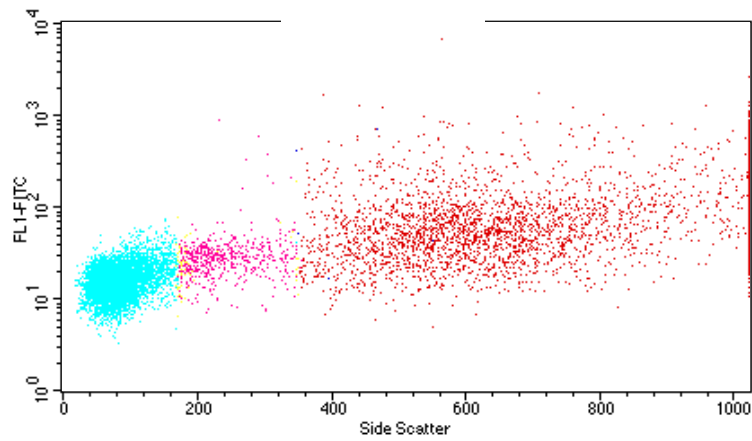


Figure 2.10 Oxidative Burst Capacity – Test Sample

To determine the percentage of cells having produced reactive oxygen metabolites (recruitment), the relevant leukocyte cluster was gated in the scatter diagram similar to figure 2.2 and the according green fluorescence histogram (FL1-FITC) was then analysed. Figures 2.11 and 2.12, respectively, represent sample histograms gated on granulocytes and monocytes as positive controls, while figure 2.13 and 2.14, respectively, represent sample histograms gated on granulocytes and monocytes when stimulated with E.coli. The sample histogram in figure 2.11 showed 77.58% positive cells (granulocytes).

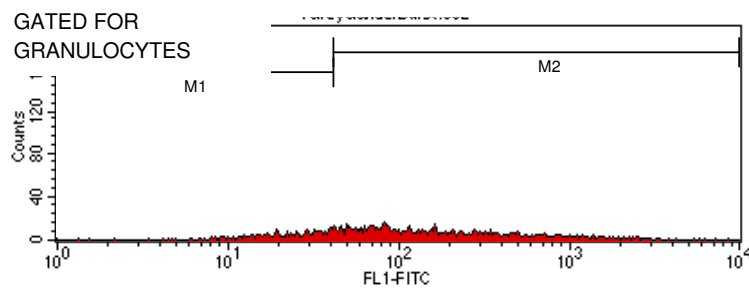


Figure 2.11 Gated granulocytes (Positive Control)

The sample histogram in figure 2.12 included 39.88% positive cells (monocytes).

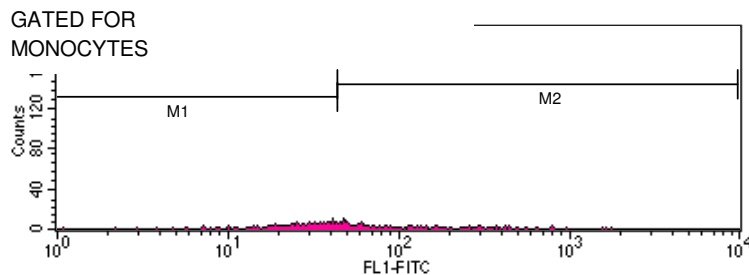


Figure 2.12 Gated monocytes (Positive Control)

The test sample displayed in figure 2.13 included 60.7% positive cells (granulocytes). The smaller percentage of positive cells than displayed in the histogram from figure 2.11 relates to the difference between the positive control and the test sample.

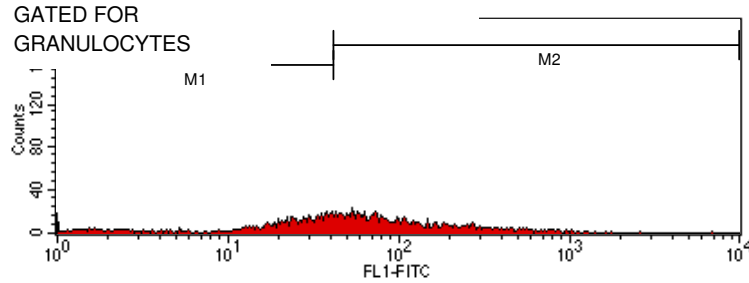


Figure 2.13 Gated granulocytes (Test Sample)

As the histogram in figure 2.14 represents the test sample as opposed to the positive control displayed in figure 2.12 only 6.06% of positive cells (monocytes) were recorded.

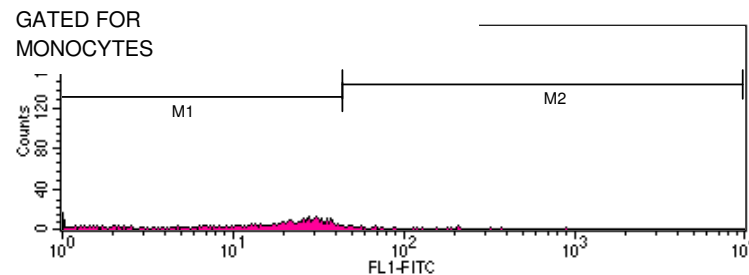


Figure 2.14 Gated monocytes (Test Sample)

Haematology Analysis

Haematological analysis was conducted by the Irish Equine Centre using the CELL-DYN 3700SL System. The number of red blood cells, packed cell volume, haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, platelet, white blood cells, neutrophils, lymphocytes, monocytes and eosinophils were measured in peripheral blood samples. The normal range as reported by Hodgson and Rose (1994) for all haematological parameters examined is displayed in Appendix A.

Biochemistry Analysis

The concentrations of total protein, albumin, globulin, aspartate transaminase, creatine kinase, gamma-glutamyl transferase, total bilirubin, sodium, potassium and

chloride were measured in peripheral blood samples using the Cobas Integra 800 System at the Irish Equine Centre. The normal range as reported by Hodgson and Rose (1994) or the Irish Equine Centre (Total Bilirubin reference values) for all biochemical parameters examined is displayed in Appendix A.

Data Analysis

SPSS 13.0 for Windows and SPSS 16.0 for Windows were used for statistical analysis. Descriptive Statistics for each variable were shown by yard to allow for a better understanding of the data, and, in case of haematology and biochemistry values, to determine whether values observed fell within the range considered as normal. Given strong differences in training and stable management (feeding, worming, vaccination, daily routine) data was analysed for each yard separately.

Nonparametric tests were performed due to small sample sizes: $n = 8$ (Yard One) and $n = 10$ (Yard Two) (Bakovic *et al.*, 2003). Furthermore, analysis was undertaken for each yard separately to avoid interference of external differences such as differences in training regime, feeding and stable management with analysis. Changes over time were analysed by Friedman's ANOVA by ranks. To present results from this analysis, χ^2 values (χ^2) and degrees of freedom (df) were reported along with the significance value (p). On rejection of the null hypothesis (when $p < 0.05$), Wilcoxon signed-rank tests were performed for nonparametric post hoc comparisons i.e. to examine the change throughout the training period based on a comparison by weeks (Bakovic *et al.*, 2003; Vassilakopoulos *et al.*, 2003, Field, 2005). All significant results were reported as T values (T) along with the significance level (p). Post hoc comparisons as examined by Wilcoxon signed-rank tests are shown in table 2.1.

Table 2.3 Post hoc comparisons

<i>Week</i>	<i>Week</i>
0	2
0	8
0	10
2	8
2	10
8	10

Oxidative Burst capacity relating to horses from yard two was analysed using the previously described technique. Only four horses from yard one had no missing values for any of the sampling dates ($n = 4$). Missing values occurred due to technical problems during laboratory analysis. This means that only values from these four horses were available for analysis by Friedman's ANOVA as cases are excluded listwise. As $n = 4$ is a relatively small sample size, individual Wilcoxon signed-rank tests (nonparametric version of paired t -tests) were performed to establish the effect of training on this variable based on a comparison by weeks as n differed from week to week. Results were reported in the format described above. Tests were performed only if $n \geq 5$.

Comparisons between yard one and yard two were made using Mann-Whitney U tests. Results were reported by stating the U value (U) along with the significance level (p). The null hypothesis was rejected when $p < 0.05$. If significant, the Median (Mdn) was provided. Due to different sample sizes in yard one and yard two for oxidative burst capacity analysis, no direct comparison between yard one and yard two was undertaken in this case.

All results in graphs were expressed as means \pm SE. The null hypothesis was rejected when $p < 0.05$.

Chapter Three

Research Findings and

Analysis

Effect of Training on Plasma Haematology, Biochemistry and Immunological Parameters in the Horse

Each of the following sections on the haematological, biochemical and immunological parameters includes measurements prior to commencement of training begin as well as at two, eight and ten weeks during a progressive training programme. Sections contain information on descriptive statistics as well as on statistical findings regarding the variations of levels of each individual parameter measured throughout the time period examined. Normal ranges for haematological and biochemical parameters can be found in appendix A. All required statistical values for the tests conducted were reported and denoted in this chapter as described in chapter two, data analysis.

Effect of Training on T-Cell Proliferation

T-cell proliferation was measured in the laboratory using the mitogen Con A, which specifically stimulates proliferative response of T-cells in vitro. Table 3.1 displays descriptive statistics on average T-cell proliferation after stimulation with Con A and six hours incubation for both yards throughout the ten week training period examined. Con A concentrations of 10, 5 and 2.5 µg/ml were included in the calculation of the average T-cell proliferation throughout the time period examined. Negative minimum values obtained from laboratory analysis using 1.25 and 0.625 µg/ml Con A lead to the exclusion of these results from this statistical analysis. These amounts of Con A in laboratory analysis were too small to produce optimum and consistent results.

Table 3.1 Descriptive Statistics – Average T-cell proliferation

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	0.247	0.266	0.094	0.033	0.073	0.363
	<i>Wk 2</i>	0.221	0.205	0.044	0.016	0.181	0.310
	<i>Wk 8</i>	0.213	0.222	0.051	0.018	0.143	0.284
	<i>Wk 10</i>	0.208	0.210	0.064	0.023	0.096	0.285
<i>Yard Two</i>	<i>Before Training</i>	0.291	0.300	0.056	0.018	0.170	0.360
	<i>Wk 2</i>	0.227	0.220	0.061	0.019	0.150	0.360
	<i>Wk 8</i>	0.240	0.255	0.086	0.027	0.100	0.380
	<i>Wk 10</i>	0.252	0.247	0.071	0.023	0.180	0.410

Average T-cell proliferation did not significantly fluctuate in either yard throughout the time period examined ($p>0.05$) and, thus, largely agreed with individual observations following statistical analysis of T-cell proliferation using individual stimulant concentrations as described below. Also, no significant differences in average T-cell proliferation between yard one and yard two were observed at any point in time ($p>0.05$). Figure 3.1 and table 3.2 illustrate these findings.

Table 3.2 Average T-cell proliferation – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	2.85	3	0.450
<i>Yard Two</i>	5.4	3	0.149
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	31.0	0.46
	2	36.0	0.762
	8	30.0	0.408
	10	26.0	0.237

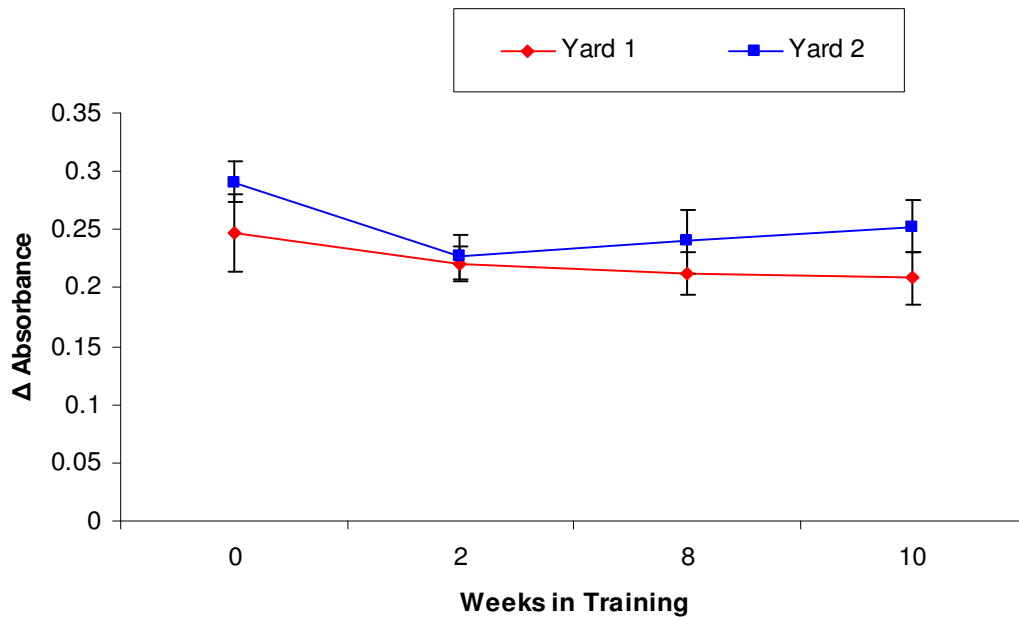


Figure 3.1 Average T-cell proliferation (mean) in response to stimulation with Con A throughout training period ($p>0.05$)

Table 3.3 displays descriptive statistics on T-cell proliferation after stimulation with 10 µg/ml Con A and six hours incubation with the mitogen Con A for both yards throughout the ten week training period examined.

Table 3.3 Descriptive Statistics – T-cell proliferation – 10 µg/ml Con A

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	0.369	0.414	0.118	0.042	0.141	0.509
	<i>Wk 2</i>	0.324	0.330	0.067	0.024	0.205	0.407
	<i>Wk 8</i>	0.307	0.301	0.032	0.011	0.270	0.361
	<i>Wk 10</i>	0.302	0.305	0.084	0.030	0.159	0.454
<i>Yard Two</i>	<i>Before Training</i>	0.342	0.350	0.064	0.020	0.222	0.429
	<i>Wk 2</i>	0.300	0.302	0.072	0.023	0.175	0.438
	<i>Wk 8</i>	0.329	0.356	0.091	0.029	0.163	0.420
	<i>Wk 10</i>	0.356	0.377	0.065	0.021	0.261	0.451

Figure 3.2 illustrates mean T-cell proliferation in response to stimulation with 10 µg/ml Con A throughout training. No significant changes in T-cell proliferation throughout the training period examined were observed in either yard ($p>0.05$). Also, no significant differences in T-cell proliferation (10 µg/ml Con A) between yard one and yard two were observed at any point in time ($p>0.05$). See table 3.4.

Table 3.4 T-cell proliferation (10 µg/ml Con A) – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	1.95	3	0.629
<i>Yard Two</i>	2.76	3	0.454
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	28.0	0.304
	2	29.0	0.360
	8	33.0	0.573
	10	25.0	0.203

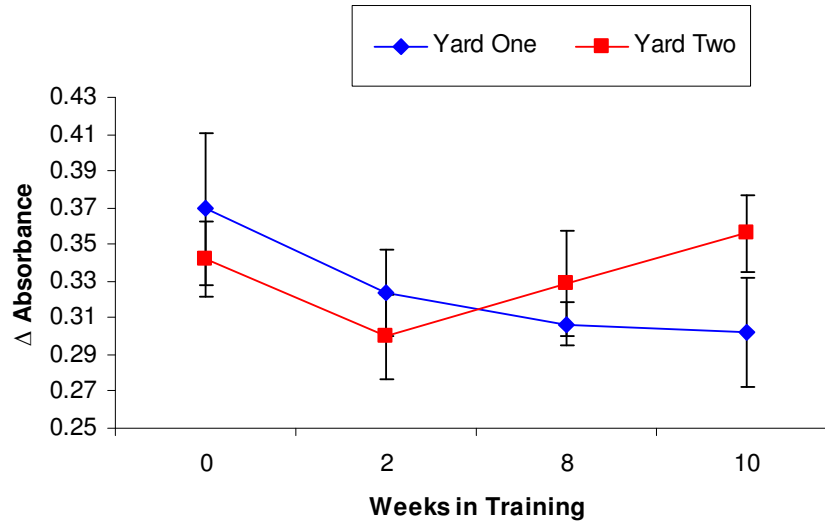


Figure 3.2 Mean T-cell proliferation in response to stimulation with 10 μ g/ml Con A throughout training period ($p>0.05$)

Table 3.5 displays descriptive statistics on T-cell proliferation after stimulation with 5 μ g/ml Con A and six hours incubation for both yards throughout the ten week training period examined.

Table 3.5 Descriptive Statistics – T-cell proliferation – 5 μ g/ml Con A

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One	Before Training	0.269	0.301	0.074	0.028	0.144	0.347
	Wk 2	0.258	0.246	0.064	0.024	0.176	0.352
	Wk 8	0.257	0.254	0.087	0.033	0.113	0.358
	Wk 10	0.254	0.262	0.085	0.032	0.130	0.364
Yard Two	Before Training	0.326	0.346	0.066	0.021	0.204	0.408
	Wk 2	0.245	0.226	0.079	0.025	0.149	0.380
	Wk 8	0.255	0.255	0.121	0.038	0.083	0.507
	Wk 10	0.256	0.243	0.098	0.031	0.109	0.471

T-cell proliferation (5 μ g/ml Con A) did not change significantly throughout training in yard one ($p>0.05$). Statistical analysis of T-cell proliferation (5 μ g/ml Con A) in yard two indicated that significant changes had occurred throughout the training period ($p<0.05$). T-cell proliferation was significantly lower after two and ten weeks of training than prior to the commencement of training ($p<0.05$). This can also be

seen in table 3.7. As before, no significant differences in T-cell proliferation (5 µg/ml Con A) between yard one and yard two were observed at any point in time ($p > 0.05$). This is also shown in table 3.6 and figure 3.3.

Table 3.6 T-cell proliferation (5 µg/ml Con A) – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	<i>N</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	7	1.629	3	0.685
<i>Yard Two</i>	10	8.28	3	0.036
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>	
<i>Yard One vs. Yard Two</i>	Pre-training	19.0	0.133	
	2	28.0	0.536	
	8	33.5	0.906	
	10	33.0	0.866	

Table 3.7 T-cell proliferation (5 µg/ml Con A) change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard Two</i>	Wk 0 – Wk 2	6	0.027
	Wk 0 – Wk 10	7.5	0.039

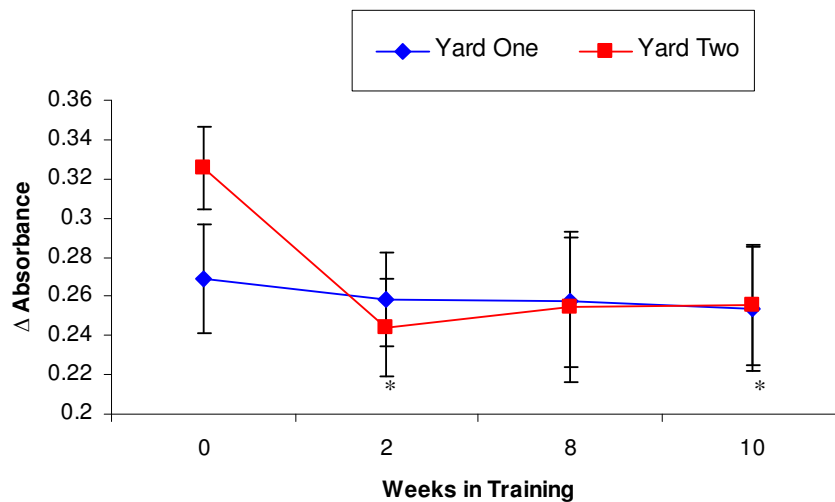


Figure 3.3 Mean T-cell proliferation in response to stimulation with 5 µg/ml Con A throughout training period ($p < 0.05$)

*Sig. different from pre-training values ($p < 0.05$)

Descriptive statistics relating to proliferation of T-cells after stimulation with 2.5 µg/ml Con A and six hours incubation throughout the period examined are shown in table 3.8.

Table 3.8 Descriptive Statistics – T-cell proliferation – 2.5 µg/ml Con A

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One	Before Training	0.154	0.142	0.060	0.023	0.078	0.233
	Wk 2	0.128	0.132	0.048	0.018	0.069	0.195
	Wk 8	0.122	0.126	0.074	0.028	0.016	0.202
	Wk 10	0.115	0.103	0.054	0.020	0.050	0.208
Yard Two	Before Training	0.205	0.212	0.063	0.020	0.083	0.291
	Wk 2	0.136	0.113	0.064	0.020	0.059	0.261
	Wk 8	0.136	0.153	0.063	0.020	0.029	0.213
	Wk 10	0.145	0.132	0.066	0.021	0.072	0.299

In yard one, T-cell proliferation (2.5 µg/ml Con A) was not found to have been significantly affected by training ($p>0.05$). On the other hand, T-cell proliferation (2.5 µg/ml Con A) in horses from yard two did change significantly during the time period examined ($p<0.01$). T-cell proliferation was significantly lower after two, eight and ten weeks of training than prior to the commencement of training (table 3.10). No significant differences in T-cell proliferation (2.5 µg/ml Con A) between yard one and yard two were observed at any point in time ($p>0.05$). This can also be observed in table 3.9 and figure 3.4.

Table 3.9 T-cell proliferation (2.5 µg/ml Con A) – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	N	χ^2	df	p
Yard One	7	2.486	3	0.524
Yard Two	10	11.64	3	0.006
Mann-Whitney U-Test	Week		U	p
Yard One vs. Yard Two	Pre-training		18.0	0.109
	2		34.0	0.962
	8		30.0	0.651
	10		25.0	0.364

Table 3.10 T-cell proliferation (2.5 µg/ml Con A) change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard Two</i>	Wk 0 – Wk 2	8	0.049
	Wk 0 – Wk 8	0	0.002
	Wk 0 – Wk 10	5	0.020

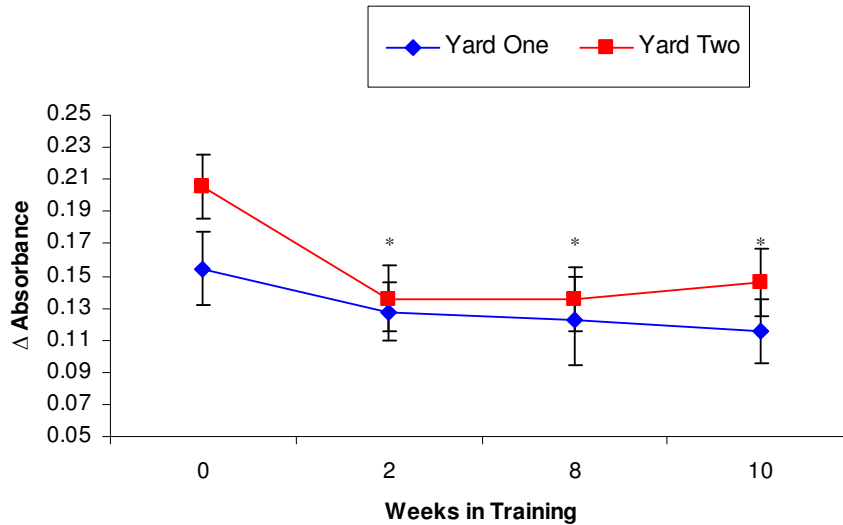


Figure 3.4 Mean T-cell proliferation in response to stimulation with 2.5 µg/ml Con A throughout training period ($p < 0.05$)

*Sig. different from pre-training values ($p < 0.05$)

Table 3.11 displays descriptive statistics on T-cell proliferation after stimulation with 1.25 µg/ml Con A and six hours incubation for both yards throughout the ten week training period examined. Negative minimum values indicate that 1.25 µg/ml Con A was an amount too small to produce optimum results.

Table 3.11 Descriptive Statistics – T-cell proliferation – 1.25 µg/ml Con A

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	0.053	0.056	0.037	0.013	-0.012	0.114
	<i>Wk 2</i>	0.065	0.071	0.030	0.011	0.005	0.106
	<i>Wk 8</i>	0.069	0.068	0.033	0.012	0.023	0.111
	<i>Wk 10</i>	0.067	0.078	0.055	0.019	-0.016	0.139
<i>Yard Two</i>	<i>Before Training</i>	0.095	0.095	0.047	0.015	0.035	0.161
	<i>Wk 2</i>	0.067	0.066	0.045	0.014	-0.006	0.147
	<i>Wk 8</i>	0.060	0.068	0.041	0.013	-0.025	0.111
	<i>Wk 10</i>	0.069	0.064	0.043	0.014	0.001	0.152

No significant changes in T-cell proliferation in response to stimulation with 1.25 µg/ml Con A were observed in yard one and yard two ($p>0.05$). Also, no significant differences in T-cell proliferation (1.25 µg/ml Con A) between yard one and yard two were observed at any point in time ($p>0.05$). See table 3.12.

Table 3.12 T-cell proliferation (1.25 µg/ml Con A) – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	1.35	3	0.757
<i>Yard Two</i>	2.28	3	0.549
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	21.5	0.106
	2	39.0	0.966
	8	36.5	0.782
	10	36.0	0.762

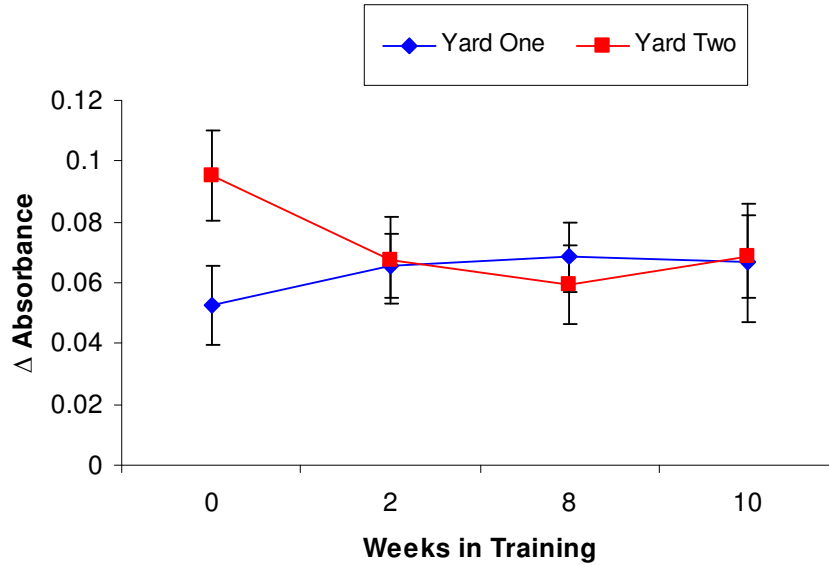


Figure 3.5 Mean T-cell proliferation in response to stimulation with 1.25 $\mu\text{g/ml}$ Con A throughout training period ($p>0.05$)

Table 3.13 displays descriptive statistics on T-cell proliferation after stimulation with 0.625 $\mu\text{g/ml}$ Con A and six hours incubation with the mitogen Con A for both yards throughout the ten week training period examined. Negative minimum values indicate that 0.625 $\mu\text{g/ml}$ Con A was an amount too small to produce optimum results.

Table 3.13 Descriptive Statistics – T-cell proliferation – 0.625 $\mu\text{g/ml}$ Con A

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	0.026	0.016	0.047	0.016	-0.022	0.128
	<i>Wk 2</i>	0.046	0.044	0.019	0.007	0.022	0.080
	<i>Wk 8</i>	0.017	0.020	0.025	0.009	-0.019	0.051
	<i>Wk 10</i>	0.027	0.025	0.028	0.010	-0.004	0.072
<i>Yard Two</i>	<i>Before Training</i>	0.049	0.044	0.030	0.010	0.002	0.089
	<i>Wk 2</i>	0.026	0.017	0.034	0.011	-0.019	0.073
	<i>Wk 8</i>	0.028	0.023	0.032	0.010	-0.023	0.085
	<i>Wk 10</i>	0.029	0.024	0.033	0.010	-0.004	0.089

As before, no significant changes in T-cell proliferation in response to stimulation with 0.625 $\mu\text{g/ml}$ Con A were observed in yard one and yard two ($p>0.05$). Also, no significant differences in T-cell proliferation (0.625 $\mu\text{g/ml}$ Con A) between yard one

and yard two were observed at any point in time ($p>0.05$). Figure 3.6 and table 3.14 illustrate these findings.

Table 3.14 T-cell proliferation (0.625 $\mu\text{g/ml}$ Con A) – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	6.75	3	0.079
<i>Yard Two</i>	4.636	3	0.207
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	21.5	0.105
	2	25.0	0.195
	8	32.0	0.515
	10	38.5	0.913

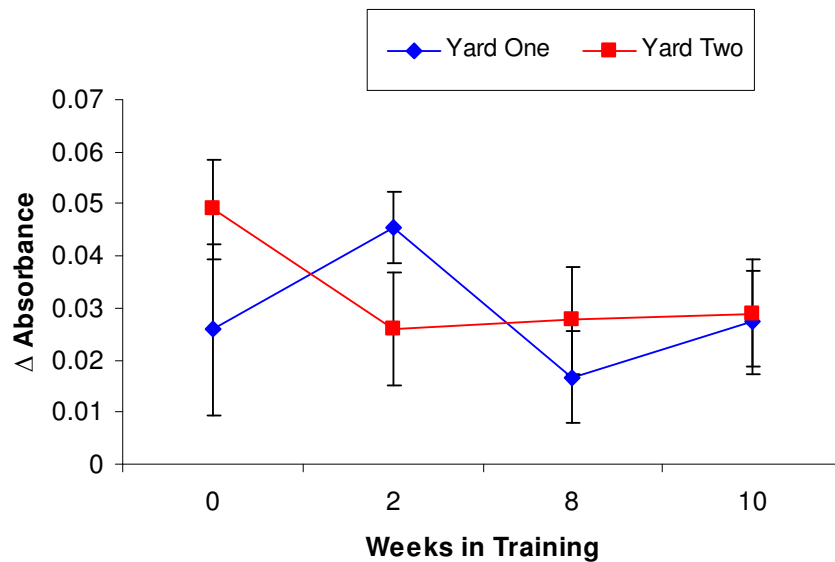


Figure 3.6 Mean T-cell proliferation in response to stimulation with 0.625 $\mu\text{g/ml}$ Con A throughout training period ($p>0.05$)

Effect of Training on Leukocyte Function – Phagocytosis

Phagocytosis of granulocytes and monocytes was analysed in the laboratory by flow cytometry and was recorded as the percentage of granulocyte or monocyte phagocytosis.

Granulocytes

Table 3.15 displays descriptive statistics on granulocyte phagocytosis for both yards throughout the ten week training period examined (%).

Table 3.15 Descriptive Statistics – Granulocyte phagocytosis

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	68.76	74.71	23.06	8.15	21.50	89.37
	<i>Wk 2</i>	72.66	72.91	10.46	3.70	60.57	87.15
	<i>Wk 8</i>	92.95	94.40	4.75	1.68	84.28	97.35
	<i>Wk 10</i>	62.16	56.20	26.48	9.36	27.62	94.46
<i>Yard Two</i>	<i>Before Training</i>	79.71	79.45	7.74	2.45	64.43	94.47
	<i>Wk 2</i>	82.53	87.51	12.72	4.02	61.30	94.86
	<i>Wk 8</i>	41.11	38.53	10.71	3.39	29.62	56.85
	<i>Wk 10</i>	69.63	74.19	14.32	4.53	48.69	86.88

Granulocyte phagocytosis significantly changed in both yards throughout the training period (Yard One: $p < 0.01$; Yard Two: $p < 0.001$). The percentage of granulocyte phagocytosis in horses from yard one was significantly higher after eight weeks of training than prior to the commencement of training and after two weeks of training. Furthermore, levels were significantly lower after ten weeks of training than after eight weeks of training ($p < 0.05$). However, by week ten of training granulocyte phagocytosis had returned to pre-training levels ($p > 0.05$). In horses from yard two, granulocyte phagocytosis was significantly lower after eight weeks of training than prior to the commencement of training and after two weeks of training, while values after ten weeks of training were lower than after two weeks of training ($p < 0.05$). Also, values after ten weeks of training were significantly higher than eight weeks of training ($p < 0.05$), which is in line with the development observed in yard one, where pre-training values were regained after ten weeks of training. Tables 3.16 and 3.17 as well as figure 3.7 display these developments.

Table 3.16 Granulocyte Phagocytosis – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	12.15	3	0.004
<i>Yard Two</i>	22.68	3	0.000
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	32.0	0.515
	2	19.0	0.068
	8	0	0.000
	10	34.0	0.633

Table 3.17 Granulocyte phagocytosis change throughout training period by weeks

Wilcoxon Signed Rank	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 8	0	0.008
	Wk 2 - Wk 8	0	0.008
	Wk 8 – Wk 10	2	0.023
<i>Yard Two</i>	Wk 0 – Wk 8	0	0.002
	Wk 2 – Wk 8	0	0.002
	Wk 2 – Wk 10	5	0.020
	Wk 8 – Wk 10	0	0.002

As can also be seen in figure 3.7, a significant difference in the percentage of phagocytosing granulocytes between the yards was observed after eight weeks of training (Yard One: Mdn=94.395; Yard Two: Mdn=38.525; $p < 0.001$). At all other sampling times, no significant differences were observed ($p > 0.05$).

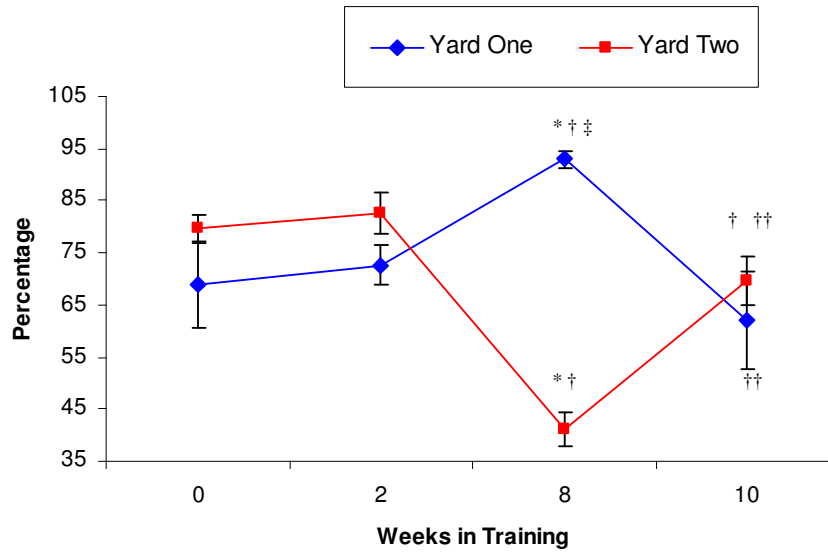


Figure 3.7 Mean percentage of phagocytosing granulocytes throughout training period ($p < 0.01$)

*Sig. different from pre-training values ($p < 0.05$) †Sig. different from wk 2 values ($p < 0.05$) ††Sig. different from wk 8 values ($p < 0.05$) ‡Yard one sig. different from yard two ($p < 0.05$)

Monocytes

Table 3.18 displays descriptive statistics relating to the percentage of phagocytosing monocytes throughout the training period examined (%).

Table 3.18 Descriptive Statistics – Monocyte phagocytosis

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	25.52	25.59	15.07	5.33	8.51	46.43
	Wk 2	29.91	29.87	11.72	4.14	9.47	49.71
	Wk 8	64.62	67.67	9.52	3.37	49.28	75.67
	Wk 10	46.80	39.74	19.60	6.93	23.44	81.52
Yard Two	Before Training	29.14	27.20	9.87	3.12	17.62	49.25
	Wk 2	33.38	35.94	12.24	3.87	10.67	46.87
	Wk 8	9.26	9.30	3.41	1.08	3.46	15.43
	Wk 10	39.66	38.68	9.63	3.05	27.96	54.11

The percentage of phagocytosing monocytes underwent significant changes during the ten week training period observed similar to the observations made with regards to the percentage of phagocytosing granulocytes (Yard One: $p < 0.01$; Yard Two: $p < 0.001$). Whereas monocyte phagocytosis had increased in yard one after eight

weeks of training with respect to pre-training values and those after two weeks of training ($p < 0.05$), the percentage of phagocytosing monocytes had regained pre-training values after ten weeks of training ($p > 0.05$); although levels were still elevated after ten weeks of training, when compared to two weeks of training ($p < 0.05$). In yard two, monocyte phagocytosis had significantly decreased after eight weeks of training, when compared to pre-training and week two values ($p < 0.05$). Also, no significant difference between values after ten weeks of training and pre-training as well as week two values was observed as values after ten weeks of training were significantly higher than after eight weeks of training ($p < 0.05$). This is also illustrated in tables 3.19 and 3.20 as well as figure 3.8.

Table 3.19 Monocyte Phagocytosis – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	14.55	3	0.001
<i>Yard Two</i>	20.28	3	0.000
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	32.0	0.515
	2	29.0	0.360
	8	0	0.000
	10	33.0	0.573

Table 3.20 Monocyte phagocytosis change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 8	0	0.008
	Wk 2 - Wk 8	0	0.008
	Wk 2 – Wk 10	2	0.023
<i>Yard Two</i>	Wk 0 – Wk 8	0	0.002
	Wk 2 – Wk 8	0	0.002
	Wk 8 – Wk 10	0	0.002

Monocyte phagocytosis was significantly higher in horses from yard one (Mdn=67.67) than in horses from yard two (Mdn=9.295) after eight weeks of training

($p < 0.001$). No significant differences were observed at all other sampling times ($p > 0.05$).

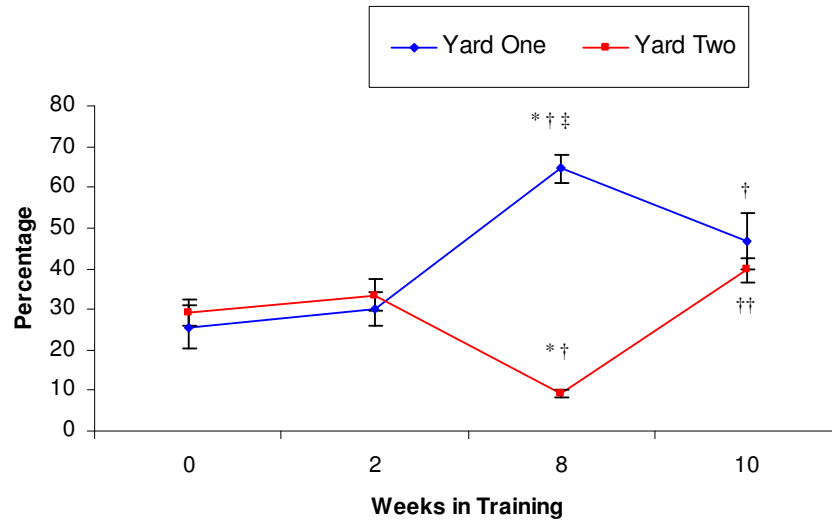


Figure 3.8 Mean percentage of phagocytosing monocytes throughout training period ($p < 0.01$)

*Sig. different from pre-training values ($p < 0.05$) †Sig. different from wk 2 values ($p < 0.05$) ††Sig. different from wk 8 values ($p < 0.05$) ‡Yard one sig. different from yard two ($p < 0.05$)

Effect of Training on Leukocyte Function – Oxidative Burst Capacity

Oxidative burst capacity of granulocytes and monocytes was analysed in the laboratory by flow cytometry and was recorded as the percentage of granulocyte or monocyte oxidative burst capacity. The stimulant PMA was used as a positive control, whereas the stimulant bacteria served for obtaining the actual result.

Oxidative Burst Capacity of Granulocytes – Yard One

Table 3.21 displays descriptive information on granulocyte oxidative burst capacity (stimulant: bacteria) in horses from yard one throughout the training period examined (%).

Table 3.21 Descriptive Statistics – Granulocyte oxidative burst capacity

	<i>n</i>	<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One Before Training</i>	10	24.07	25.30	13.07	4.13	3.83	45.69
<i>Wk 2</i>	9	44.35	46.58	15.73	5.24	23.35	61.26
<i>Wk 8</i>	6	53.74	57.86	13.49	5.51	27.46	64.80
<i>Wk 10</i>	6	46.11	45.67	9.74	3.98	29.72	57.60

Table 3.22 Granulocyte oxidative burst capacity change throughout training period by weeks

	<i>n</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	9	Wk 0 – Wk 2	2	0.012
	6	Wk 0 – Wk 8	0	0.031
	6	Wk 0 – Wk 10	0	0.031

As shown in table 3.22 and figure 3.9 granulocyte oxidative burst capacity in response to stimulation with bacteria in horses from yard one had significantly increased after two weeks of training when compared to pre-training values ($p < 0.05$).

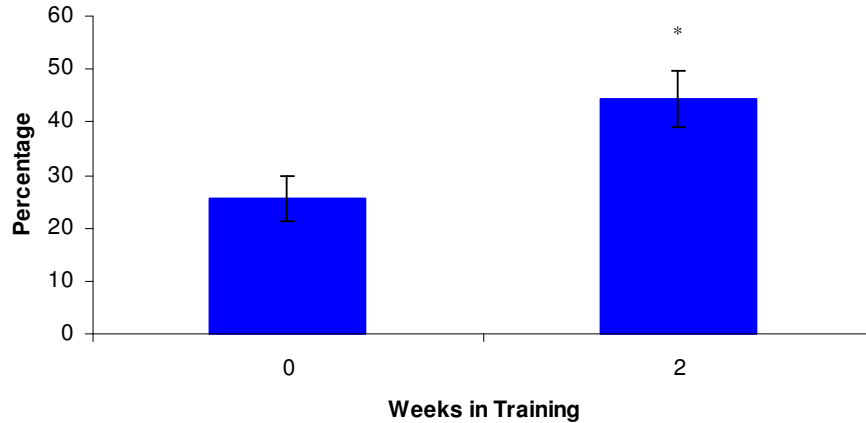


Figure 3.9 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria in week zero and week two of training – Yard One (* $p < 0.05$; $n = 9$)

Statistical analysis showed a significant difference between pre-training values and values after eight weeks of training ($p < 0.05$). Figure 3.10 and table 3.22 display this finding.

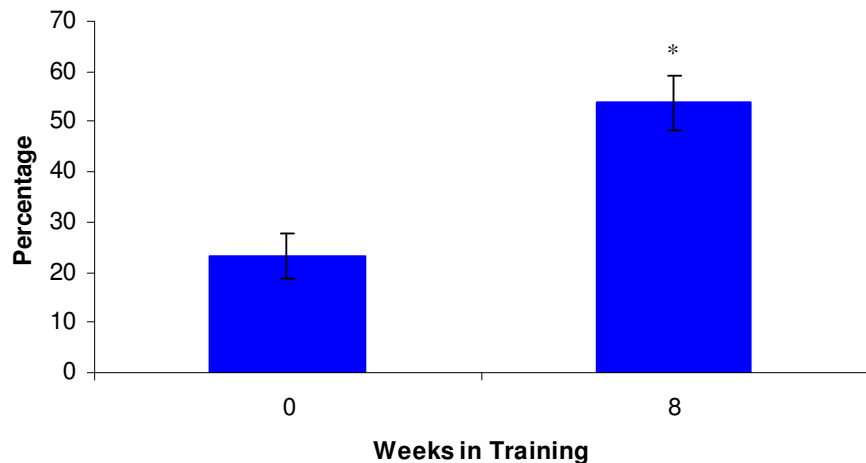


Figure 3.10 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria in week zero and week eight of training – Yard One (* $p < 0.05$; $n = 6$)

A significant difference between oxidative burst capacity of granulocytes in horses from yard one after ten weeks of training when compared with pre-training values was observed ($p < 0.05$). This can also be taken from figure 3.11 and table 3.22.

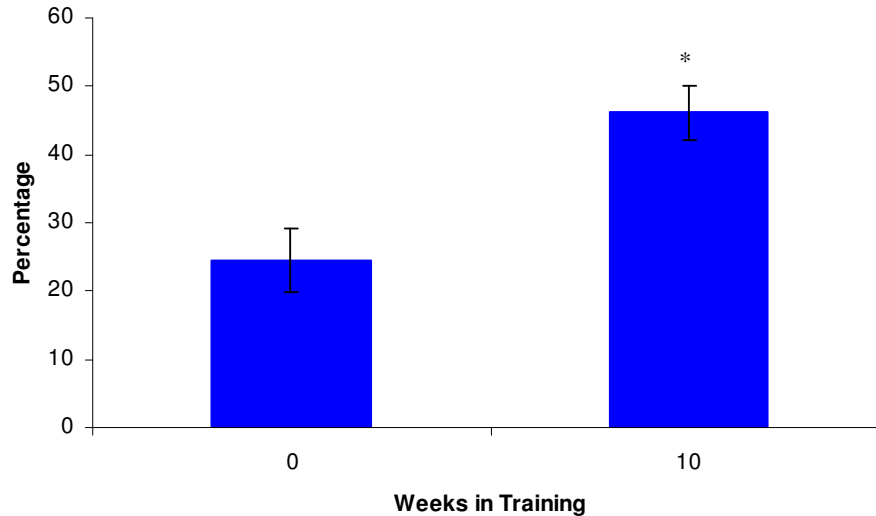


Figure 3.11 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria in week zero and week ten of training – Yard One (* $p < 0.05$; $n = 6$)

Figure 3.12 illustrates the general trend of granulocyte oxidative burst capacity (stimulant: bacteria) in horses from yard one. A similar trend can be observed in figure 3.19 illustrating granulocyte oxidative burst capacity (stimulant: bacteria) in horses from yard two. Although the overall statistical analysis in this case indicated a significant change in granulocyte oxidative burst capacity in response to stimulation with bacteria, ($p < 0.05$), post hoc analysis did not confirm this finding likely due to the small sample size not providing sound data for statistical analysis ($p > 0.05$). Hence, figure 3.12 and table 3.23 solely serve as illustrations of the overall trend observed. This can also be taken from table 3.23.

Table 3.23 Granulocyte oxidative burst capacity – Change throughout training period ($n = 4$)

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard Two</i>	9.3	3	0.012

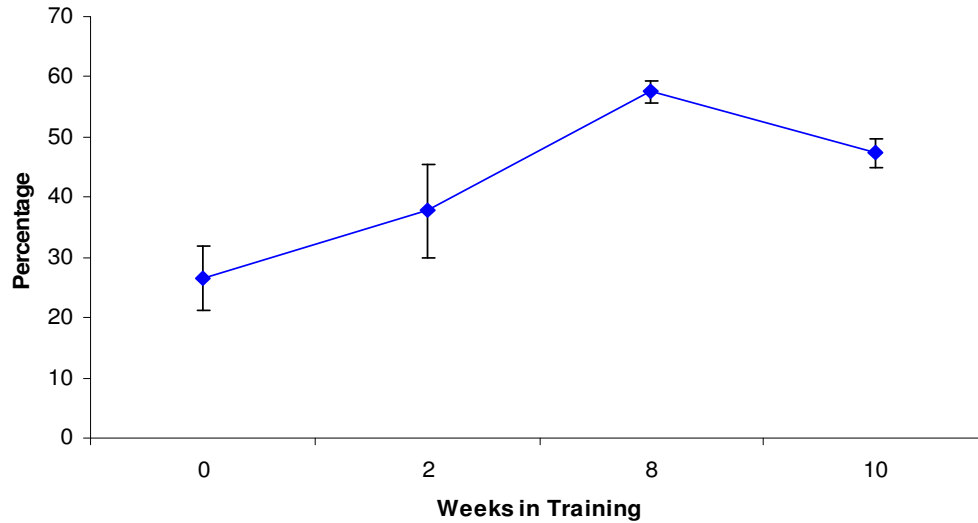


Figure 3.12 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria throughout training period – Yard One ($p < 0.05$; post hoc n.s.; $n = 4$)

Table 3.24 displays descriptive information on granulocyte oxidative burst capacity (stimulant: PMA) in horses from yard one throughout the training period examined (%).

Table 3.24 Descriptive Statistics – Granulocyte oxidative burst capacity

	<i>n</i>	<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One							
Before Training	9	63.94	62.39	13.76	4.59	37.79	82.04
Wk 2	9	80.92	78.68	11.04	3.68	61.83	96.22
Wk 8	4	86.87	88.92	5.90	2.95	78.54	91.10
Wk 10	6	62.13	65.00	14.82	6.05	39.74	78.88

Only one comparison examining granulocyte oxidative burst capacity in response to stimulation with PMA in horses from yard one was made due to experimental reasons as explained before. It was observed that values had not significantly changed from week zero to week two of training ($p > 0.05$). Figure 3.13 and table 3.25 illustrate this finding.

Table 3.25 Granulocyte oxidative burst capacity change throughout training period by weeks

	<i>n</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	8	Wk 0 – Wk 2	6	0.109

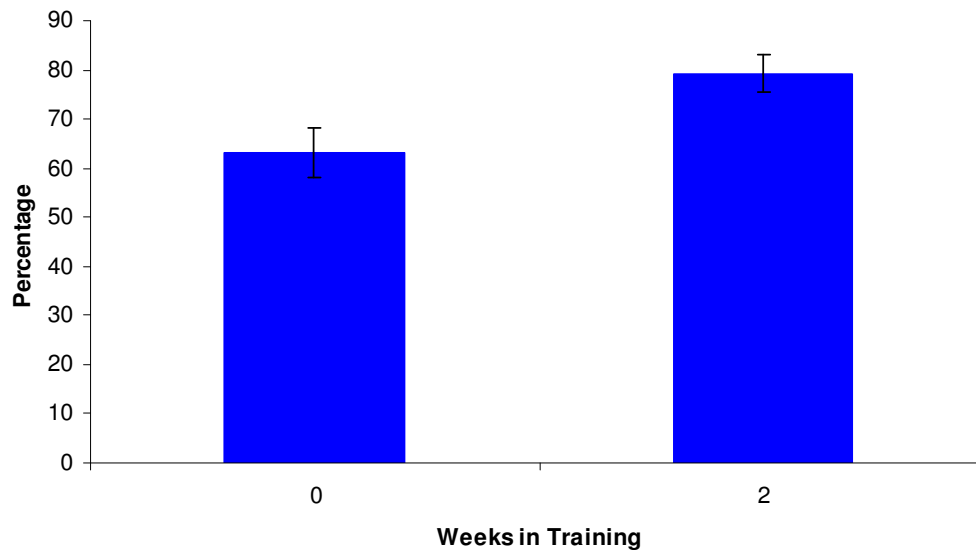


Figure 3.13 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with PMA in week zero and week two of training – Yard One ($p>0.05$; $n=8$)

Oxidative Burst Capacity of Monocytes – Yard One

Table 3.26 shows descriptive statistics relating to monocyte oxidative burst capacity after stimulation with bacteria in horses from yard one (%).

Table 3.26 Descriptive Statistics – Monocyte oxidative burst capacity

	<i>n</i>	<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One Before Training</i>	10	1.97	1.72	1.37	0.43	0.21	4.73
<i>Wk 2</i>	9	9.34	6.39	6.20	2.07	3.44	21.72
<i>Wk 8</i>	6	22.61	18.70	12.39	5.06	14.20	46.46
<i>Wk 10</i>	6	24.57	29.77	12.44	5.08	5.17	38.16

Table 3.27 Monocyte oxidative burst capacity change throughout training period by weeks

	<i>n</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	9	Wk 0 – Wk 2	0	0.004
	6	Wk 0 – Wk 8	0	0.031
	6	Wk 0 – Wk 10	0	0.031

As shown in table 3.27 and figure 3.14, oxidative burst capacity of monocytes in response to stimulation with bacteria increased significantly from week zero to week two of training ($p < 0.01$).

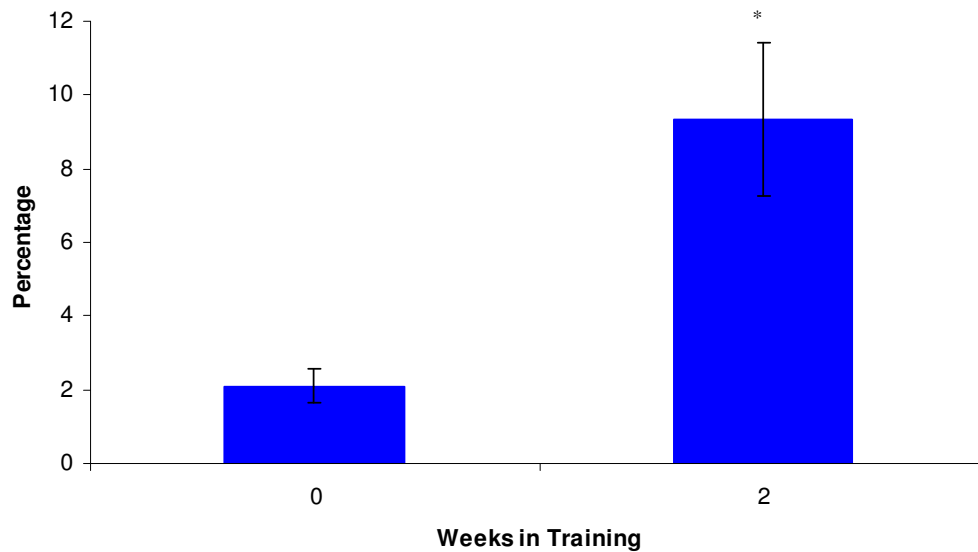


Figure 3.14 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria in week zero and week two of training – Yard One (* $p < 0.01$; $n = 9$)

Furthermore, a significant difference between values in week zero and week eight of training was observed ($p < 0.05$). Figure 3.15 and table 3.27 demonstrate this finding.

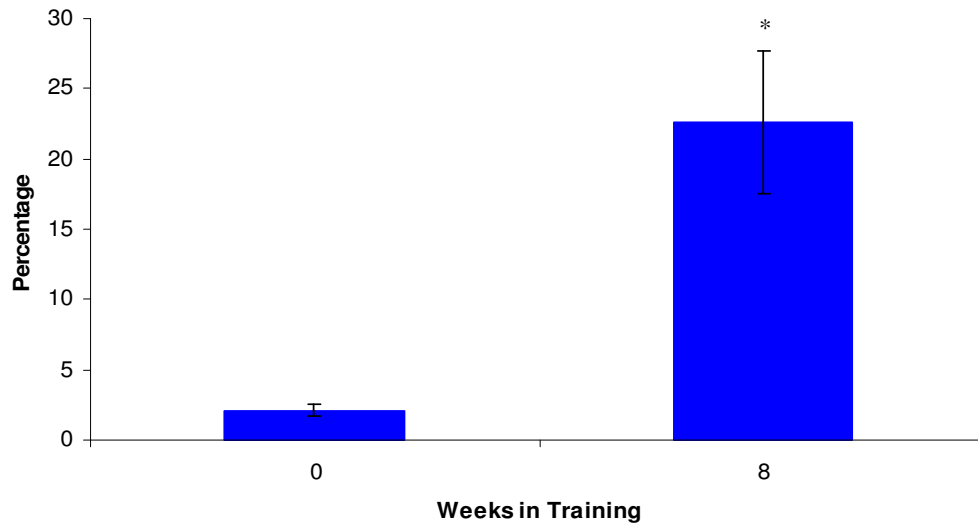


Figure 3.15 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria in week zero and week eight of training – Yard One ($p < 0.05$; $n = 6$)

Similar to previously shown differences, a significant difference between monocyte oxidative burst capacity (stimulant: bacteria) in horses from yard one at sampling in week zero and sampling in week ten of training was observed ($p < 0.05$). See figure 3.16 and table 3.27.

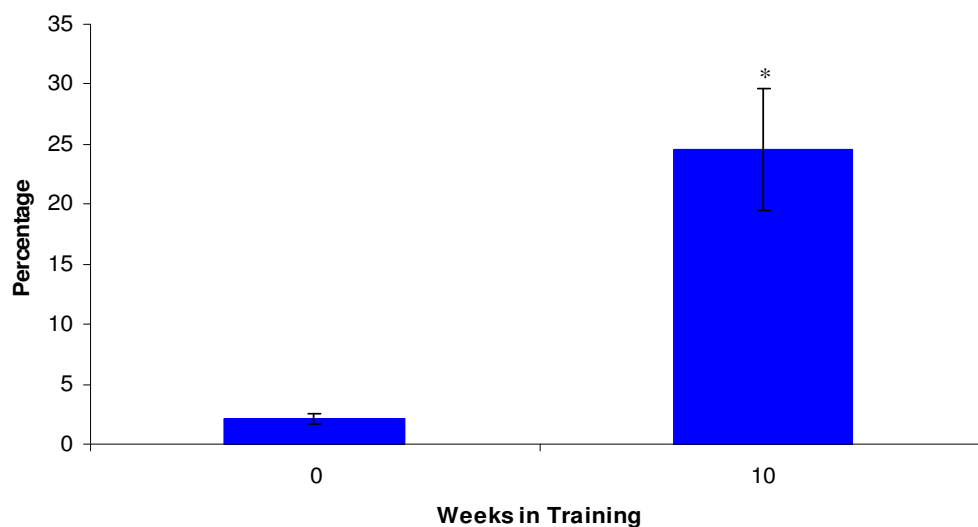


Figure 3.16 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria in week zero and week ten of training – Yard One ($p < 0.05$; $n = 6$)

While the initial statistical analysis indicates that monocyte oxidative burst capacity of horses in yard one changed significantly throughout training ($p < 0.05$), post hoc analysis showed that it could not be concluded that changes occurred due to any other reason than chance ($p > 0.05$). This finding possibly occurred due to the small sample size and information taken from table 3.28 and figure 3.17 should, hence, only be considered for illustrative purposes.

Table 3.28 Monocyte oxidative burst capacity – Change throughout training period ($n = 4$)

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	8.1	3	0.033

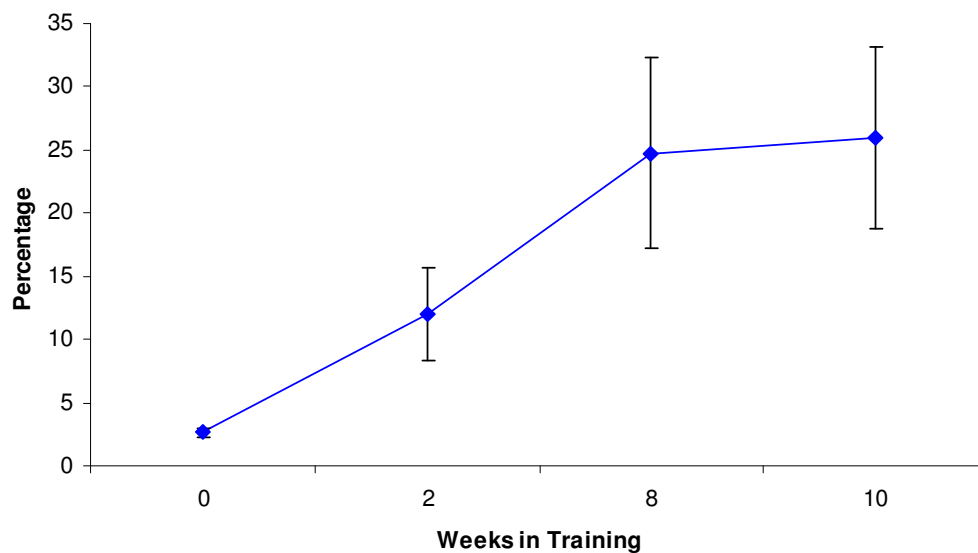


Figure 3.17 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria throughout training period – Yard One ($p < 0.05$; post hoc *n.s.*; $n=4$)

Table 3.29 displays descriptive information on monocyte oxidative burst capacity (stimulant: PMA) in horses from yard one throughout the training period examined (%).

Table 3.29 Descriptive Statistics – Monocyte oxidative burst capacity

	<i>n</i>	<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One							
<i>Before Training</i>	9	21.60	23.26	8.97	2.99	8.84	34.08
<i>Wk 2</i>	9	29.00	31.03	18.98	6.33	4.92	60.65
<i>Wk 8</i>	4	16.57	16.50	7.63	3.81	7.40	25.89
<i>Wk 10</i>	7	10.99	8.98	5.42	2.05	4.25	17.58

Due to previously described circumstances only three comparisons regarding monocyte oxidative burst capacity in response to stimulation with PMA in yard one were made. No significant differences between pre-training values and those after two and ten weeks of training were observed ($p>0.05$). However, values observed were significantly elevated after ten weeks of training when compared to values two weeks into the training programme. Table 3.30 and figures 3.18, 3.19 and 3.20 demonstrate these findings.

Table 3.30 Monocyte oxidative burst capacity change throughout training period by weeks

	<i>n</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	8	Wk 0 – Wk 2	11	0.383
	6	Wk 0 – Wk 10	2	0.094
	6	Wk 2 – Wk 10	0	0.031

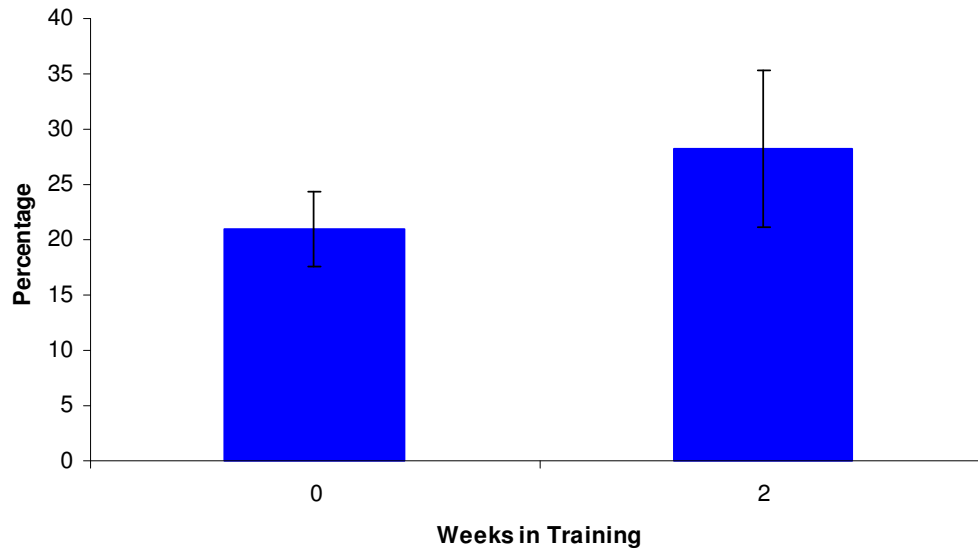


Figure 3.18 Mean oxidative burst capacity (%) of monocytes in response to stimulation with PMA in week zero and week two of training – Yard One ($p>0.05$; $n=8$)

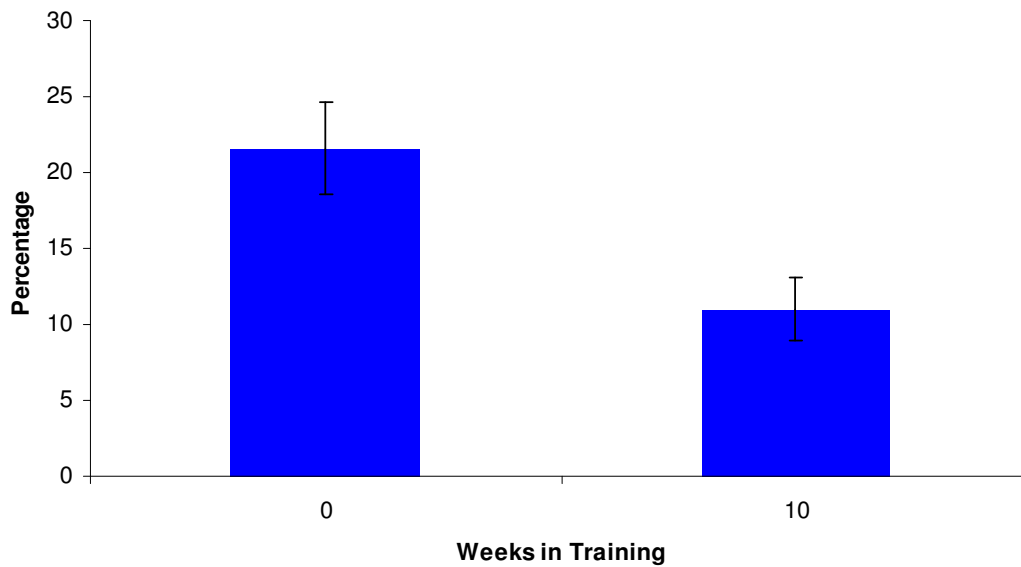


Figure 3.19 Mean oxidative burst capacity (%) of monocytes in response to stimulation with PMA in week zero and week ten of training – Yard One ($p>0.05$; $n=6$)

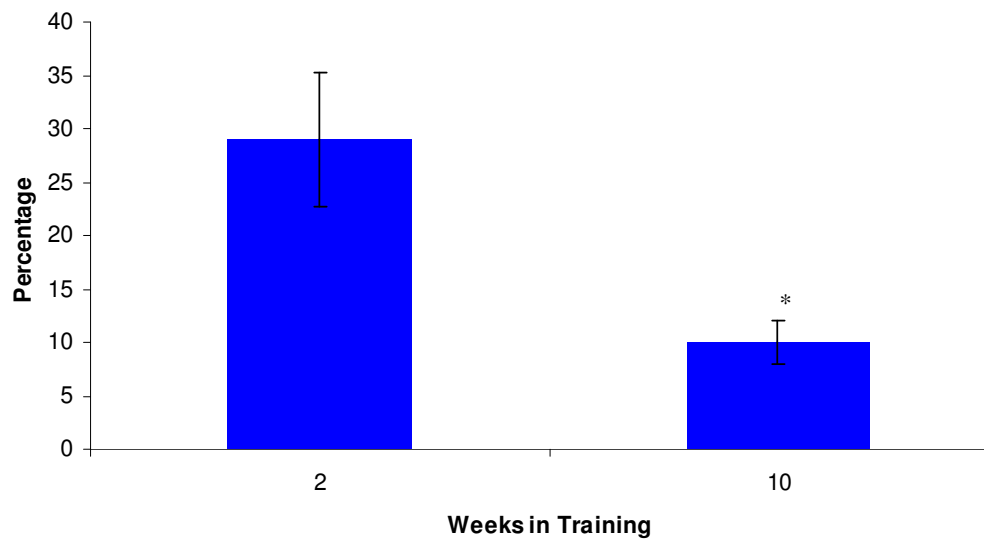


Figure 3.20 Mean oxidative burst capacity (%) of monocytes in response to stimulation with PMA in week two and week ten of training – Yard One (* $p < 0.05$; $n = 6$)

Oxidative Burst Capacity of Granulocytes – Yard Two

Descriptive statistics relating to the oxidative burst capacity of granulocytes in response to stimulation with bacteria are presented in table 3.31 (%).

Table 3.31 Descriptive Statistics – Granulocyte oxidative burst capacity

		Mean	Median	S.D.	S.E.	Min	Max
Yard Two	Before Training	41.83	41.67	12.56	3.97	21.42	60.70
	Wk 2	43.03	43.83	15.47	4.89	19.83	65.70
	Wk 8	65.53	64.92	14.02	4.43	42.60	85.99
	Wk 10	49.19	51.47	11.39	3.60	23.48	61.95

Following a similar trend as the percentage of phagocytosing granulocytes, granulocyte oxidative burst capacity changed significantly throughout the training period examined ($p < 0.001$). Values observed after eight weeks of training were significantly higher than prior to the start of training and after two weeks of training ($p < 0.05$). Furthermore, values decreased again between eight and ten weeks into training ($p < 0.05$). This can also be seen in tables 3.32 and 3.33 and figure 3.21.

Table 3.32 Granulocyte oxidative burst capacity – Change throughout training period

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard Two</i>	16.68	3	0.000

Table 3.33 Granulocyte oxidative burst capacity change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard Two</i>	Wk 0 – Wk 8	0	0.002
	Wk 2 – Wk 8	2	0.006
	Wk 8 – Wk 10	1	0.004

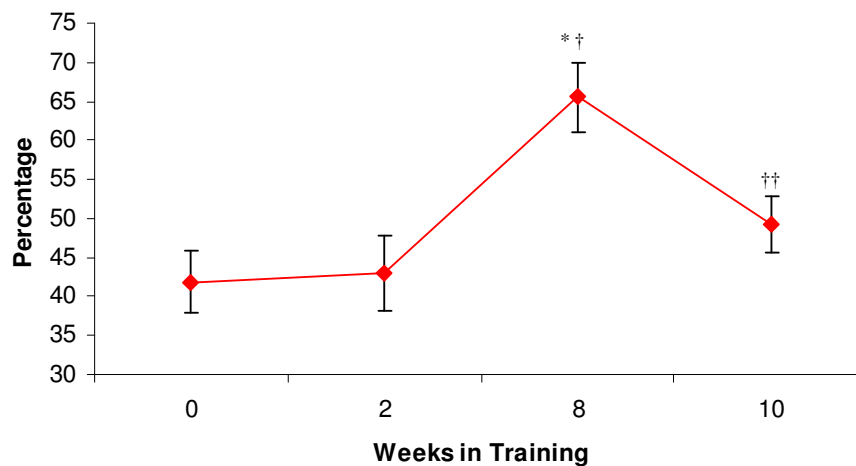


Figure 3.21 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with bacteria throughout training period – Yard Two ($p < 0.001$)

*Sig. different from pre-training values ($p < 0.05$) †Sig. different from wk 2 values ($p < 0.05$) ††Sig. different from wk 8 values ($p < 0.05$)

Descriptive statistics relating to the oxidative burst capacity of granulocytes in response to stimulation with PMA, acting as a positive control, are presented in table 3.34 (%).

Table 3.34 Descriptive Statistics – Granulocyte oxidative burst capacity

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard Two</i>	<i>Before Training</i>	50.84	55.87	20.45	6.47	17.66	77.58
	<i>Wk 2</i>	61.92	61.32	23.89	7.56	18.57	94.48
	<i>Wk 8</i>	86.64	88.74	8.93	2.82	74.67	97.64
	<i>Wk 10</i>	80.35	84.68	14.87	4.70	46.37	95.91

Granulocyte oxidative burst capacity (Stimulant: PMA) significantly changed throughout the ten week training period examined ($p < 0.001$), as shown in table 3.35. As seen previously, oxidative burst capacity of granulocytes increased significantly between the start of training and week eight of training ($p < 0.001$). After ten weeks of training, percentage oxidative burst capacity of granulocytes in response to stimulation with PMA was still significantly higher than prior to the commencement of training as well as after eight weeks of training. Also, a significant increase from week two to week eight of training was observed. Although, it can be seen in figure 3.20 that values in week ten of training were slightly lower than after eight weeks of training. Table 3.36 displays the output from post hoc analysis.

Table 3.35 Granulocyte oxidative burst capacity – Change throughout training period

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard Two</i>	20.76	3	0.000

Table 3.36 Granulocyte oxidative burst capacity change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard Two</i>	Wk 0 – Wk 8	0	0.002
	Wk 0 – Wk 10	0	0.002
	Wk 2 – Wk 8	3	0.010
	Wk 8 – Wk 10	8	0.049

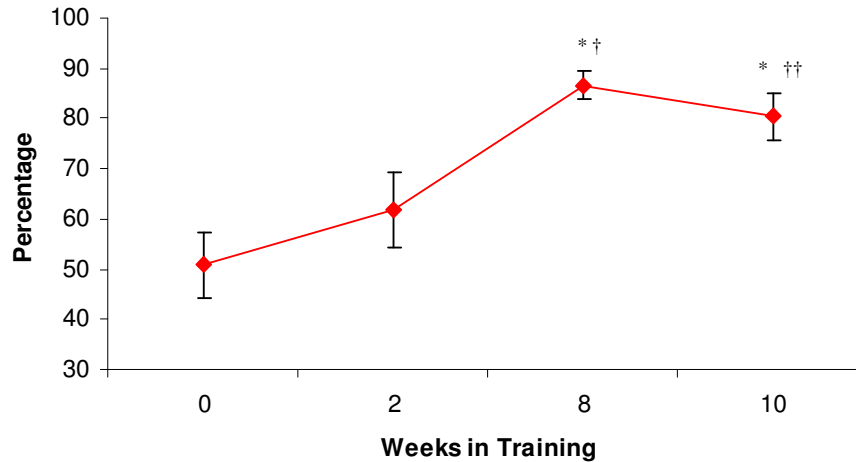


Figure 3.22 Mean oxidative burst capacity (%) of granulocytes in response to stimulation with PMA throughout training period – Yard Two ($p < 0.001$)

*Sig. different from pre-training values ($p < 0.05$) †Sig. different from wk 2 values ($p < 0.05$) ††Sig. different from wk 8 values ($p < 0.05$)

Oxidative Burst Capacity of Monocytes – Yard Two

Table 3.37 shows descriptive statistics relating to monocyte oxidative burst capacity after stimulation with bacteria (%).

Table 3.37 Descriptive Statistics – Monocyte oxidative burst capacity

		Mean	Median	S.D.	S.E.	Min	Max
Yard Two	Before Training	5.06	3.97	3.56	1.13	1.06	11.00
	Wk 2	7.75	6.10	7.35	2.33	0.49	22.29
	Wk 8	9.05	10.22	5.56	1.76	1.83	20.44
	Wk 10	15.93	15.04	7.54	2.38	5.94	29.96

Monocyte oxidative burst capacity in response to stimulation with bacteria appeared to have significantly changed throughout the training period observed ($p < 0.01$). Similar to observations regarding levels of granulocyte oxidative burst capacity during training, values had increased significantly after eight weeks of training when compared to pre-training levels as well as after ten weeks of training when compared to pre-training, week two and week eight levels ($p < 0.05$). These findings are also illustrated in tables 3.38 and 3.39 as well as in figure 3.23.

Table 3.38 Monocyte oxidative burst capacity – Change throughout training period

Friedman's ANOVA	χ^2	df	p
Yard Two	12.84	3	0.003

Table 3.39 Monocyte oxidative burst capacity change throughout training period by weeks

Wilcoxon Signed Rank	Comparison	T	p
Yard Two	Wk 0 – Wk 8	7	0.037
	Wk 0 – Wk 10	3	0.010
	Wk 2 – Wk 10	3	0.010
	Wk 8 – Wk 10	5	0.020

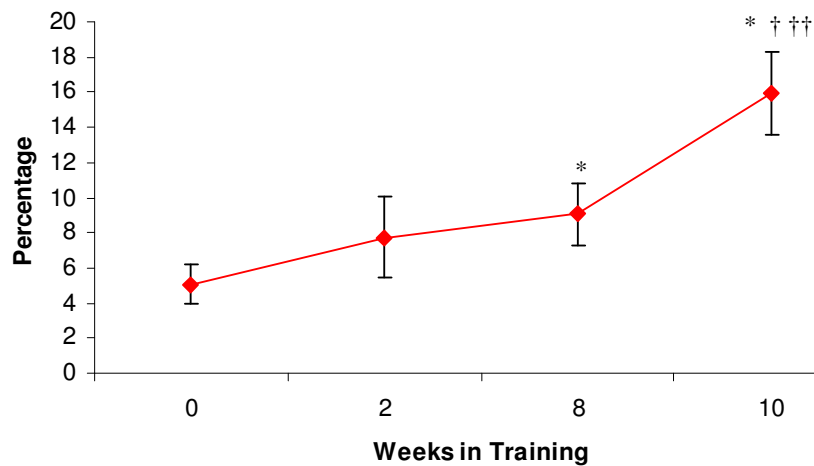


Figure 3.23 Mean oxidative burst capacity (%) of monocytes in response to stimulation with bacteria throughout training period – Yard Two ($p < 0.01$)

*Sig. different from pre-training values ($p < 0.05$) †Sig. different from wk 2 values ($p < 0.05$) ††Sig. different from wk 8 values ($p < 0.05$)

Table 3.40 shows descriptive statistics relating to monocyte oxidative burst capacity in response to stimulation with PMA, which acted as a positive control (%).

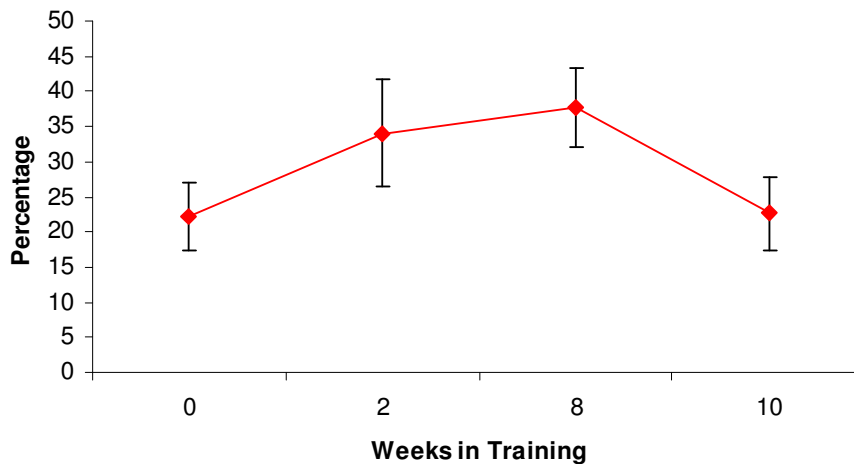
Table 3.40 Descriptive Statistics – Monocyte oxidative burst capacity

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard Two</i>	<i>Before Training</i>	22.14	17.32	15.19	4.80	6.43	56.12
	<i>Wk 2</i>	34.06	37.91	23.99	7.59	2.20	76.59
	<i>Wk 8</i>	37.67	29.03	17.79	5.63	21.70	67.48
	<i>Wk 10</i>	22.60	18.44	16.43	5.20	4.49	56.11

Confirming the previously described finding, monocyte oxidative burst capacity in response to stimulation with PMA did not change significantly throughout the ten week training period examined ($p>0.05$). Figure 3.24 and table 3.41 display this finding.

Table 3.41 Monocyte oxidative burst capacity – Change throughout training period

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard Two</i>	7.56	3	0.053

**Figure 3.24 Mean oxidative burst capacity (%) of monocytes in response to stimulation with PMA throughout training period – Yard Two ($p>0.05$)**

Effect of Training on Plasma Haematology

Red Blood Cells

Descriptive statistical results for the number of red blood cells are presented for each yard separately in table 3.42. It is important to note that the minimum resting values prior to training in both yards lay slightly below the normal range of adult thoroughbred horses. Furthermore, minimum values in yard one eight weeks into training and in yard two at two weeks into training were below the normal range. Also, maximum resting values of yard one in week eight of training were above the normal range of adult thoroughbred horses ($\times 10^{12}/l$).

Table 3.42 Descriptive Statistics – Total erythrocytes

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	8.05	7.98	1.06	0.37	6.53	9.65
	<i>Wk 2</i>	8.53	8.52	0.87	0.31	7.07	10.00
	<i>Wk 8</i>	9.12	9.56	1.61	0.57	6.85	11.20
	<i>Wk 10</i>	8.58	8.56	0.79	0.28	7.59	10.10
<i>Yard Two</i>	<i>Before Training</i>	7.93	7.94	0.65	0.21	6.98	8.97
	<i>Wk 2</i>	7.90	7.89	0.79	0.25	6.94	9.63
	<i>Wk 8</i>	8.65	8.93	0.87	0.28	7.16	10.20
	<i>Wk 10</i>	8.49	8.61	0.86	0.27	7.25	9.91

The results show that total erythrocyte levels did not appear to be significantly affected by training in either yard one ($p>0.05$) or yard two ($p>0.05$) during the time period examined as can be seen in figure 3.25. Furthermore, no significant differences between total erythrocyte levels were observed between yard one and yard two at any point in time measured ($p>0.05$). Table 3.43 refers to these findings.

Table 3.43 Total Erythrocytes – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	3.152	3	0.388
<i>Yard Two</i>	5.4	3	0.149
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	37.5	0.846
	2	21.0	0.101
	8	30.0	0.408
	10	40.0	1.000

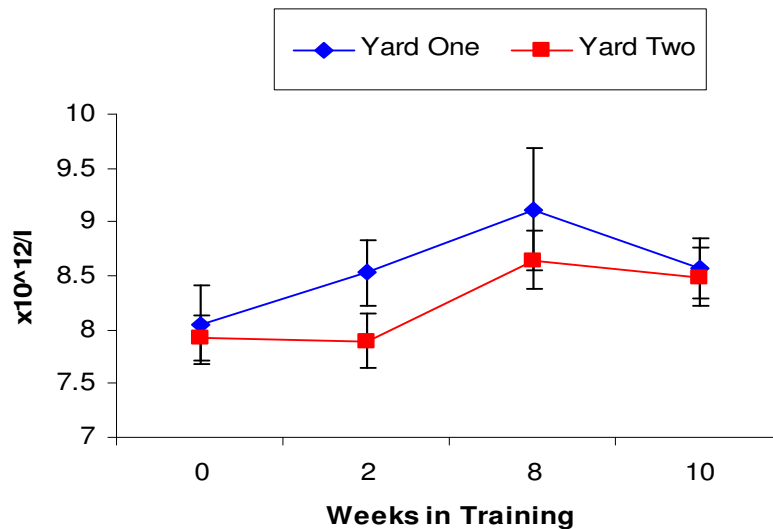


Figure 3.25 Mean total erythrocyte levels throughout training period ($p > 0.05$)

Packed Cell Volume (PCV)

Table 3.44 presents descriptive statistical results for both yards. Minimum values below the normal range were observed prior to the beginning of training and eight weeks into training in yard one as well as prior to the beginning of training and after two weeks of training in yard two. After eight weeks of training a maximum value above the normal range was seen in yard one (%).

Table 3.44 Descriptive Statistics – Packed cell volume

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	36.26	35.55	3.74	1.32	31.50	41.20
	<i>Wk 2</i>	38.50	38.75	2.52	0.89	34.20	43.10
	<i>Wk 8</i>	40.68	42.25	6.27	2.22	30.00	48.40
	<i>Wk 10</i>	38.40	38.05	2.69	0.95	33.40	41.60
<i>Yard Two</i>	<i>Before Training</i>	35.61	35.65	3.47	1.10	31.10	42.10
	<i>Wk 2</i>	35.50	34.55	3.75	1.19	30.80	43.20
	<i>Wk 8</i>	38.54	39.20	3.55	1.12	32.10	44.30
	<i>Wk 10</i>	38.06	37.40	3.43	1.09	33.20	43.80

Packed Cell Volume did not significantly change in yard one ($p>0.05$) or yard two ($p>0.05$) throughout the ten week period examined. This can also be observed in figure 3.24. Packed cell volume levels did not differ significantly between yard one and yard two prior to the start of training or after two, eight and ten weeks of training ($p>0.05$). These findings can also be observed in table 3.45.

Table 3.45 Packed Cell Volume – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	3.9	3	0.287
<i>Yard Two</i>	5.667	3	0.131
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	37.5	0.715
	2	21.0	0.052
	8	30.0	0.326
	10	40.0	0.650

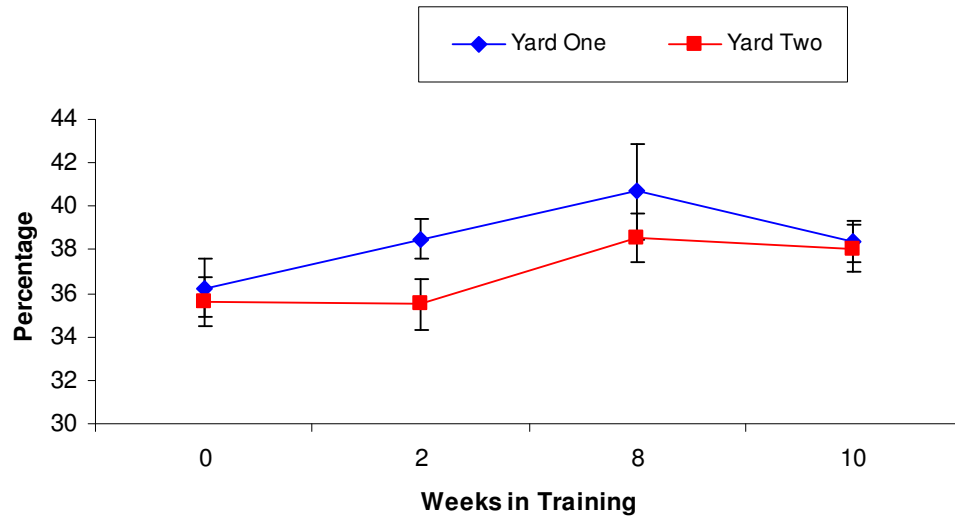


Figure 3.26 Mean packed cell volume levels throughout training period ($p>0.05$)

Haemoglobin

Single cases of low and high haemoglobin concentration were observed in yard one after eight weeks of training as can be taken from table 3.46 displaying descriptive statistics when taking the normal range given in appendix A into account (g/l).

Table 3.46 Descriptive Statistics – Haemoglobin concentration

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	12.79	12.55	1.36	0.48	11.20	14.80
	Wk 2	13.54	13.55	0.98	0.35	11.90	15.30
	Wk 8	14.95	15.55	2.39	0.85	10.90	17.80
	Wk 10	14.05	14.00	1.01	0.36	12.30	15.60
Yard Two	Before Training	12.68	12.65	1.18	0.37	11.10	14.70
	Wk 2	12.94	12.55	1.30	0.41	11.40	15.50
	Wk 8	14.18	14.35	1.36	0.43	11.80	16.60
	Wk 10	14.08	14.00	1.33	0.42	12.30	16.30

A significant increase in haemoglobin concentrations throughout the ten week period examined was observed in both yards. Haemoglobin levels had significantly increased after ten weeks of training when compared to pre-training levels in both yards and also after eight and ten weeks in yard two when compared to levels after two weeks of

training ($p < 0.05$). There were no significant differences observed between yard one and yard two prior to training or after two, eight and ten weeks of training ($p > 0.05$). Figure 3.27 displays these findings alongside tables 3.47 and 3.48.

Table 3.47 Haemoglobin – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	8.7	3	0.028
<i>Yard Two</i>	8.88	3	0.025
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	37.5	1.000
	2	21.0	0.304
	8	30.0	0.304
	10	40.0	0.984

Table 3.48 Haemoglobin change throughout training period by weeks

Wilcoxon Signed Rank	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 10	3	0.039
<i>Yard Two</i>	Wk 0 – Wk 10	8	0.047
	Wk 2 – Wk 8	7	0.037
	Wk 2 – Wk 10	5.5	0.023

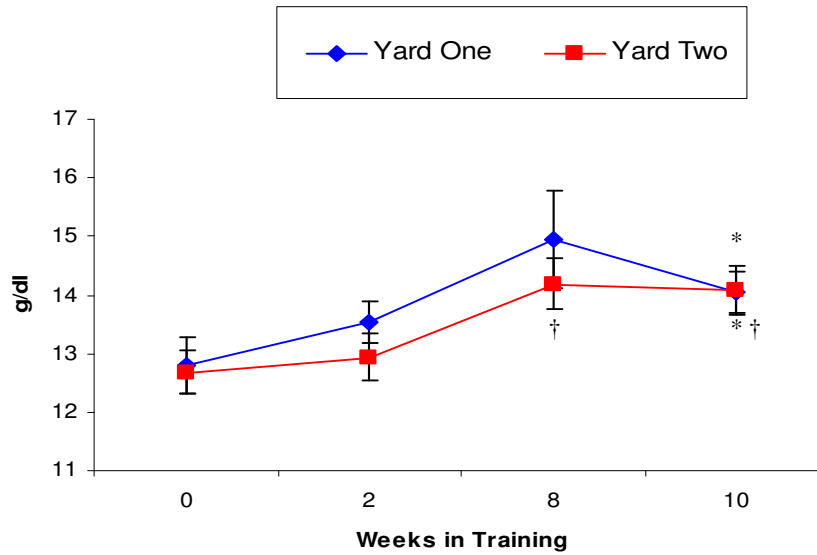


Figure 3.27 Mean haemoglobin levels throughout training period ($p < 0.05$)

*Sig. different from pre-training values ($p < 0.05$) †Sig. different from wk 2 values ($p < 0.05$)

Mean Corpuscular Volume (MCV)

All minimum and maximum values observed in yard one except after two weeks of training (=42.6 fl) were outside the normal range. Values reported from yard two appeared to fall within the normal range. See table 3.49 (fl).

Table 3.49 Descriptive Statistics – MCV

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	45.26	45.65	2.33	0.82	41.70	48.20
	Wk 2	45.26	45.40	2.27	0.80	42.60	48.40
	Wk 8	44.84	44.95	2.37	0.84	41.50	47.90
	Wk 10	44.89	45.10	2.38	0.84	41.10	47.60
Yard Two	Before Training	44.89	44.55	1.37	0.43	43.10	46.90
	Wk 2	44.94	44.75	1.15	0.36	43.00	47.00
	Wk 8	44.58	44.55	1.16	0.37	42.60	46.30
	Wk 10	44.87	45.00	1.12	0.35	43.00	46.30

The results show that MCV was not significantly affected by training during the time period examined ($p > 0.05$) as can be observed in figure 3.28. No significant differences in MCV were observed between yard one and yard two ($p > 0.05$). This is also shown in table 3.50.

Table 3.50 MCV – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	5.051	3	0.175
<i>Yard Two</i>	4.516	3	0.217
Mann-Whitney U-Test	Week	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	35.5	0.712
	2	36.5	0.779
	8	37.0	0.810
	10	37.5	0.846

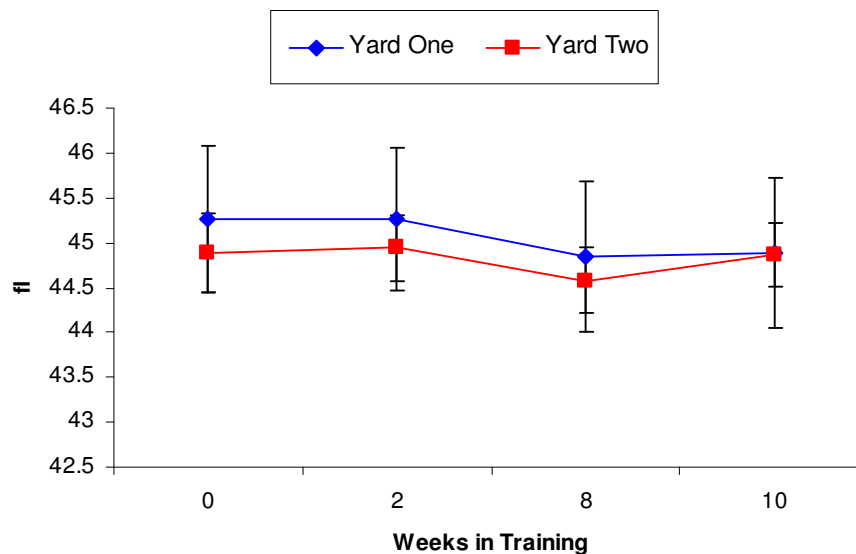


Figure 3.28 Mean MCV levels throughout training period ($p>0.05$)

Mean Corpuscular Haemoglobin Concentration (MCHC)

Descriptive Statistics as shown in table 3.51 demonstrated that mean MCHC values observed fell within the normal range (g/l).

Table 3.51 Descriptive Statistics – MCHC

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	35.23	35.35	0.69	0.24	33.80	36.00
	<i>Wk 2</i>	35.11	35.10	0.42	0.15	34.50	35.80
	<i>Wk 8</i>	36.75	36.65	0.60	0.21	35.90	37.80
	<i>Wk 10</i>	36.64	36.75	0.67	0.24	35.20	37.50
<i>Yard Two</i>	<i>Before Training</i>	35.61	35.75	0.47	0.15	34.90	36.30
	<i>Wk 2</i>	36.45	36.35	0.36	0.11	35.90	37.20
	<i>Wk 8</i>	36.81	36.85	0.41	0.13	36.10	37.50
	<i>Wk 10</i>	36.94	37.00	0.41	0.13	36.10	37.50

The results shown in table 3.52 demonstrate that MCHC levels changed significantly throughout the time period examined in yard one and yard two ($p < 0.001$). Wilcoxon signed-rank tests were performed to follow up this significant finding. Significant results for the post-hoc test are shown in table 3.53 ($p < 0.05$). In both yards a trend towards rising MCHC levels throughout the training period was observed, while MCHC levels remained within the normal range. This can also be observed in figure 3.29.

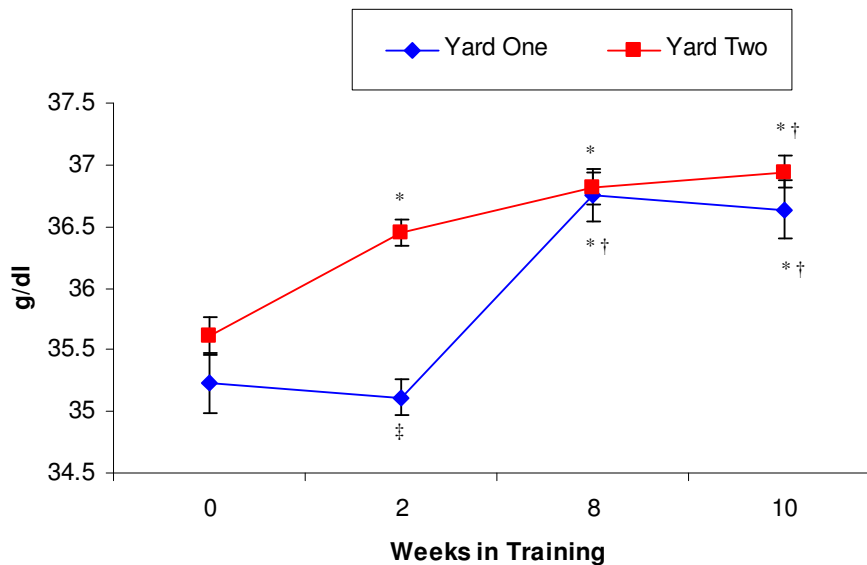
Table 3.52 MCHC – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	20.922	3	0.000
<i>Yard Two</i>	20.505	3	0.000
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	26.5	0.242
	2	0.00	0.000
	8	35.5	0.710
	10	28.5	0.325

Table 3.53 MCHC change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 8	0	0.008
	Wk 0 – Wk 10	0	0.008
	Wk 2 – Wk 8	0	0.008
	Wk 2 - Wk 10	0	0.008
<i>Yard Two</i>	Wk 0 – Wk 2	0	0.004
	Wk 0 – Wk 8	0	0.002
	Wk 0 - Wk 10	0	0.002
	Wk 2 - Wk 10	4	0.031

Two weeks after training commenced, MCHC values in yard one (Mdn = 35.10) were found to be significantly different to MCHC values in yard two (Mdn = 36.35) ($p < 0.001$). At all other times of sampling MCHC values did not appear to differ significantly between yard one and yard two ($p > 0.05$). Table 3.52 and figure 3.29 illustrate these findings.

**Figure 3.29 Mean MCHC levels throughout training period ($p < 0.001$)**

*Sig. different from pre-training values ($p < 0.05$) † Sig. different from wk 2 values ($p < 0.05$) ‡Yard one sig. different from yard two ($p < 0.05$)

Mean Corpuscular Haemoglobin (MCH)

Table 3.54 displays descriptive statistics for MCH levels throughout the time period examined (pg).

Table 3.54 Descriptive Statistics – Mean corpuscular haemoglobin

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One	Before Training	15.95	16.05	0.90	0.32	14.80	17.10
	Wk 2	15.91	15.95	0.78	0.28	14.90	17.00
	Wk 8	16.49	16.40	0.75	0.27	15.40	17.50
	Wk 10	16.44	16.40	0.73	0.26	15.40	17.30
Yard Two	Before Training	15.98	15.90	0.47	0.15	15.20	16.80
	Wk 2	16.39	16.30	0.44	0.14	15.70	17.10
	Wk 8	16.40	16.35	0.43	0.14	15.70	17.20
	Wk 10	16.57	16.65	0.52	0.16	15.50	17.30

MCH levels changed significantly throughout the training period in yard one and yard two ($p < 0.001$) (table 3.55). Wilcoxon signed-rank tests as displayed in table 3.56 lead to the following findings. MCH values in yard one were significantly lower at sampling prior to the beginning of training than at sampling after eight and ten weeks of training. Furthermore, MCH values in yard one were significantly lower at time of sampling after two weeks of training than after eight and ten weeks of training ($p < 0.05$). In yard two, MCH values were significantly lower at time of sampling prior to the beginning of training than during any of the successive times of sampling examined ($p < 0.05$). Also, levels after ten weeks of training had significantly increased when compared to levels after two weeks of training. Similarly to MCHC levels, MCH levels increased along with training duration, while remaining largely within the normal range. This can also be observed in tables 3.55 and 3.56 as well as figure 3.30.

Table 3.55 MCH – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	21.0	3	0.000
<i>Yard Two</i>	19.531	3	0.000
Mann-Whitney U-Test	Week	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	39.5	0.982
	2	26.0	0.227
	8	39.0	0.948
	10	36.0	0.745

Table 3.56 MCH change throughout training period by weeks

Wilcoxon Signed Rank	Comparison	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 8	0	0.008
	Wk 0 – Wk 10	0	0.008
	Wk 2 – Wk 8	0	0.008
	Wk 2 - Wk 10	0	0.008
<i>Yard Two</i>	Wk 0 – Wk 2	1	0.004
	Wk 0 – Wk 8	0	0.002
	Wk 0 – Wk 10	0	0.002
	Wk 2 – Wk 10	3.5	0.023

MCH values were not significantly different between yard one and yard two at any time of sampling ($p > 0.05$). See table 3.55 for exact findings.

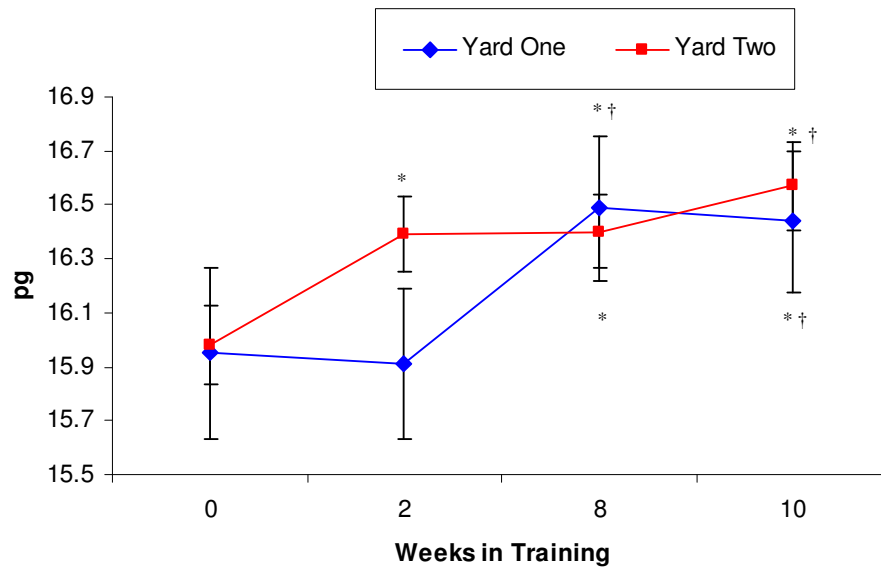


Figure 3.30 Mean MCH levels throughout training period ($p < 0.001$)

*Sig. different from pre-training values ($p < 0.05$) † Sig. different from wk 2 values ($p < 0.05$)

Platelets

Table 3.57 displays descriptive statistics for platelet counts. All mean and median values in both yards were below the normal range due to very low minimum values in both yards at all times of sampling. Maximum values were found to be within the normal range ($\times 10^9/l$).

Table 3.57 Descriptive Statistics – Platelet counts

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	128.00	136.50	40.12	14.19	48.00	183.00
	Wk 2	101.63	106.00	27.81	9.83	41.00	133.00
	Wk 8	124.75	131.50	41.54	14.69	36.00	170.00
	Wk 10	100.88	110.50	30.46	10.77	32.00	129.00
Yard Two	Before Training	110.10	103.50	34.51	10.91	69.00	181.00
	Wk 2	127.90	131.00	28.43	8.99	71.00	168.00
	Wk 8	128.30	121.00	33.63	10.64	75.00	183.00
	Wk 10	122.30	118.00	28.61	9.05	89.00	164.00

Platelet levels were found to significantly fluctuate throughout training in both yards (Yard One: $p < 0.01$; Yard Two: $p < 0.05$) (table 3.58). Post hoc analysis showed that

platelet counts, of horses in yard one, were significantly lower after ten weeks of training than after eight weeks of training and prior to the beginning of training. However, platelet counts after two weeks of training were significantly lower after two weeks of training than pre-training counts ($p < 0.05$). Platelet counts of horses in yard two had increased after two and eight weeks of training when compared to pre-training levels ($p < 0.05$). Table 3.59 and figure 3.31 display this information.

Table 3.58 Platelets – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	df	p
<i>Yard One</i>	11.051	3	0.007
<i>Yard Two</i>	8.212	3	0.037
Mann-Whitney U-Test	Week	U	p
<i>Yard One vs. Yard Two</i>	Pre-training	25.5	0.211
	2	17.5	0.046
	8	36.0	0.745
	10	29.5	0.372

Table 3.59 Platelets change throughout training period by weeks

Wilcoxon Signed Rank	Comparison	T	p
<i>Yard One</i>	Wk 0 – Wk 2	1	0.016
	Wk 0 – Wk 10	1	0.016
	Wk 8 – Wk 10	0	0.008
<i>Yard Two</i>	Wk 0 – Wk 2	7	0.037
	Wk 0 – Wk 8	6	0.027

At time of sampling after two weeks of training platelet counts differed significantly between yard one (Mdn=106.00) and yard two (Mdn=131.00) ($p < 0.05$). At all other times of sampling no significant differences between yard one and yard two were observed ($p > 0.05$). This is also shown in table 3.58.

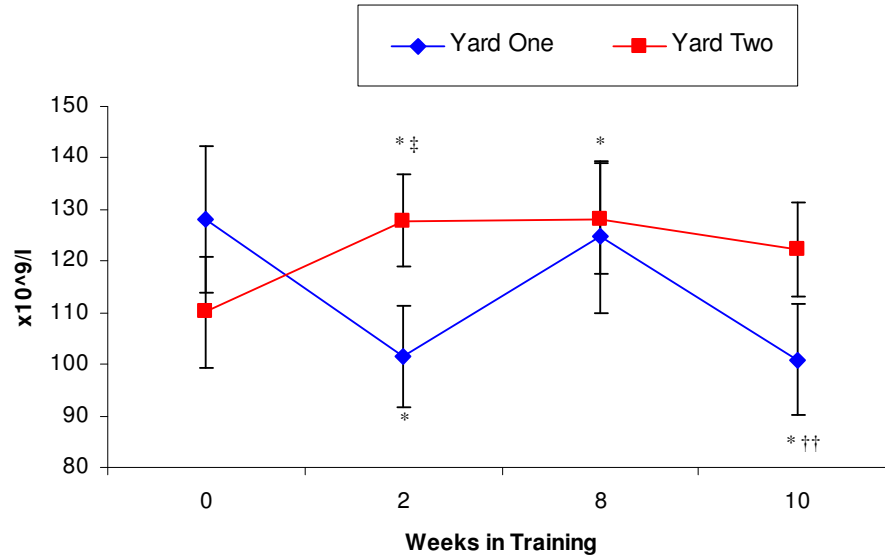


Figure 3.31 Mean platelet levels throughout training period ($p < 0.05$)

*Sig. different from pre-training values ($p < 0.05$) ††Sig. different from wk 8 values ($p < 0.05$)
 ‡Yard one sig. different from yard two ($p < 0.05$)

Total leukocytes

Minimum values outside the normal range were reported in both yards at all times of sampling as can be observed in table 3.60. Maximum values observed fell within the normal range at all times of sampling ($\times 10^9/l$).

Table 3.60 Descriptive Statistics – Total leukocytes

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	6.45	6.55	1.33	0.47	4.80	8.10
	Wk 2	6.66	6.65	0.83	0.30	5.70	8.10
	Wk 8	6.96	7.25	1.25	0.44	4.60	8.30
	Wk 10	6.09	6.05	0.66	0.23	5.40	7.20
Yard Two	Before Training	6.11	6.10	0.80	0.25	5.00	7.90
	Wk 2	6.04	5.80	1.13	0.36	5.00	8.40
	Wk 8	6.66	6.15	1.37	0.43	4.90	8.50
	Wk 10	6.47	6.90	1.37	0.43	4.20	8.00

Total leukocyte levels did not significantly change throughout the training period examined in either yard ($p > 0.05$). Figure 3.32 illustrates mean total leukocyte counts at all sampling times in yard one and yard two. Total leukocyte count did not

significantly vary between yard one and yard two ($p>0.05$). These findings are displayed in table 3.61.

Table 3.61 Total Leukocytes – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	5.026	3	0.172
<i>Yard Two</i>	3.796	3	0.292
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	37.0	0.811
	2	24.5	0.180
	8	37.5	0.847
	10	29.0	0.346

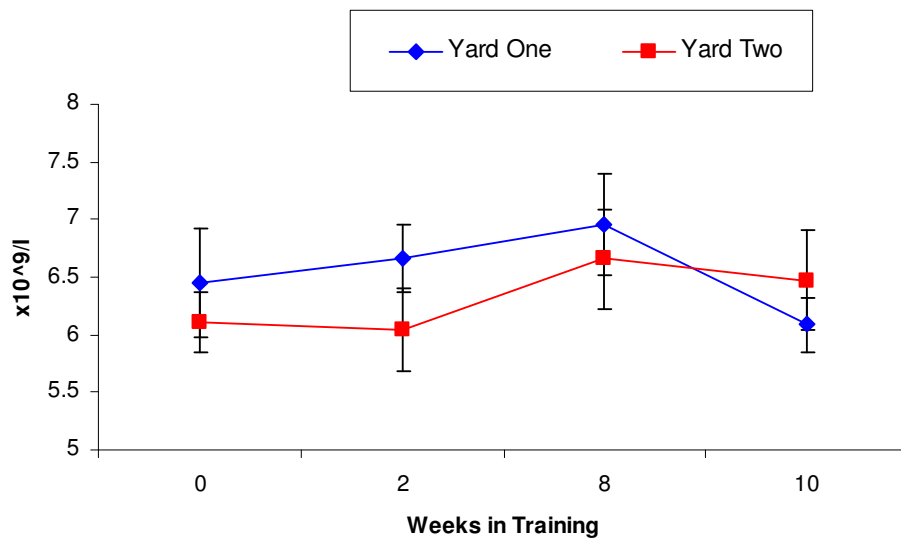


Figure 3.32 Mean total leukocyte levels throughout training period ($p>0.05$)

Neutrophils

Table 3.62 provides descriptive information on neutrophil levels throughout the time period examined. Whereas maximum neutrophil counts in both yards remained within the normal range, minimum counts in both yards were found to be outside the normal range except during week ten in yard one and week eight in yard two.

Although it should be noted that at sampling in weeks two and eight in yard one, minimum values were only marginally below normal minimum values ($\times 10^9/l$).

Table 3.62 Descriptive Statistics – Neutrophils

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	3.53	3.53	1.25	0.44	2.17	5.75
	<i>Wk 2</i>	3.84	3.67	0.97	0.34	2.48	5.59
	<i>Wk 8</i>	4.11	4.47	0.87	0.31	2.44	4.81
	<i>Wk 10</i>	3.35	3.46	0.40	0.14	2.75	3.89
<i>Yard Two</i>	<i>Before Training</i>	3.28	3.22	0.82	0.26	1.85	4.98
	<i>Wk 2</i>	3.06	3.02	0.81	0.26	2.09	4.87
	<i>Wk 8</i>	4.08	4.34	0.99	0.31	2.75	5.27
	<i>Wk 10</i>	3.88	4.17	0.99	0.31	2.39	5.08

Neutrophil counts did not appear to have changed significantly throughout the period examined in yard one or yard two ($p>0.05$). This is demonstrated in figure 3.33 and table 3.63. Neutrophil levels observed did not show any significant difference between the two yards in this study ($p>0.05$).

Table 3.63 Neutrophils – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	1.95	3	0.629
<i>Yard Two</i>	7.424	3	0.055
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	35.0	0.696
	2	19.0	0.068
	8	39.0	0.945
	10	25.5	0.211

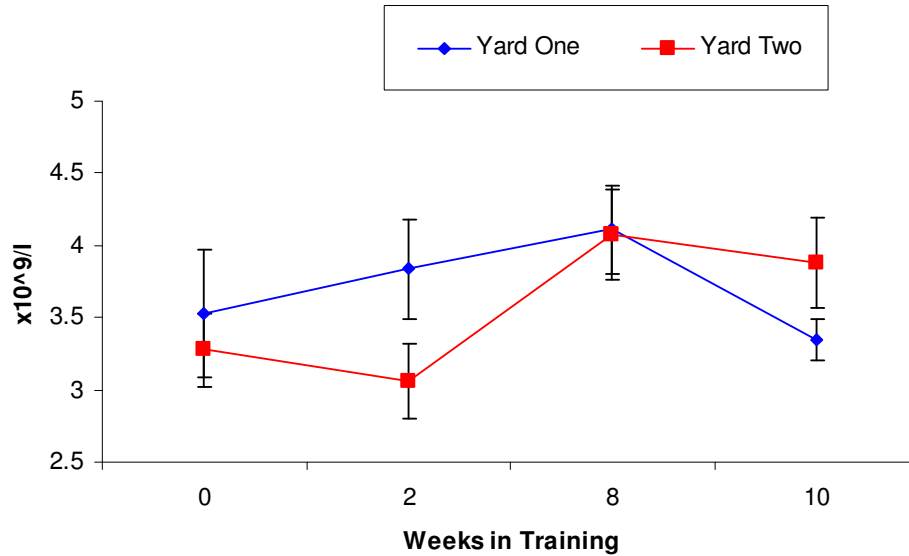


Figure 3.33 Mean neutrophil levels throughout training period ($p>0.05$)

Table 3.64 displays descriptive information for the percentage of neutrophils from total leukocytes throughout the ten week training period (%).

Table 3.64 Descriptive Statistics – Neutrophils

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One	Before Training	53.75	51.00	10.71	3.79	41.00	71.00
	Wk 2	57.13	57.50	9.58	3.39	42.00	69.00
	Wk 8	58.75	59.00	5.04	1.78	53.00	67.00
	Wk 10	55.13	53.00	5.57	1.97	49.00	64.00
Yard Two	Before Training	53.10	52.50	8.23	2.60	37.00	63.00
	Wk 2	50.10	51.00	4.72	1.49	41.00	58.00
	Wk 8	61.10	61.00	7.88	2.49	50.00	75.00
	Wk 10	59.60	60.50	5.68	1.80	48.00	66.00

Neutrophil percentage in yard one did not fluctuate significantly throughout training ($p>0.05$). However, neutrophil percentage in yard two varied significantly throughout the ten week period examined ($p<0.001$). As detailed in table 3.65 and table 3.66 as well as figure 3.34, neutrophil percentage had increased significantly after eight and ten weeks of training when compared to two weeks of training as well as after eight weeks of training when compared to two weeks of training in yard two ($p<0.05$).

Table 3.65 Neutrophil % – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	1.808	3	0.637
<i>Yard Two</i>	17.156	3	0.000
Mann-Whitney U-Test	Week	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	40.0	1.000
	2	20.0	0.079
	8	32.5	0.526
	10	21.5	0.105

Table 3.66 Neutrophil % change throughout training period by weeks

Wilcoxon Signed Rank	Comparison	<i>T</i>	<i>p</i>
<i>Yard Two</i>	Wk 0 – Wk 8	2	0.012
	Wk 2 – Wk 8	0	0.002
	Wk 2 – Wk 10	3	0.010

No significant differences between yard one and yard two were observed at any point of sampling ($p > 0.05$). This can also be seen in table 3.65.

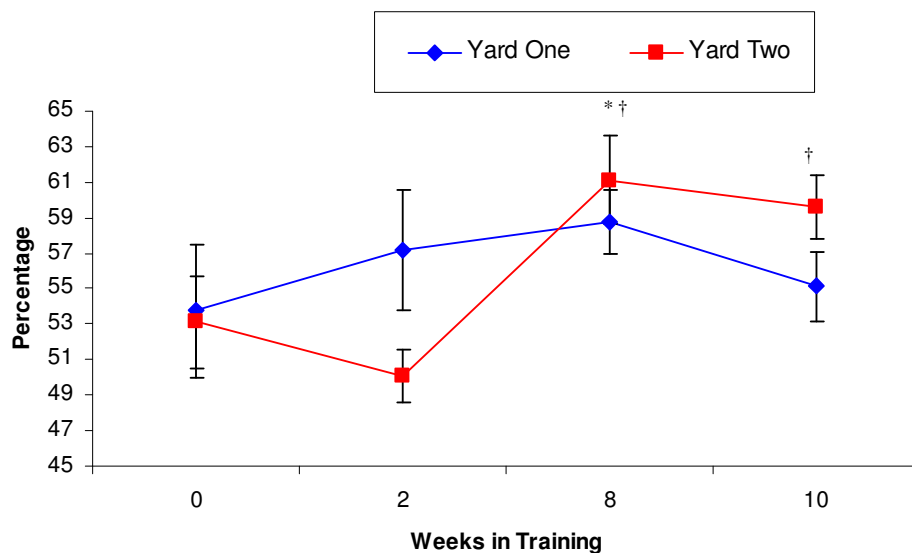


Figure 3.34 Mean neutrophil percentage levels throughout training period (Y1: >0.05 ; Y2: $p < 0.001$)

*Sig. different from pre-training values ($p < 0.05$) †Sig. different from wk 2 values ($p < 0.05$)

Lymphocytes

As can be observed from table 3.67, maximum levels of lymphocytes fell within the normal range, while minimum levels outside the normal range were seen in both yards except during sampling in yard one eight weeks after training begin and in yard two after two weeks of training. However, the minimum value in yard one after two weeks of training was only slightly lower than normal minimum values ($\times 10^9/l$).

Table 3.67 Descriptive Statistics – Lymphocytes

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One	Before Training	2.50	2.44	0.59	0.21	1.62	3.36
	Wk 2	2.56	2.38	0.56	0.20	1.99	3.50
	Wk 8	2.63	2.56	0.52	0.18	2.02	3.65
	Wk 10	2.57	2.58	0.50	0.18	1.88	3.12
Yard Two	Before Training	2.58	2.75	0.38	0.12	1.66	2.90
	Wk 2	2.82	2.74	0.40	0.13	2.45	3.63
	Wk 8	2.39	2.54	0.62	0.20	1.45	3.08
	Wk 10	2.37	2.52	0.49	0.16	1.51	3.20

As illustrated in figure 3.35 and table 3.68 lymphocyte levels did not significantly fluctuate throughout training ($p>0.05$). Also, lymphocyte levels did not appear to differ between yard one and yard two ($p>0.05$).

Table 3.68 Lymphocytes – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	4.443	3	0.223
<i>Yard Two</i>	6.96	3	0.070
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	38.5	0.915
	2	27.5	0.284
	8	36.0	0.762
	10	32.0	0.501

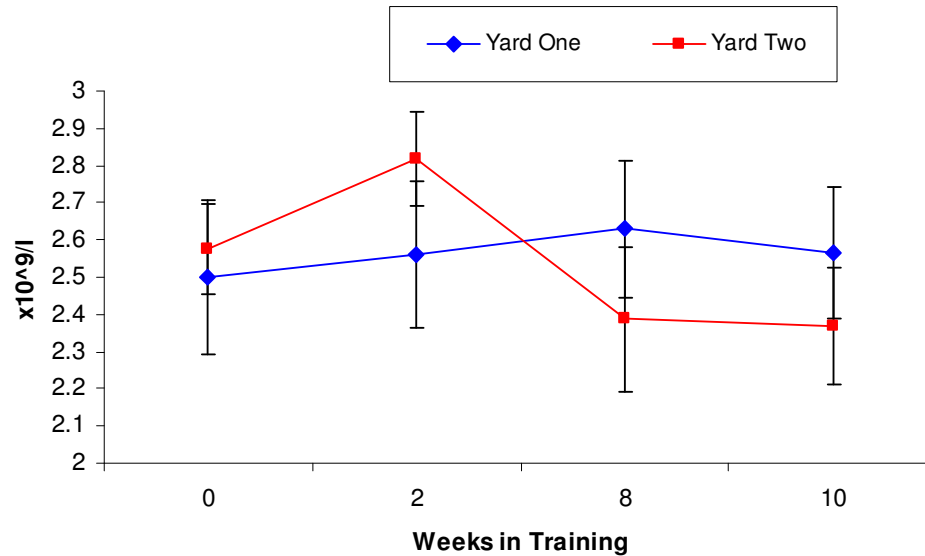


Figure 3.35 Mean lymphocyte levels throughout training period ($p>0.05$)

Descriptive information on the percentage of lymphocytes from total leukocytes can be taken from table 3.69 (%).

Table 3.69 Descriptive Statistics – Lymphocytes

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One	Before Training	39.88	40.50	11.00	3.89	25.00	56.00
	Wk 2	39.00	39.50	9.64	3.41	27.00	54.00
	Wk 8	38.13	37.50	5.46	1.93	31.00	45.00
	Wk 10	42.00	43.50	6.09	2.15	33.00	49.00
Yard Two	Before Training	42.50	42.00	7.01	2.22	32.00	57.00
	Wk 2	47.10	48.00	4.70	1.49	38.00	56.00
	Wk 8	35.90	36.00	6.74	2.13	25.00	47.00
	Wk 10	36.90	35.50	4.51	1.43	33.00	48.00

Whereas lymphocyte percentage in yard one did not appear to have changed significantly throughout the ten week period examined ($p>0.05$), lymphocyte percentage in yard two fluctuated significantly ($p<0.001$). This is displayed in table 3.70. Wilcoxon signed rank tests showed that the percentage of lymphocytes after two weeks of training was significantly higher than prior to training begin as well as after eight and ten weeks of training. Also, lymphocyte percentage was significantly

lower after eight weeks of training when compared to pre-training levels ($p < 0.05$). See table 3.71.

Table 3.70 Lymphocyte % – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	2.013	3	0.595
<i>Yard Two</i>	18.212	3	0.000
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	33.0	0.557
	2	17.5	0.045
	8	34.5	0.648
	10	23.0	0.137

Table 3.71 Lymphocyte % change throughout training period by weeks

	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard Two</i>	Wk 0 – Wk 2	8	0.047
	Wk 0 – Wk 8	2.5	0.016
	Wk 2 – Wk 8	0	0.002
	Wk 2 – Wk 10	2	0.006

Furthermore, as shown in figure 3.36 and table 3.70, a significantly higher percentage of lymphocytes was observed in yard two (Mdn = 48.0) than in yard one (Mdn = 39.5) after two weeks of training ($p < 0.05$). During all other weeks of training no significant differences between yard one and yard two were observed ($p > 0.05$).

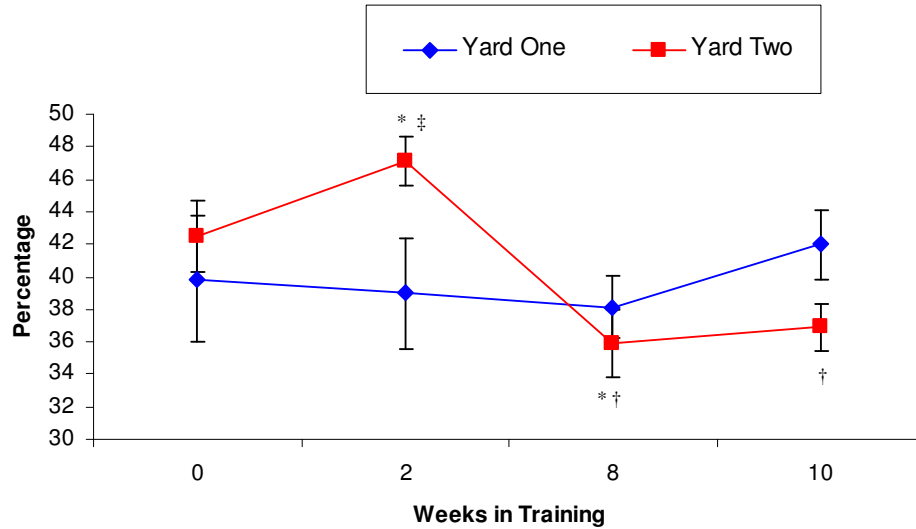


Figure 3.36 Mean lymphocyte percentage levels throughout training period (Y1: $p > 0.05$; Y2: $p < 0.001$)

*Sig. different from pre-training values ($p < 0.05$) †Sig. different from wk 2 values ($p < 0.05$)
‡Yard one sig. different from yard two ($p < 0.05$)

Monocytes

Table 3.72 shows descriptive statistics of monocyte levels throughout training. Monocyte mean and median numbers in both yards at all times of sampling were below the minimum value of the normal range due to minimum values being as low as zero at time of sampling ($\times 10^9/l$).

Table 3.72 Descriptive Statistics – Monocytes

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	0.16	0.14	0.13	0.04	0.00	0.38
	Wk 2	0.11	0.09	0.07	0.03	0.00	0.24
	Wk 8	0.17	0.09	0.15	0.05	0.00	0.42
	Wk 10	0.15	0.15	0.09	0.03	0.00	0.26
Yard Two	Before Training	0.10	0.11	0.04	0.01	0.05	0.20
	Wk 2	0.11	0.09	0.08	0.03	0.00	0.25
	Wk 8	0.16	0.15	0.11	0.03	0.00	0.38
	Wk 10	0.18	0.15	0.14	0.04	0.00	0.48

No significant change in monocyte levels was observed throughout the training period examined as can be seen from figure 3.37 and table 3.73 ($p > 0.05$). Furthermore, monocyte levels did not vary significantly between the two yards included in this study ($p > 0.05$) as illustrated in figure 3.37 and table 3.73.

Table 3.73 Monocytes – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	1.066	3	0.806
<i>Yard Two</i>	3.194	3	0.375
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	30.5	0.419
	2	37.0	0.810
	8	34.0	0.619
	10	35.0	0.681

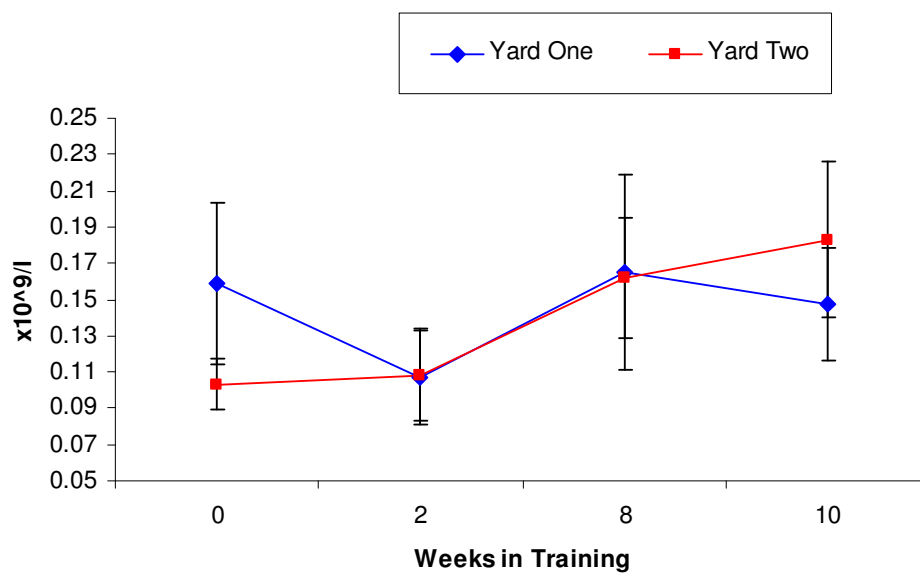


Figure 3.37 Monocyte levels throughout training period ($p > .05$)

Information on descriptive statistics for the percentage of monocytes from total leukocytes is displayed in table 3.74 (%).

Table 3.74 Descriptive Statistics – Monocytes

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	2.38	2.00	1.85	0.65	0.00	5.00
	<i>Wk 2</i>	1.63	1.50	1.19	0.42	0.00	4.00
	<i>Wk 8</i>	2.25	1.50	1.83	0.65	0.00	5.00
	<i>Wk 10</i>	2.38	2.50	1.41	0.50	0.00	4.00
<i>Yard Two</i>	<i>Before Training</i>	1.70	2.00	0.67	0.21	1.00	3.00
	<i>Wk 2</i>	1.70	1.50	1.06	0.34	0.00	3.00
	<i>Wk 8</i>	2.50	2.00	1.84	0.58	0.00	7.00
	<i>Wk 10</i>	2.90	2.50	1.97	0.62	0.00	6.00

The percentage of monocytes throughout training did not appear to have changed significantly throughout training ($p>0.05$). This can be observed in figure 3.38 and table 3.75. Also, monocyte percentage did not vary significantly between yard one and yard two at any stage of training ($p>0.05$).

Table 3.75 Monocyte % – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	1.348	3	0.743
<i>Yard Two</i>	3.25	3	0.367
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	33.5	0.605
	2	37.5	0.822
	8	36.0	0.736
	10	36.0	0.756

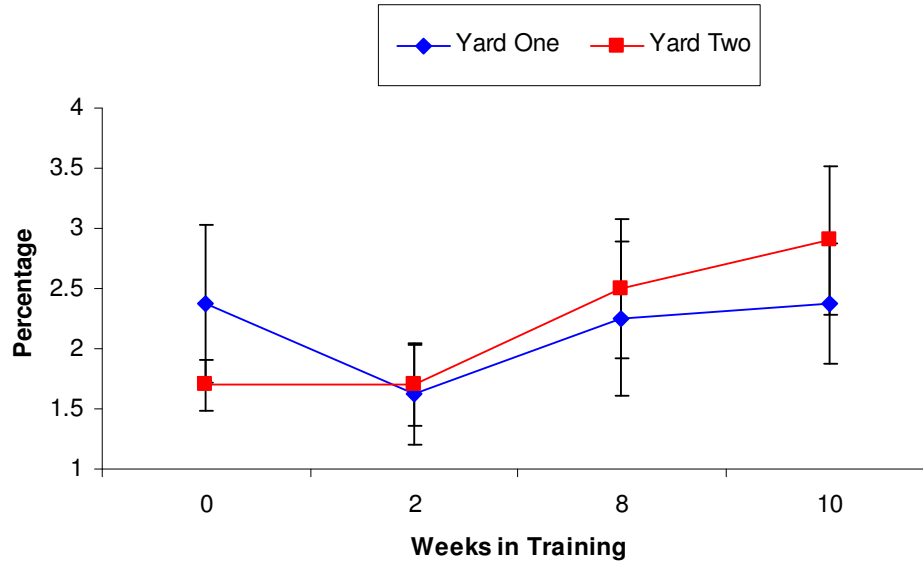


Figure 3.38 Mean monocyte percentage levels throughout training period ($p>0.05$)

Eosinophils

As demonstrated in table 3.76 eosinophil counts below the minimum normal value (<0.1) as described by Hodgson and Rose (1994) were frequently observed in both yards. As a result, median and mean counts outside the normal range appeared repeatedly in both yards ($\times 10^9/l$).

Table 3.76 Descriptive Statistics – Eosinophils

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	0.27	0.16	0.25	0.09	0.05	0.84
	<i>Wk 2</i>	0.16	0.14	0.13	0.05	0.00	0.43
	<i>Wk 8</i>	0.06	0.07	0.05	0.02	0.00	0.14
	<i>Wk 10</i>	0.03	0.03	0.03	0.01	0.00	0.07
<i>Yard Two</i>	<i>Before Training</i>	0.16	0.06	0.22	0.07	0.00	0.71
	<i>Wk 2</i>	0.06	0.03	0.08	0.03	0.00	0.25
	<i>Wk 8</i>	0.03	0.03	0.04	0.01	0.00	0.09
	<i>Wk 10</i>	0.04	0.02	0.05	0.02	0.00	0.14

Whereas eosinophil counts in yard two did not appear to have significantly changed throughout the training period examined ($p>0.05$), eosinophil counts in yard one appeared to have been significantly affected by training ($p<0.01$). Eosinophil count in

yard one had significantly decreased after eight and ten weeks of training when compared to pre-training counts ($p < 0.05$). This can be seen in table 3.77 and 3.78. It can also be observed from figure 3.39 that eosinophil counts in both yards followed a similar trend and as such appeared to progressively get lower as training advanced. Eosinophil counts did not show any variation between yard one and yard two ($p > 0.05$). See table 3.77.

Table 3.77 Eosinophils – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	11.282	3	0.006
<i>Yard Two</i>	1.052	3	0.808
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	26.0	0.225
	2	20.5	0.081
	8	29.5	0.344
	10	37.0	0.817

Table 3.78 Eosinophils change throughout training period by weeks

	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 8	2	0.023
	Wk 0 – Wk 10	1	0.016

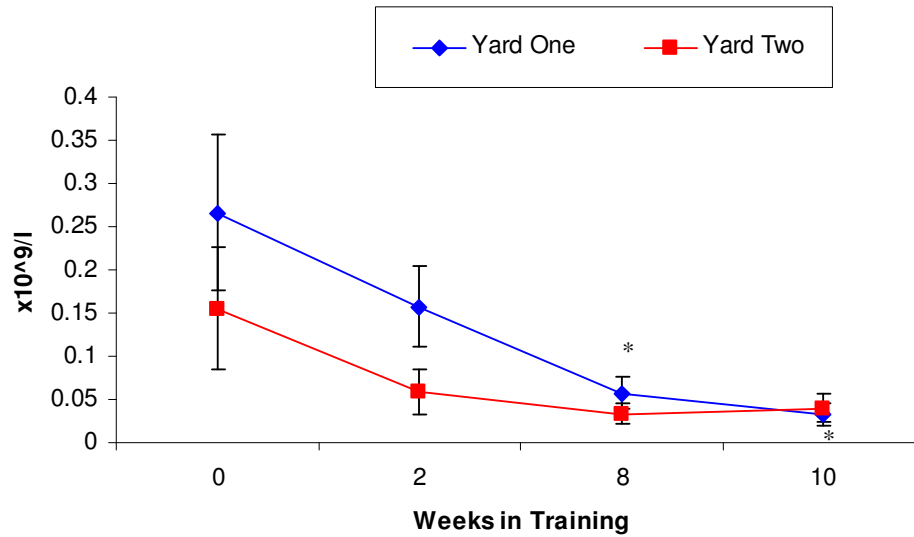


Figure 3.39 Mean eosinophil levels throughout training period (Y1: $p>0.01$; Y2: $p>0.05$)

*Sig. different from pre-training values ($p<0.05$)

Table 3.79 displays descriptive information on the percentage of eosinophils of total leukocytes throughout the ten week training period examined (%).

Table 3.79 Descriptive Statistics – Eosinophils

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	4.00	3.00	3.34	1.18	1.00	11.00
	Wk 2	2.25	2.00	1.83	0.65	0.00	6.00
	Wk 8	0.88	1.00	0.83	0.30	0.00	2.00
	Wk 10	0.50	0.50	0.53	0.19	0.00	1.00
Yard Two	Before Training	2.70	1.00	3.80	1.20	0.00	12.00
	Wk 2	1.10	0.50	1.60	0.50	0.00	5.00
	Wk 8	0.50	0.50	0.53	0.17	0.00	1.00
	Wk 10	0.60	0.50	0.70	0.22	0.00	2.00

Eosinophil percentage in yard two did not significantly vary throughout training ($p>0.05$). On the other hand, eosinophil percentage in yard one appeared to have changed significantly throughout the time period examined ($p<0.001$). Table 3.80 displays this information. Similar to eosinophil counts, eosinophil percentage had significantly decreased after eight and ten weeks of training when compared to pre-

training levels ($p < 0.05$). No difference in eosinophil percentage between yard one and yard two was observed ($p > 0.05$). These observations are also displayed in tables 3.80 and 3.81 as well as figure 3.40.

Table 3.80 Eosinophil % – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	15.333	3	0.000
<i>Yard Two</i>	1.901	3	0.615
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	27.0	0.258
	2	21.5	0.096
	8	30.0	0.385
	10	38.0	1.000

Table 3.81 Eosinophil % change throughout training period by weeks

	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 8	0	0.031
	Wk 0 – Wk 10	0	0.016

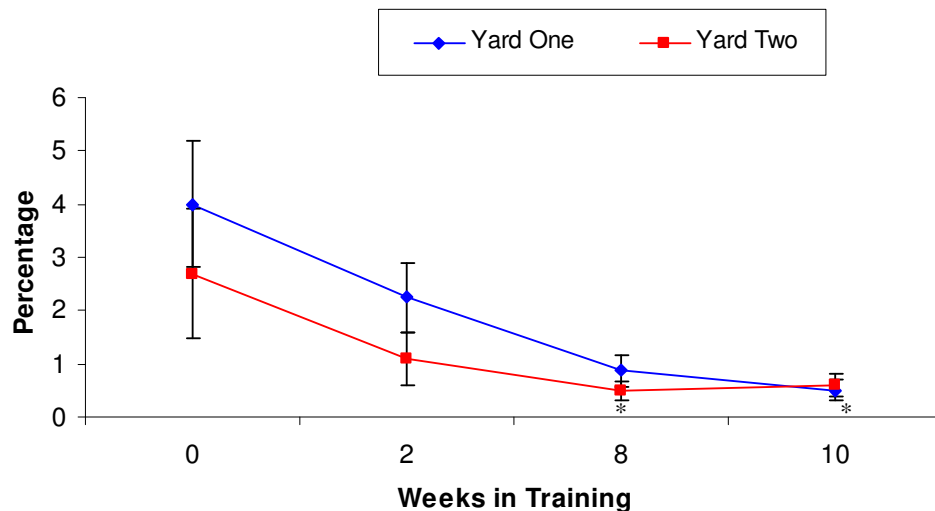


Figure 3.40 Mean eosinophil percentage levels throughout training period (Y1: $p < 0.001$; Y2: $p > 0.05$)

*Sig. different from pre-training values ($p < 0.05$)

Effect of Training on Plasma Biochemistry

Total Protein

Table 3.82 displays descriptive statistics for total protein levels throughout the time period examined. No values below the normal range were observed, however, at sampling before training begin in yard one and two as well as at sampling after two weeks of training in yard one, values slightly above the normal range were observed (g/l).

Table 3.82 Descriptive Statistics – Total Protein

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
<i>Yard One</i>	<i>Before Training</i>	72.83	71.35	4.31	1.52	67.90	81.10
	<i>Wk 2</i>	70.56	70.65	5.08	1.79	61.70	77.30
	<i>Wk 8</i>	68.81	69.30	4.96	1.76	59.90	74.10
	<i>Wk 10</i>	65.90	67.55	4.32	1.53	59.10	70.60
<i>Yard Two</i>	<i>Before Training</i>	71.34	70.80	3.80	1.20	65.70	76.60
	<i>Wk 2</i>	64.78	65.40	3.43	1.08	59.20	68.50
	<i>Wk 8</i>	62.91	62.45	4.34	1.37	56.60	71.20
	<i>Wk 10</i>	64.64	65.30	3.74	1.18	57.70	70.10

Total Protein levels appeared to have changed significantly throughout the time period examined in both yards (Yard One: $p < 0.01$; Yard Two: $p < 0.001$). See table 3.83. As can be seen in table 3.84, total protein levels in yard one had significantly decreased after ten weeks of training when compared to levels prior to the beginning of training and after eight weeks of training, while levels in yard two were found to have decreased significantly after two, eight and ten weeks of training, when compared levels prior to the start of training ($p < 0.05$).

Table 3.83 Total Protein – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	10.5	3	0.009
<i>Yard Two</i>	18.36	3	0.000
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	32.0	0.515
	2	13.0	0.015
	8	14.0	0.021
	10	30.5	0.421

Table 3.84 Total protein change throughout training period by weeks

Wilcoxon Signed Rank	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 10	3	0.039
	Wk 8 – Wk 10	2	0.023
<i>Yard Two</i>	Wk 0 – Wk 2	0	0.002
	Wk 0 – Wk 8	0	0.002
	Wk 0 – Wk 10	0	0.002

At time of sampling after two weeks of training total protein levels differed significantly between yard one (Mdn=70.65) and yard two (Mdn=65.40) ($p < 0.05$). Total Protein levels also differed significantly between yard one (Mdn=69.30) and yard two (Mdn=62.45) after eight weeks of training ($p < 0.05$). At sampling prior to training begin and after ten weeks of sampling no significant differences between yard one and yard two were observed ($p > 0.05$). Table 3.83 and figure 3.41 illustrate these findings.

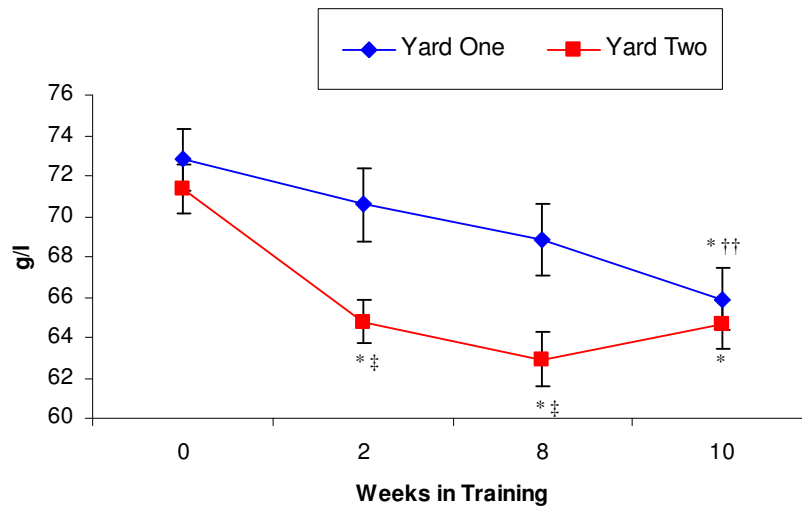


Figure 3.41 Mean total protein levels throughout training period ($p < 0.01$)

*Sig. different from pre-training values ($p < 0.05$) ††Sig. different from wk 8 values ($p < 0.05$)

‡Yard one sig. different from yard two ($p < 0.05$)

Albumin

Descriptive statistics information regarding the levels of albumin throughout the time period examined can be taken from table 3.85. All albumin values were within the normal range at all times of sampling (g/l).

Table 3.85 Descriptive Statistics – Albumin

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	32.71	32.65	1.60	0.57	31.20	36.00
	Wk 2	32.61	32.35	0.80	0.28	31.80	33.70
	Wk 8	33.25	33.95	2.21	0.78	28.00	34.90
	Wk 10	32.88	33.25	2.09	0.74	28.30	35.30
Yard Two	Before Training	33.77	33.80	1.85	0.58	31.50	36.90
	Wk 2	31.68	31.80	1.70	0.54	28.80	34.60
	Wk 8	31.51	32.15	1.22	0.39	29.70	33.10
	Wk 10	31.70	31.65	1.78	0.56	28.30	34.80

Albumin levels appeared to have fluctuated significantly in both yards throughout the training period examined ($p < 0.05$). This is also shown in table 3.86. However, post hoc tests on results from yard one, it could not be concluded that changes in yard one were due to any other reason than chance. This finding was in line with the exact significance value of the Friedman's ANOVA, which was a borderline value

($p=0.049$). On the other hand, values for albumin in horses from yard two were significantly lower after two, eight and ten weeks of training than prior to the commencement of training ($p<0.0083$). This is also shown in table 3.87 and figure 3.42.

Table 3.86 Albumin – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	7.65	3	0.049
<i>Yard Two</i>	8.939	3	0.025
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	25.0	0.211
	2	25.5	0.212
	8	11.0	0.008
	10	20.0	0.079

Table 3.87 Albumin change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard Two</i>	Wk 0 – Wk 2	1	0.004
	Wk 0 – Wk 8	1	0.008
	Wk 0 – Wk 10	6	0.027

Values for plasma albumin showed a significant difference between yard one (Mdn=33.95) and yard two (Mdn=32.15) at sampling after eight weeks of training ($p<0.05$). At all other times of sampling albumin levels did not significantly differ between yard one and yard two ($p>0.05$).

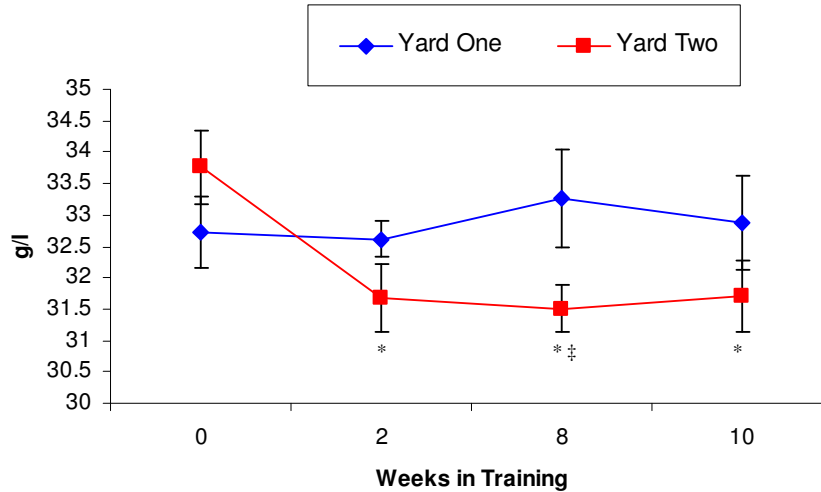


Figure 3.40 Mean albumin levels throughout training period ($p < 0.05$)

*Sig. different from pre-training values ($p < 0.05$) ‡Yard one sig. different from yard two ($p < 0.05$)

Globulin

Table 3.88 displays descriptive statistics for globulin values throughout the ten week training period examined. Whereas no values below the normal range were observed at any stage, maximum values above the normal range appeared at all times of sampling. This is also reflected in the median and mean values lying above the normal range in week zero (both yards), week two (yard one) and week eight (yard one) (g/l).

Table 3.88 Descriptive Statistics – Globulin

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	40.11	38.95	4.27	1.51	34.60	47.90
	Wk 2	37.95	37.75	5.33	1.89	28.40	45.40
	Wk 8	35.56	35.35	5.78	2.04	25.90	45.00
	Wk 10	33.03	33.60	4.63	1.64	26.40	40.50
Yard Two	Before Training	37.57	37.90	3.13	0.99	31.70	41.30
	Wk 2	33.10	32.85	3.03	0.96	28.10	37.20
	Wk 8	31.40	30.60	3.96	1.25	24.40	39.00
	Wk 10	32.94	32.55	3.41	1.08	28.70	39.20

On initial statistical analysis, significant values indicating that changes in globulin occurred throughout the time period examined were observed in both yards (Yard

One: $p < 0.05$; Yard Two: $p < 0.001$) (table 3.89). As can be seen in table 3.90, globulin values in horses from yard were significantly lower after ten weeks of training than prior to the beginning of training and after eight weeks of training. Also, globulin levels in horses from yard two after two, eight and ten weeks of training were significantly different to globulin values prior to the commencement of training ($p < 0.05$). Figure 3.43 shows that globulin values in both yards decreased throughout training with respect to pre-training values.

Table 3.89 Globulin – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	10.05	3	0.014
<i>Yard Two</i>	18.582	3	0.000
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	27.0	0.264
	2	16.5	0.037
	8	21.5	0.106
	10	39.0	0.965

Table 3.90 Globulin change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 10	2	0.023
	Wk 8 – Wk 10	3	0.039
<i>Yard Two</i>	Wk 0 – Wk 2	0	0.002
	Wk 0 – Wk 8	0	0.002
	Wk 0 – Wk 10	0	0.002

Globulin values were significantly higher in yard one (Mdn=37.75) than in yard two (Mdn=32.85) after two weeks of training ($p < 0.05$). No differences in globulin levels between both yards were observed at sampling prior to the start of training as well as at eight and ten weeks into the training programme respectively ($p > 0.05$).

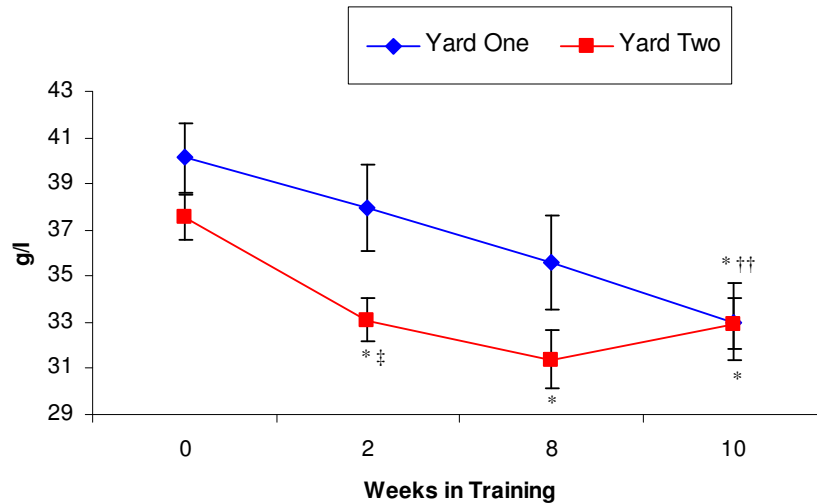


Figure 3.43 Mean globulin levels throughout training period ($p < 0.05$)

*Sig. different from pre-training values ($p < 0.05$) ††Sig. different from wk 8 values ($p < 0.05$)

‡Yard one sig. different from yard two ($p < 0.05$)

Aspartate Transaminase (AST)

As shown in table 3.91, minimum and maximum AST values outside the normal range were observed at various sampling times, while mean and median lay within the normal range at all sampling dates. AST minimum values lay below the normal range in yard one after eight and ten weeks of training, whereas maximum values above the normal range were observed in yard one at sampling in week zero, two and eight as well as in yard two after ten weeks of training (iu/l).

Table 3.91 Descriptive Statistics – AST

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	379.88	348.50	119.60	42.28	215.00	584.00
	Wk 2	289.88	253.50	132.08	46.70	191.00	603.00
	Wk 8	301.88	294.50	100.92	35.68	132.00	468.00
	Wk 10	301.88	316.50	70.35	24.87	142.00	389.00
Yard Two	Before Training	305.30	311.50	50.32	15.91	219.00	393.00
	Wk 2	281.50	275.00	30.59	9.67	246.00	347.00
	Wk 8	299.20	311.00	53.55	16.93	199.00	358.00
	Wk 10	328.30	322.00	84.00	26.56	207.00	457.00

Whereas AST levels of horses in yard two were not significantly affected by training ($p>0.05$), AST levels of horses in yard one appeared to have significantly changed throughout the ten week training period examined ($p<0.05$). This finding is also displayed in table 3.87. AST levels after ten weeks of training were significantly lower when compared to pre-training levels of horses in yard one ($p<0.05$). No significant differences between AST levels of horses in yard one and yard two were found at any time of sampling ($p>0.05$). Figure 3.44 as well as tables 3.92 and 3.93 illustrate these findings.

Table 3.92 AST – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	df	p
<i>Yard One</i>	10.05	3	0.014
<i>Yard Two</i>	4.44	3	0.230
Mann-Whitney U-Test	Week	U	p
<i>Yard One vs. Yard Two</i>	Pre-training	22.5	0.128
	2	27.0	0.274
	8	39.0	0.965
	10	36.0	0.762

Table 3.93 AST change throughout training period by weeks

Wilcoxon Signed Rank	Comparison	T	p
<i>Yard One</i>	Wk 0 – Wk 10	2	0.023

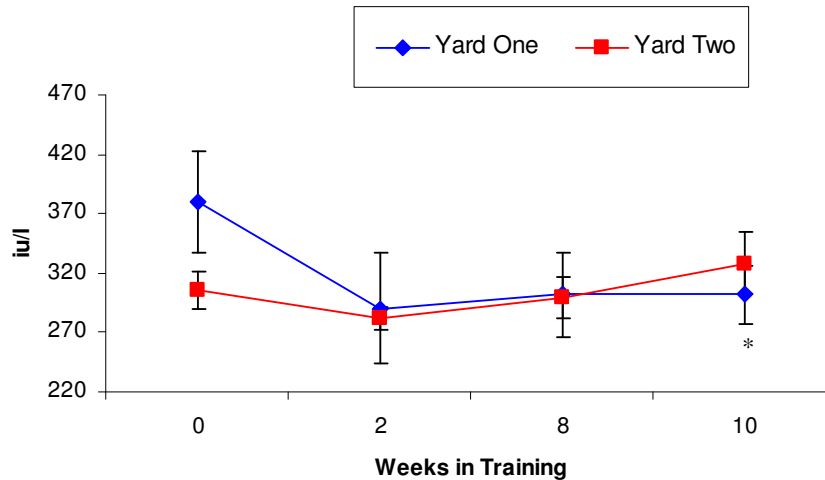


Figure 3.44 Mean AST levels throughout training period (Y1: $p < 0.05$; Y2: $p > 0.05$)

*Sig. different from pre-training values ($p < 0.05$)

Creatine Kinase (CK)

As can be taken from table 3.94, displaying descriptive statistics relating to CK values in horses from both yards, minimum values below the normal range only occurred in yard one after eight weeks of training. On the other hand, CK values that lay above the normal range occurred at time of sampling in week zero (yard one), week two (yard two), week eight (both yards) and week ten (both yards). Furthermore, a median value just above the normal range was observed in yard one prior to the commencement of sampling (iu/l).

Table 3.94 Descriptive Statistics – CK

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One	Before Training	276.00	301.50	62.98	22.27	160.00	343.00
	Wk 2	205.63	210.50	46.95	16.60	127.00	267.00
	Wk 8	229.13	218.50	102.04	36.08	69.00	429.00
	Wk 10	205.50	195.50	77.44	27.38	104.00	366.00
Yard Two	Before Training	223.60	216.00	42.11	13.32	168.00	295.00
	Wk 2	236.60	201.50	93.50	29.57	144.00	434.00
	Wk 8	218.50	192.50	93.53	29.58	140.00	423.00
	Wk 10	190.90	181.50	52.30	16.54	134.00	305.00

Following a similar trend as AST levels, CK levels in yard two were not found to have been significantly affected by training ($p>0.05$), while CK levels in yard one were found to have changed significantly during training ($p<0.05$). CK levels of horses in yard one had significantly reduced after two and ten weeks of training when compared to pre-training levels ($p<0.05$). Also, CK levels did not significantly vary between horses in yard one and yard two at any time of sampling ($p>0.05$). Figure 3.45 and table 3.95 demonstrate these findings.

Table 3.95 CK – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	8.55	3	0.031
<i>Yard Two</i>	3.96	3	0.288
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	19.0	0.068
	2	39.0	0.946
	8	29.0	0.360
	10	35.0	0.681

Table 3.96 CK change throughout training period by weeks

Wilcoxon Signed Rank	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 2	2	0.023
	Wk 0 – Wk 10	2	0.023

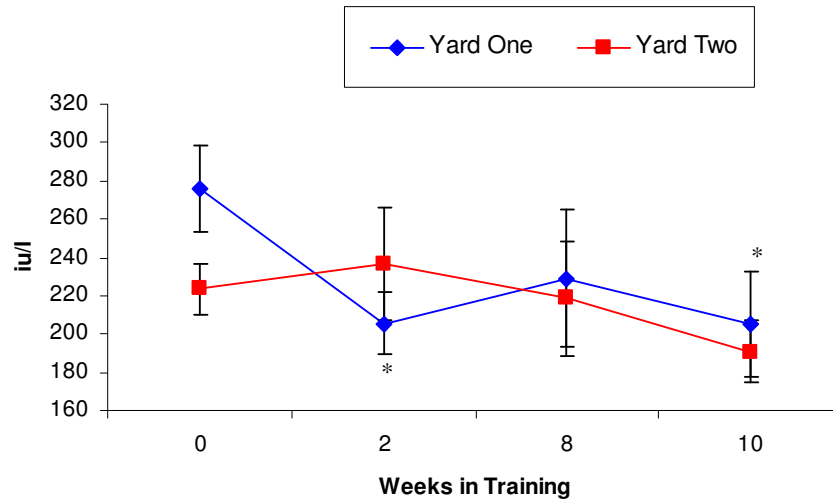


Figure 3.45 Mean CK levels throughout training period (Y1: $p < 0.05$; Y2: $p > 0.05$)
 *Sig. different from pre-training values ($p < 0.05$)

Gamma-Glutamyl Transferase (GGT)

As table 3.97 shows, values for GGT in horses from yard two lay within the normal range at all sampling times, whereas maximum values of horses from yard one lay above the normal range at all sampling times (iu/l).

Table 3.97 Descriptive Statistics – GGT

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	33.66	28.95	16.89	5.97	20.00	73.20
	Wk 2	24.81	23.45	8.39	2.97	15.30	40.90
	Wk 8	25.53	20.25	14.19	5.02	12.90	57.40
	Wk 10	24.83	23.00	9.65	3.41	16.00	45.50
Yard Two	Before Training	23.02	21.25	5.21	1.65	16.00	32.70
	Wk 2	22.87	22.75	5.82	1.84	15.00	33.50
	Wk 8	21.14	20.85	5.81	1.84	14.70	29.90
	Wk 10	23.29	23.00	6.40	2.02	15.40	35.80

Figure 3.46 and table 3.98 illustrate that GGT levels did not change significantly throughout the time period examined in either yard ($p > 0.05$). Furthermore, GGT levels did not significantly vary between horses in yard one and yard two at any time of sampling ($p > 0.05$).

Table 3.98 GGT – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	7.05	3	0.068
<i>Yard Two</i>	1.848	3	0.627
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	19.0	0.064
	2	36.0	0.762
	8	35.0	0.680
	10	37.0	0.829

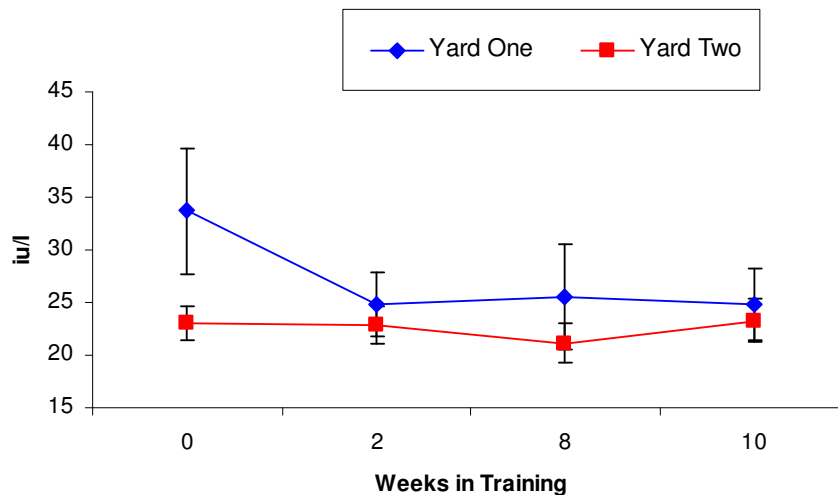


Figure 3.46 Mean GGT levels throughout training period ($p>0.05$)

Total Bilirubin

As shown in table 3.99, maximum values for total bilirubin of horses in yard one lay more than four times above the normal range at all sampling times with minimum values lying below the normal range, also at all times of sampling. Whereas the mean values in yard one lay mainly above the normal range, the median values lay inside the normal range at all sampling times. Also, minimum and maximum values outside the normal range were observed in yard two, although not to the same extent ($\mu\text{mol/l}$).

Table 3.99 Descriptive Statistics – Total Bilirubin

		<i>Mean</i>	<i>Median</i>	<i>S.D.</i>	<i>S.E.</i>	<i>Min</i>	<i>Max</i>
Yard One	Before Training	71.96	49.30	60.70	21.46	24.10	205.90
	Wk 2	44.61	33.65	30.45	10.77	25.20	118.00
	Wk 8	56.01	46.45	39.08	13.82	33.00	151.30
	Wk 10	53.91	39.50	43.50	15.38	31.90	160.80
Yard Two	Before Training	39.46	34.80	8.29	2.62	33.00	58.30
	Wk 2	38.23	35.35	7.49	2.37	30.40	55.30
	Wk 8	42.27	40.35	10.13	3.20	28.90	58.00
	Wk 10	46.64	46.15	11.89	3.76	33.60	68.10

Total bilirubin levels of horses in yard one did not change significantly throughout the training period examined ($p > 0.05$). Total bilirubin levels in yard two, however, were found to increase significantly as the duration of training increased ($p < 0.05$). This finding can be observed in figure 3.47 and table 3.100. Table 3.101 further demonstrates the change in total bilirubin levels as observed in yard two.

Table 3.100 Total Bilirubin – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	4.05	3	0.278
<i>Yard Two</i>	8.76	3	0.030
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	28.0	0.304
	2	34.0	0.633
	8	34.0	0.633
	10	30.0	0.395

Table 3.101 Total Bilirubin change throughout training period by weeks

Wilcoxon Signed Rank	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard Two</i>	Wk 0 – Wk 10	2.50	0.008
	Wk 2 – Wk 10	3.50	0.012

Total bilirubin levels did not significantly vary between horses in yard one and yard two at any time of sampling ($p>0.05$).

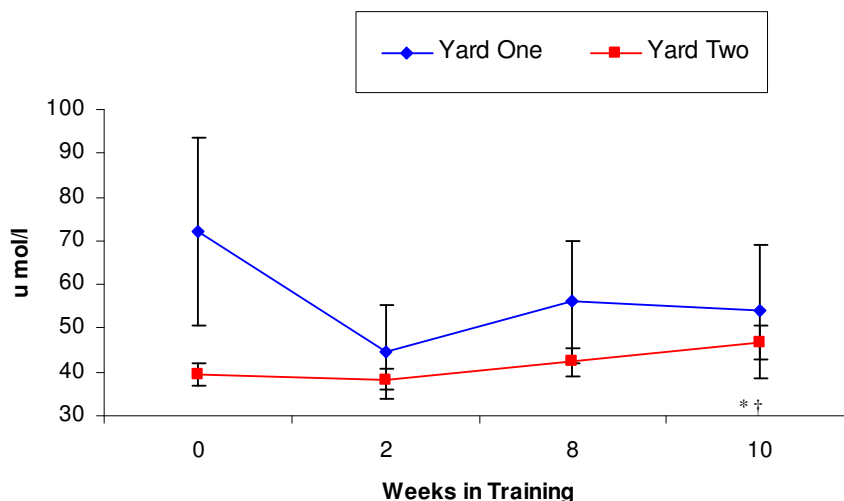


Figure 3.47 Mean total bilirubin levels throughout training (Y1: $p>0.05$; Y2: $p<0.05$)

*Sig. different from pre-training values ($p<0.05$) †Sig. different from wk 2 values ($p<0.05$)

Sodium (Na)

Table 3.102 below shows that sodium levels of horses in both yards appeared to have been elevated throughout the time period examined. Both mean and median values were found to lie above the normal range after two and eight weeks of training in yard one and two as well as after ten weeks of training in yard two. This was reflected in elevated minimum and maximum values at these times of sampling (mmol/l).

Table 3.102 Descriptive Statistics – Sodium

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	141.63	141.50	1.06	0.38	140.00	143.00
	Wk 2	144.50	144.00	1.07	0.38	144.00	147.00
	Wk 8	144.38	144.50	1.41	0.50	143.00	147.00
	Wk 10	142.75	143.00	0.71	0.25	142.00	144.00
Yard Two	Before Training	143.30	143.00	1.95	0.62	141.00	148.00
	Wk 2	144.70	144.00	1.42	0.45	143.00	147.00
	Wk 8	144.70	145.00	0.67	0.21	144.00	146.00
	Wk 10	147.10	147.50	2.73	0.86	143.00	151.00

According to statistical analysis sodium levels, changed significantly in both yards throughout the ten week training period examined (Yard One: $p < 0.001$; Yard Two: $p < 0.01$). Figure 3.48 and table 3.104 show that sodium levels of horses in yard one first increased after two and eight weeks of training, while decreasing again between week two and ten of training ($p < 0.05$). Plasma sodium levels of horses in yard two had significantly increased throughout the training period examined ($p < 0.05$).

Table 3.103 Sodium – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	16.986	3	0.000
<i>Yard Two</i>	11.677	3	0.005
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	16.5	0.032
	2	39.5	0.999
	8	31.5	0.449
	10	3.5	0.000

Table 3.104 Sodium change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 2	0	0.008
	Wk 0 – Wk 8	0	0.016
	Wk 2 – Wk 10	0	0.008
<i>Yard Two</i>	Wk 0 – Wk 2	3	0.016
	Wk 0 – Wk 8	5	0.035
	Wk 0 – Wk 10	1.5	0.012
	Wk 2 – Wk 10	2.5	0.031
	Wk 8 – Wk 10	6	0.031

As indicated by table 3.103 and figure 3.48 sodium levels differed significantly between yard one (Mdn=141.50) and yard two (Mdn=143.00) prior to the commencement of training ($p < 0.05$) as well as after ten weeks of training (Yard One: Mdn=143.00; Yard Two: Mdn=147.50; $p < 0.001$). After two and eight weeks of

training respectively, no significant differences between plasma sodium levels of horses in either yard were observed ($p>0.05$).

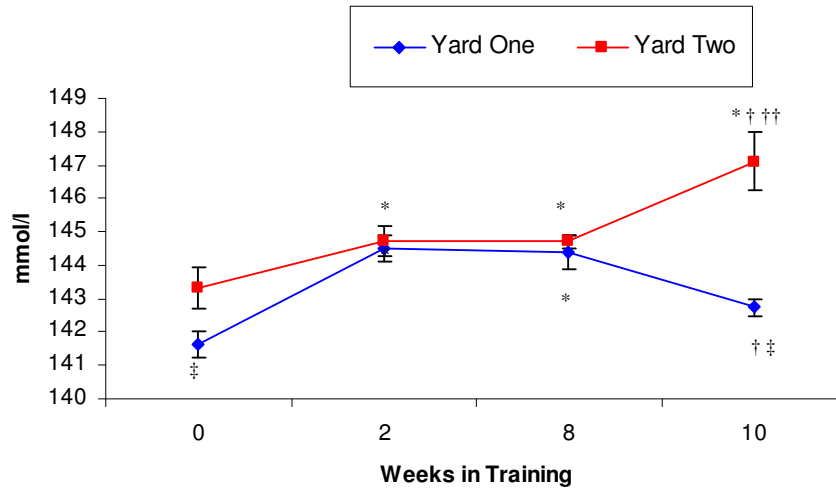


Figure 3.48 Mean sodium levels throughout training period ($p<0.01$)

*Sig. different from pre-training values ($p<0.053$) †Sig. different from wk 2 values ($p<0.05$)
 ††Sig. different from wk 8 values ($p<0.05$) ‡Yard one sig. different from yard two ($p<0.05$)

Potassium (K)

Potassium values slightly above the normal range were observed at all times of sampling in both yards except after ten weeks of training in yard one (table 3.105). Minimum, mean and median values lay inside the normal range at all sampling times in yard one. Minimum levels below the normal range were observed in yard two at all sampling times except after eight weeks of training (mmol/l).

Table 3.105 Descriptive Statistics – Potassium

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	3.97	4.03	0.28	0.10	3.33	4.26
	Wk 2	3.58	3.54	0.38	0.13	3.25	4.38
	Wk 8	3.68	3.63	0.34	0.12	3.22	4.37
	Wk 10	3.60	3.52	0.23	0.08	3.28	3.95
Yard Two	Before Training	3.55	3.57	0.52	0.16	2.52	4.31
	Wk 2	3.65	3.46	0.59	0.19	2.84	4.85
	Wk 8	3.84	3.85	0.24	0.08	3.50	4.33
	Wk 10	3.86	4.02	0.49	0.15	2.69	4.46

Potassium levels in yard two did not change significantly throughout the training period examined ($p>0.05$). In contrast to this finding, plasma potassium levels of horses in yard one were found to have changed significantly throughout the ten week training period ($p<0.05$). Refer to table 3.106 and figure 3.49 for this finding. Plasma potassium levels of horses in yard one were significantly lower at sampling time in week two and ten than at sampling time prior to the commencement of training. This is also shown in table 3.107.

Table 3.106 Potassium – Change throughout training period and differences between yard one and yard two

<i>Friedman's ANOVA</i>	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	9.15	3	0.022
<i>Yard Two</i>	3.48	3	0.327
<i>Mann-Whitney U-Test</i>	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	19.0	0.065
	2	39.5	0.984
	8	23.5	0.151
	10	17.0	0.043

Table 3.107 Potassium change throughout training period by weeks

<i>Wilcoxon Signed Rank</i>	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard One</i>	Wk 0 – Wk 2	3	0.039
	Wk 0 – Wk 10	0	0.008

Potassium levels were significantly higher in yard two (Mdn=4.02) than in yard one (Mdn=3.52) after ten weeks of training ($p<0.05$), whereas no significant differences between the yards were observed at all other times of sampling ($p>0.05$).

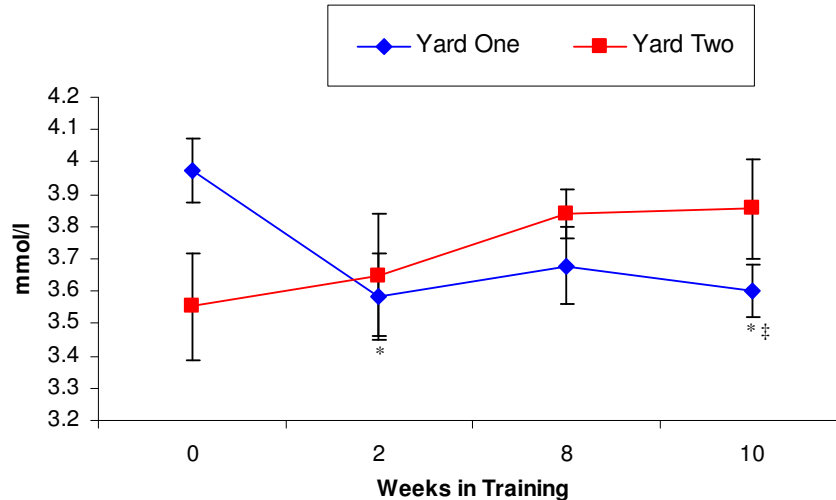


Figure 3.49 Mean potassium levels throughout training period (Y1: $p<0.05$; Y2: $p>0.05$)

*Sig. different from pre-training values ($p<0.05$) ‡Yard one sig. different from yard two ($p<0.05$)

Chloride (Cl)

As can be taken from table 3.108 chloride values remained within the normal range at all times of sampling in both yards except maximum values after ten weeks of training in yard two (mmol/l).

Table 3.108 Descriptive Statistics – Chloride

		Mean	Median	S.D.	S.E.	Min	Max
Yard One	Before Training	98.88	98.00	2.03	0.72	97.00	102.00
	Wk 2	98.63	98.50	1.51	0.53	96.00	101.00
	Wk 8	98.75	98.50	1.49	0.53	97.00	101.00
	Wk 10	98.75	98.50	1.04	0.37	98.00	101.00
Yard Two	Before Training	97.50	98.00	1.35	0.43	95.00	100.00
	Wk 2	99.60	99.50	1.17	0.37	98.00	101.00
	Wk 8	101.10	101.00	1.10	0.35	100.00	103.00
	Wk 10	102.20	102.00	2.10	0.66	99.00	105.00

As tables 3.109 and 3.110 as well as figure 3.50 show, plasma chloride levels of horses in yard one did not significantly change during training ($p>0.05$), while levels of horses in yard two significantly increased throughout the time period examined ($p<0.001$).

Table 3.109 Chloride – Change throughout training period and differences between yard one and yard two

Friedman's ANOVA	χ^2	<i>df</i>	<i>p</i>
<i>Yard One</i>	0.043	3	0.999
<i>Yard Two</i>	23.905	3	0.000
Mann-Whitney U-Test	<i>Week</i>	<i>U</i>	<i>p</i>
<i>Yard One vs. Yard Two</i>	Pre-training	25.5	0.190
	2	24.5	0.177
	8	8.5	0.003
	10	4.5	0.001

Table 3.110 Chloride change throughout training period by weeks

Wilcoxon Signed Rank	<i>Comparison</i>	<i>T</i>	<i>p</i>
<i>Yard Two</i>	Wk 0 – Wk 2	0	0.008
	Wk 0 – Wk 8	0	0.002
	Wk 0 – Wk 10	0	0.002
	Wk 2 – Wk 8	2	0.031
	Wk 2 – Wk 10	0	0.002

As also illustrated in figure 3.50 and table 3.109, plasma chloride levels were significantly higher in yard two (Mdn=101.00) than in yard one (Mdn=98.50) after eight weeks of training ($p < 0.01$) and also after ten weeks of training (Yard One: Mdn=98.50; Yard Two: Mdn=102.00; $p < 0.01$). However, prior to the start of training and after two weeks of training, no differences between the yards were observed ($p > 0.05$).

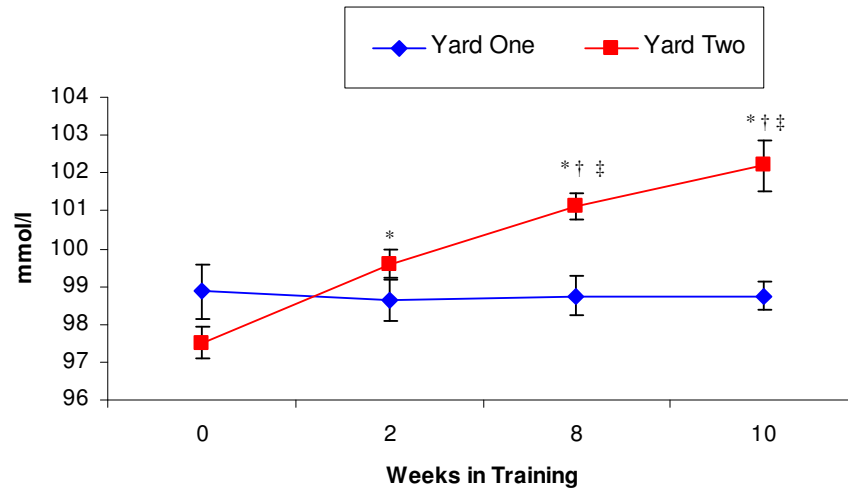


Figure 3.48 Mean chloride levels throughout training (Y1: $p > 0.05$; Y2: $p < 0.001$)
*Sig. different from pre-training values ($p < 0.05$) †Sig. different from wk 2 values ($p < 0.05$) ‡Yard one sig. different from yard two ($p < 0.05$)

Chapter Four

Discussion

Introduction

The increased susceptibility of racehorses in training to respiratory infections has been widely recognised within the industry (Morley *et al.*, 2000a; Wood *et al.*, 2005). This is particularly significant as poor racing performance has been linked to respiratory conditions (Morris and Seeherman, 1991; Bourke, 1995; Knight and Evans, 2008). Aspects of both the non-specific and the specific immune systems may be affected by both exercise and training (Nieman and Pedersen, 1999; Mackinnon, 2000; Hines *et al.*, 2008). A connection between exercise, disease and in vitro measurements of immune performance has been suggested (Nieman, 1995; Folsom *et al.*, 2001). Several studies have examined both innate and adaptive aspects of the immune system with respect to acute exercise and training in both humans and horses. Reviews to date have summarised that aspects of the immune system as well as plasma haematology and biochemistry which have been shown to change as a response to acute and chronic exercise training. Mechanisms underlying these changes are complex and multifactorial, but have been linked to changes in the endocrine system as a response to the stress imposed on the body by exercise (Nieman, 1997; Nieman and Pedersen, 1999; Mackinnon, 1999; Nieman, 2000; Gleeson, 2007; Hines *et al.*, 2008). However, to the knowledge of the researcher no study published to date has taken immunological parameters of the innate and specific immune system as well as haematological and biochemical parameters into consideration. Thus, the current study presents new results on the effects of introduction to training on immunological parameters in the racehorse, which is considered of particular importance due to the importance of respiratory infections in racehorses in training.

Biological rhythms can influence both exercise tolerance and immune function mediated by a variety of endogenous and exogenous factors, which are subject to diurnal variation (Thuma *et al.*, 1995). For this reason, sampling time was kept consistent throughout this study and could thus be excluded as a possible reason for the any reported variations in the functioning of the immune system. To allow for better evaluation of the clinical relevance of the status of the immune system as well as plasma haematology and biochemistry, further research including close monitoring

of the health of the horses in the research study for clinical signs of illness may be required.

Effect of Training on Lymphocyte Function

Lymphocytes become activated to enter the cell cycle and proliferate as a response to exposure to antigen or other activation mechanisms (Mackinnon, 2000). The stimulation of lymphocytes using mitogens in vitro is thought to mimic events which occur after antigen stimulation of lymphocytes in vivo (Nehlsen-Cannarella *et al.*, 1991). Hence, the ability of lymphocytes to proliferate in response to stimulation with a mitogen in vitro has been used to provide information about the functioning of the adaptive immune response (Gogal *et al.*, 1997 and Ansar Ahmed *et al.*, 1994).

Limited data have been reported on the effect of chronic exercise training on lymphocyte proliferation as a measure of the functioning of the adaptive immune system in the equine athlete (Buschmann and Baumann, 1991; Malinowski *et al.*, 2004). Furthermore, to the knowledge of the researcher no studies to date have examined the effect of training on lymphocyte proliferation in racehorses in training. To investigate the status of the adaptive immune system of racehorses upon introduction to training, lymphocyte proliferative response was assessed in this study using the mitogen Con A to cause activation of the immune cell signalling pathways in the absence of pathogen-derived antigens that can be employed to assess immunocompetence (Folsom *et al.*, 2001). In this study, chronic exercise training of the horses had no effect on average proliferative response of T-lymphocytes to stimulation with the mitogen Con A. Lymphocyte proliferation against the mitogen Con A at the individual concentrations of 10 µg/ml in both yards and 5 µg/ml in yard one remained unchanged throughout the ten week training period examined, while stimulation of T-lymphocytes with 5 µg/ml Con A demonstrated a significant decrease in lymphoproliferative response of horses in yard two after two, eight and ten weeks of training; however, levels did not fluctuate significantly between week two and ten of training. Similarly, elevated levels of T-lymphocyte proliferation in response to stimulation with 2.5 µg/ml of Con A at pre-training were observed in yard two, while levels did not significantly change between weeks two and ten of training. Since the dose response rate to using 1.25 and 0.625 µg/ml Con A lead to the occurrence of negative minimum values, analysis of these results will not be

considered at this stage. As the significant effect observed was confined to one yard and overall lymphocyte proliferation remained unchanged during the training period examined, it was concluded that chronic exercise training did not affect this aspect of the adaptive immune system. This study differed from other studies in the type of horse used as well as the type of exercise challenge, intensity and duration (Buschmann and Baumann, 1991; Malinowski *et al.*, 2004). Furthermore, a non-radioactive assay was used in this study (Ansar Ahmed *et al.*, 1994; Nakayama *et al.*, 1997; Saker *et al.*, 2001; Witonsky *et al.*, 2003) to assess lymphocyte proliferation as opposed to assays incorporating radiolabelled biochemicals such as [³H]thymidine, which have been traditionally employed (Buschmann and Baumann, 1991; Nesse *et al.*, 2002; Malinowski *et al.*, 2004). However, as research findings from this study agreed with findings from Buschmann and Baumann (1991) and Malinowski *et al.* (2004) in that proliferative response of T-lymphocytes remained unchanged in horses during training, it appears that the response of this aspect of the adaptive immune system responds more uniformly in horses to chronic exercise stress than has been implied in previous studies in human subjects. In humans, studies have suggested that the response of the adaptive immune system to chronic exercise is quite inconsistent such that lymphocyte proliferation has been found to decrease (Papa *et al.*, 1989), increase (Verde *et al.*, 1992; Nieman *et al.*, 2000) or remain unchanged (Nehlsen-Cannarella *et al.*, 1991) with training.

Although the significance of changes in lymphocyte activation and proliferation are not clear at present (Mackinnon *et al.*, 2000), it is interesting to note that research has shown that lymphoproliferative response in horses following acute exercise was found to be transiently suppressed in several studies (Kurcz *et al.*, 1988; Keadle *et al.*, 1993; Horohov *et al.*, 1999; Lunn *et al.*, 2001; Nesse *et al.*, 2002; Folsom *et al.*, 2001). Only one study by Wong *et al.* (1992) observed no changes in lymphoproliferative response after acute exercise. Taking research findings from this study into consideration, it has therefore been indicated that acute exercise leads to a transient reduction in lymphoproliferative response, whereas chronic exercise training does not appear to significantly affect lymphocyte proliferation in horses. However, further research is required to assess the effect of training on the immune response to acute exercise. Although this research study could not examine the mechanisms underlying the effect of exercise on immunity, other studies largely attributed the effect of

transient suppression of the adaptive immune system following exercise to increases in circulating corticosteroids following acute exercise stress (Kurcz *et al.*, 1988; Wong *et al.*, 1992; Keadle *et al.*, 1993; Horohov *et al.*, 1999). As lymphocyte function is essential to initiate effective responses of the adaptive immune system to pathogens, a potential decrease in this capacity might expose the individual to an increased risk of respiratory infection for a period following acute exercise rather than due to the effect of chronic exercise as indicated by findings of this research study. On this note, it appears interesting that exercise-induced changes in lymphoproliferative responses were reported to be more pronounced in younger horses than in older horses (Horohov *et al.*, 1999), which might also explain the increased incidence of upper respiratory horses in young racehorses upon introduction to training.

Effect of Training on Leukocyte Function – Phagocytosis

Granulocytes

This research study observed significant changes in phagocytic activity of granulocytes an aspect of innate immunity during training ($p < 0.01$). Whereas an increase of the percentage of phagocytosing granulocytes after eight weeks of training was observed in yard one, a decrease in the percentage of phagocytosing granulocytes was reported after eight weeks of training in yard two. It should be noted that after ten weeks of training pre-training values were regained in both yards. Due to the contradictory developments after eight weeks of training in each yard, a significant difference between the percentages of phagocytosing granulocytes was observed at that stage. This finding initially indicated that external factors other than training led to the results obtained after eight weeks of training, leading to the conclusion that chronic exercise itself did not affect phagocytic activity of granulocytes.

Only a few research studies to date have examined the effect of training on granulocyte function in the horse (Buschmann und Baumann, 1991; Raidal *et al.*, 2000a; Raidal *et al.*, 2001; Escribano *et al.*, 2002; Escribano *et al.*, 2005a). The present study differed from these in the type of horses used as well as in training duration, exercise mode and intensity and, further, in the research design employed. Findings from this research study agreed with findings by Escribano *et al.* (2002,

2005a) as well as with findings examining the effect of exercise on granulocyte function in human athletes (Hack *et al.*, 1992; Nieman *et al.*, 2000; Wolach *et al.*, 2000) in that no significant differences in granulocyte response directly linked to training or in athletes compared with non-athletes were reported. However, several other studies on equine or human athletes observed significant reductions in granulocyte activity during high-intensity training periods (Buschmann and Baumann, 1991; Hack *et al.*, 1994; Yamamoto *et al.*, 2008), indicating that training intensity does affect granulocyte function. Also, Raidal *et al.* (2001) found that phagocytic activity decreased only during a period of overtraining. Results on plasma haematology, which indicated that horses in yard two showed signs typical of horses undergoing more intensive training, the reduction in phagocytic activity of granulocytes observed in yard two might, thus, be explained. Differing findings observed in the present study and differing observations described in other studies might hence relate back to different training intensities or duration. The present study observed the effect of introduction to training and observations ceased after the initial ten week period; changes observed in phagocytic activity of granulocytes might be attributed to the fact that training intensity was more moderate during the training period examined due to the progressive nature of training at least in yard one.

Several mechanisms have been associated with changes in phagocytic activity during training and after exercise. Exercise has been widely recognised as a stress, which may affect the immune system and, thus, an individual's susceptibility to disease (Pedersen and Hoffman-Goetz, 2000; Raidal *et al.*, 2000a; Hines, *et al.*, 2008). Stress can lead to a number of physiological changes taking place in the endocrine system of the human or equine athlete. These changes have been thought to affect the immune system of the human and horse. Increased plasma epinephrine and norepinephrine levels at rest during intensive training periods were associated with decreased in phagocytic activity in one study (Hack *et al.*, 1994), while Raidal *et al.* (2000a) linked increases in circulating cortisol following acute exercise to decreased phagocytic activity of granulocytes. The latter could not be confirmed in a later study by Raidal *et al.* (2001), where changes in phagocytic activity could not be associated with changes in basal cortisol or β -endorphin levels in the horses examined. Also, enhanced neutrophil function during moderate exercise has been attributed to increases in growth hormone, and amongst others possibly prolactin (Raidal *et al.*,

2000a). The contradicting findings reported in this study after eight and ten weeks of training could possibly be attributed to the above underlying mechanisms with varying effects on granulocyte function.

Findings indicate that high-intensity exercise might have suppressive effects on the immune system of the human or equine athlete. A weakness in the first-line of defence against invading pathogens as indicated by reduced phagocytic activity at rest during high-intensity training periods or after high-intensity exercise might represent the link between exercise and an increased risk of infection in athletes, although athletes are not clinically immune deficient (Gleeson, 2007). Hack *et al.* (1994) further described that even a transient suppression of the innate immune system, as was described in horses of yard two after eight weeks of training, might present an opportunity for microorganisms to evade early immunologic recognition and thus might establish infections in the athlete. As previously stated, results from this study did not indicate changes in phagocytic activity of granulocytes occurred solely as a response to introduction to training, but rather as a response to external factors such as the length of the training period examined, training intensity as well as differing exercise and training regimes and management. Contradictory results between the two yards observed in the present study appear to relate back to differences in training intensity as also suggested by observations relating to the effect of training on plasma haematology in the present study.

Monocytes

Most research studies have focused on the effect of acute and chronic exercise on phagocytic activity of granulocytes in equine and human subjects. It is thought that monocytes respond similarly to neutrophils in terms of the phagocytic process (Robson *et al.*, 2003); however, no research has been published to date to examine the effect of chronic exercise on monocyte function in horses. As monocytes play an important role within the first line of defence against invading pathogens, this study sought to assess the effect of training on phagocytic activity of monocytes.

This research study established that monocyte phagocytosis follows similar trends as granulocyte phagocytic activity ($p < 0.01$). While the percentage of phagocytosing

monocytes increased after eight weeks of training in yard one, the percentage of phagocytosing monocytes in yard two had decreased after eight weeks of training. Phagocytic activity of monocytes had returned to pre-training values after ten weeks of training in both yards. These contradictory findings indicate the effect of external factors other than training on phagocytic activity of monocytes after eight weeks of training considering that pre-training values had been regained at the time of sampling after ten weeks of training. Nonetheless, observations on the effect of training on plasma haematology in this study suggested that training intensity was higher in horses from yard two and could, thus, have contributed to the reported reduction in monocyte phagocytosis in horses from yard two after eight weeks of training.

However, enhanced neutrophil function during moderate exercise had been attributed to increases in growth hormone, and possibly prolactin and substance P (Raidal *et al.*, 2000a), while reductions in phagocytosis of bacteria and oxidative cell functions were attributed to increased levels of circulating cortisol, released in response to increased adrenocorticotrophic hormone (ACTH) following acute exercise. These mechanisms underlying changes in the innate immune system following exercise could also explain the differing findings reported in this study throughout the training period examined. Generally these changes have been more closely linked to the effects of acute exercise on immunity. Research as presented in the literature review has shown that intensive exercise can lead to a longer lasting impact on immunological parameters than moderate exercise. Hence, repetitive bouts of exercise with increasing intensity throughout a training period could have caused a long-lasting effect on immunological parameters linked to the last bout of exercise, which affected immune status at time of consecutive blood sampling as described by Mackinnon (2000) and Gleeson (2007). Changes observed in the present study might therefore not represent true resting values, but might rather be linked to recent bouts of acute exercise. This might be linked to the reported decrease in phagocytic activity of monocytes of horses in yard two after eight weeks of training.

In the absence of other studies examining the effect of training on monocyte function in horses, it was observed that studies reviewing the effect of exercise on granulocyte or monocyte function were quite inconsistent in that it was either suppressed or remained unchanged in response to acute exercise (Pedersen and Hoffman-Goetz,

2000), or was enhanced following exercise (Nieman, 1997; Nieman, 2000; Woods *et al.*, 2000). Furthermore, Raidal *et al.* (2000b) indicated that training lead to decreased phagocytic activity of pulmonary alveolar macrophages in horses following acute exercise when compared to pre-exercise values. These findings, in conjunction with results from the present study relating to the functioning of the adaptive and innate immune system, indicate that the immune system is transiently suppressed following exercise rather than as a result of training. Further research to examine the effect chronic exercise might have on the response of the innate immune system to acute exercise is required to make definitive conclusions.

Effect of Training on Leukocyte Function – Oxidative Burst Capacity

Granulocytes

This study observed a marked tendency for granulocyte oxidative burst capacity to increase in horses in both yards throughout training (Y1: $p < 0.05$); Y2: $p < 0.01$). However, due to laboratory difficulties only certain weeks and horses from yard one could be compared to analyse the effect of training on oxidative burst capacity. Values after two, eight and ten weeks of training were significantly higher than prior to the commencement of training. Oxidative burst capacity of granulocytes in response to stimulation with bacteria of horses in yard two had significantly increased after eight weeks of training, however, after ten weeks of training levels showed a decrease in oxidative burst capacity of granulocytes compared to levels after eight weeks of training. The effect was even more pronounced in oxidative burst capacity of granulocytes in response to stimulation with PMA as a positive control ($p < 0.001$) in that a significant increase in oxidative burst capacity was observed after eight and ten weeks of training compared to pre-training levels. Overall, a tendency towards enhanced oxidative burst capacity after ten weeks of training was noted.

Other research studies in horses have produced similar results such that enhanced oxidative burst capacity appeared to have been associated with the effect of training on the immune system (Escribano *et al.*, 2002; Escribano *et al.*, 2005a; Raidal *et al.*, 2001). Hack *et al.* (1994) found that resting levels in athletes undergoing intensive training were lower than in athletes in moderate training but not lower than in non-athletes, indicating that moderate exercise might be linked to elevated levels of

oxidative burst capacity, as observed in the present study. In contrast other studies reported decreased levels of oxidative burst capacity after endurance training (Raidal *et al.*, 2000a) and throughout a period of overtraining (Raidal *et al.*, 2001). Also, several studies found no difference between resting superoxide anion production in human athletes and non-athletes (Hack *et al.*, 1992; Nieman *et al.*, 2000; Wolach *et al.*, 2000). These initially contrasting findings indicate a correlation between exercise intensity and duration and oxidative burst capacity of granulocytes. Taking previous research findings into consideration, the present study showed that oxidative burst capacity of granulocytes shows a marked increase upon introduction of thoroughbreds to race training. This finding, however, appeared to be strongly related to training intensity and duration. As the described increase was only apparent when considering oxidative burst capacity rather than phagocytosis, it appears reasonable to suggest that oxidative burst capacity is more sensitive to the effects of chronic exercise than phagocytic activity.

Oxidative burst capacity of leukocytes is part of the first line of defence of the immune system and, as such, is important in establishing the immune status of the equine or human athlete. Alterations in oxidative metabolism were likely to have been mediated by training induced alterations in the endocrine response to exercise (Hack *et al.*, 1994; Raidal *et al.*, 2000a). Although these alterations have been more closely linked to the effects of acute exercise on immunity, repetitive bouts of increasing exercise throughout the training period examined could have caused a long-lasting stimulation linked to the last bout of exercise, which affected immune status at time of consecutive blood sampling as described by Mackinnon (2000) and Gleeson (2007).

Monocytes

Monocyte oxidative burst capacity showed a strong trend towards increasing levels throughout the training period examined in both yards ($p < 0.01$). In horses of yard one, an increase of monocyte oxidative burst activity during training was reported. Similar to the non-significant increase in overall granulocyte oxidative burst capacity, non-significant findings in spite of marked apparent increases in oxidative burst capacity as reported when analysing all weeks at once can, in this case, be linked to large inter-individual variability obtained in this study, from which a large S.E.

resulted (Escribano *et al.*, 2002). Wilcoxon signed-rank analysis of oxidative burst capacity in yard one showed significant increases after two weeks of training with respect to pre-training levels. Similar to the overall oxidative burst capacity of monocytes in response to stimulation with bacteria in horses from yard one, a trend towards increasing levels of monocyte oxidative burst capacity over the training period in yard two became apparent. Results after stimulation of monocytes with PMA as a positive control confirmed these findings.

Limited research on the effects of acute and chronic exercise on monocyte oxidative burst activity has been published to date (Nieman *et al.*, 2000; Raidal *et al.*, 2000b, Raidal *et al.*, 2001, Robson *et al.*, 2003), but it is thought that monocyte function responds similarly to granulocyte function in terms of phagocytic killing (Robson *et al.*, 2003), as observed in this research study. Findings from this research study were consistent with reports by Raidal *et al.* (2001) in that oxidative burst activity of pulmonary alveolar macrophages increased during a 16 week training programme in horses. Overtraining, on the other hand, caused an initial decrease in monocyte function, which supports the suggestion of training intensity and duration affecting the nature of the immune response. This could explain the results by Nieman *et al.* (2000) and Raidal *et al.* (2000b), which contrasted with findings reported in this study, reporting unchanged monocyte and pulmonary alveolar macrophage oxidative burst capacity in human athletes and during training in horses. Transient reductions in monocyte and pulmonary alveolar macrophage oxidative burst capacity were only noticed as a response to acute exercise (Robson *et al.*, 2003). Robson *et al.* (2003) described changes in stress hormones, cytokines and haematological factors as possible mechanisms underlying the effect of exercise on oxidative burst capacity of granulocytes and monocytes. Due to the important role of oxidative burst capacity in the first line of defence against invading pathogens, the apparent transient suppression of innate immunity reportedly linked to acute exercise could expose the athletic horse (or athlete) to an increased risk of contracting common infections such as upper respiratory infection or influenza following acute exercise. On the other hand, this study showed a marked trend of an enhancement in granulocyte and monocyte oxidative burst capacity at rest as a response to chronic exercise training. Further research could clarify whether chronic exercise training influences the effect of acute

exercise on innate immunity in a similar way to find a mechanism to explain the increased incidence of upper respiratory infection of racehorses in training.

Effect of Training on Plasma Haematology

Total Erythrocytes

Descriptive statistics indicated that total erythrocyte levels outside the normal range (6.53 – 11.20 x 10¹²/l) were observed, however, this was not likely to have been of clinical relevance, as Hodgson (1994) described values as high as 11.6 x 10¹²/l in different populations of thoroughbreds and minimum values only lay marginally below the normal range as described by Hodgson (1994). Also, Rose and Allen (1985) stated that higher normal haematological resting values were found in younger horses upon introduction to training, as applies to this research study.

Similar to research findings reported by Rose and Hodgson (1982), no significant changes in total erythrocyte counts of horses were observed during the ten week training period examined in this study ($p>0.05$). On the other hand, a study by Tyler-McGowan *et al.* (1999) detected significant increases in total erythrocyte levels during a 34 week training period ($p<0.05$). It is interesting to note, that a non-significant tendency towards increasing total erythrocyte counts at rest was observed throughout the ten week period examined. These different findings probably arose due to differences in study design, in this case, in particular the duration of training. An increase in erythrocyte numbers during a training programme in horses has been associated with an improved nutrition upon introduction to training and an increase in sympathetic tone with training stimulating erythropoiesis (Tyler-McGowan *et al.*, 1999). However, as previously specified, changes in total erythrocyte levels have generally been associated with acute exercise (Hodgson, 1994; Krumrych, 2006), due to the fact that the erythrocyte pool in the horse has been linked directly to variations in catecholamine concentrations, which, in turn, have been strongly associated with acute exercise stress (Hodgson, 1994). On the other hand, the non-significant increase observed in this study might imply that changes in total erythrocytes are not solely linked to the effect of acute exercise, but also to non-exercise factors such as nutrition (Tyler-McGowan *et al.*, 1999) as well as the previously described ‘overreaching’ effect of acute exercise bouts (Mackinnon, 1999; Gleeson, 2007).

Packed Cell Volume (PCV)

Values slightly below and above the normal range (32.0 – 46.0 %) as described by Hodgson (1994) were observed throughout the training period examined. However, mean and median of PCV levels at sampling time lay within the normal range. These findings were not likely of clinical relevance in the absence of clinical signs.

In line with findings by Rose and Hodgson (1982), who investigated PCV at rest in endurance horses during a twelve week training programme, this study reported no significant alterations in PCV throughout the training period examined ($p>0.05$). Furthermore, a cross-sectional study in humans reported no difference between elite athletes and untrained control subjects ($p>0.05$) (Nieman *et al.*, 2000). Similar to total erythrocyte levels, however, a non-significant increase in PCV at rest could be seen throughout training in this study. A marked increase in PCV with training was further observed by Tyler-McGowan *et al.* (1999) ($p<0.05$). However, research as described in the literature review indicates that alterations in PCV have been connected with changes in the endocrine system following acute exercise (Hodgson, 1994; Krumrych, 2006) rather than chronic exercise training (Robson *et al.*, 2003; Krumrych, 2006; Piccione *et al.*, 2007). The observed increase of PCV at rest during training could have been caused by the ‘overreaching’ effect of acute exercise, associated with increasing intensity of single exercise bouts, lasting until consecutive blood sampling (Mackinnon, 2000; Gleeson, 2007).

Haemoglobin

Descriptive statistics reported single cases of minimum and maximum values (10.90 – 17.80 g/dl) of plasma haemoglobin below and above the normal range (11.0 – 17.0 g/dl) as described by Hodgson (1994) in yard one after eight weeks of training. Due to values only varying slightly from normal values, this was not regarded as clinically relevant.

The present research study found that haemoglobin levels fluctuated during the training period examined. Haemoglobin levels were significantly elevated after ten weeks of training when compared to pre-training values ($p<0.05$). Research findings thus agreed with results from Tyler-McGowan *et al.* (1999), which found that

haemoglobin increased in line with other red blood cell indices during training. Findings from the present study contrasted with results from other studies, which reported no differences between haemoglobin levels in elite human athletes and untrained control subjects (Nieman *et al.*, 2000) and that of Hodgson and Rose (1982) which observed unchanged haemoglobin levels of horses throughout training. At the same time, training intensity was not reported to affect haemoglobin, or any of the previously described red blood cell indices in studies by Tyler-McGowan *et al.* (1999) and Padalino *et al.* (2007). Only total erythrocytes were found to have been higher in hypothetically overtrained horses than in normally trained ones (Padalino *et al.*, 2007). It should be noted that the reported elevation in haemoglobin concentrations during training was in line with non-significant increases in other red blood cell indices during training described beforehand. Notwithstanding, as previously indicated, changes in red blood cell indices, such as haemoglobin levels, were more commonly associated with acute exercise rather than with the effects of long-term training (Krumrych, 2006; Piccione *et al.*, 2007). Changes in red blood cell parameters as observed in the present study might hence be linked to the previously described ‘overreaching’ effect of acute exercise bouts on blood samples taken at rest during long-term exercise training.

Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH)

Minimum and maximum MCV values (41.1 – 48.4 fl) were observed which were outside the normal range (42.0 – 47.0 fl) described by Hodgson (1994). However, means and medians observed in this study were very similar to the normal mean suggested by Hodgson (1994), indicating large individual variation of MCV levels within the sample population. All MCHC values reported in this study fell within the normal range reported by Hodgson (1994). MCH values just marginally above the normal range (>17 pg) were observed, but these were so close to normal values that no clinical significance was attributed to these levels.

It was reported that few changes in erythrocytes themselves appear to take place during chronic exercise (Hodgson, 1994). This study reported significantly higher levels of MCHC and MCH of horses in both yards as training progressed compared to

levels in the first weeks of training ($p < 0.001$), whereas MCV levels remained unchanged throughout the training period examined ($p > 0.05$). However, as MCHC and MCH levels of horses in both yards appeared to be within the normal range, this trend towards rising MCHC and MCH levels was not considered to be clinically relevant. Padalino *et al.* (2007) suggested MCV and MCH as possible markers for overtraining due to having observed lower levels in conjunction with higher average total erythrocyte distribution weight in hypothetically overtrained horses. This was thought to have been a sign of anisocytosis.

Platelets

Very low platelet counts ($32.0 - 89.0 \times 10^9/l$) were observed throughout the time period examined. The normal range described by Hodgson (1994) reported values between 141 and $191 \times 10^9/l$. However, many technical problems have been associated with the work on equine platelets; it is therefore difficult to comment either on platelet counts or on the effect of training on platelets (Kingston *et al.*, 2001).

Platelet counts of horses in yard one fluctuated significantly during the training period examined ($p < 0.05$). Values were significantly lower after two and ten weeks of training than after eight weeks of training; however, figure 3.7 indicated that platelet levels varied throughout the entire training period. On the other hand, post hoc analysis of platelet levels of horses in yard two specified that platelet levels increased following introduction to training ($p > 0.05$). These variations were not likely to have occurred due to the effect of training as significant variations were only reported in one yard. Padalino *et al.* (2007) did not observe any differences between hypothetically overtrained horses and those undergoing less intensive training. This also leads to the conclusion that differences in potential training intensity between yard one and yard two did not induce changing platelet counts during the time period examined.

Total Leukocytes

Throughout the training period examined minimum total leukocytes counts just below the normal range ($6-11 \times 10^9/l$) described by Hodgson (1994) were observed. However, as mean and median values of the sample fell within the normal range,

deviations from the normal range of total leukocyte counts were not considered clinically relevant.

Similar to studies in humans (Nehlsen-Cannarella *et al.*, 1991; Hack *et al.*, 1994; Gleeson *et al.*, 1995; Nieman *et al.*, 2000; Yamamoto *et al.*, 2008) and horses (Tyler-McGowan *et al.*, 1999), equine total leukocyte count in this study did not increase during the ten week training programme ($p>0.05$). In contrast, Rose and Hodgson (1982) reported a slight significant increase in total leukocyte count in endurance horses in week ten and 12 of the training period examined. This was not considered clinically relevant as values fell within the normal range. Research findings by Padalino *et al.* (2007) indicated that a reported increase in total leukocyte count in horses during training might be related to overtraining. Changes in total leukocyte counts appear to be related to acute exercise (Hack *et al.*, 1994; Krumrych, 2006) with transitory leukocytosis following exercise having been associated with an increase in lymphocytes released mostly from the spleen (Krumrych, 2006). It should be taken into account, as also partly seen in this study, that possible changes in differential leukocyte counts might not be reflected in total leukocyte counts. The magnitude of the increase in lymphocytes following exercise was found to be related to exercise intensity (Krumrych, 2006). During long-lasting endurance exercise, the initial increase in lymphocytes was followed by transient lymphopenia and neutrophilia. This increase in the N/L ratio was related to the increase in cortical-adrenal hormones (mainly cortisol) in blood as a response to acute exercise.

Neutrophils

Neutrophil counts below the normal range ($2.5-6.5 \times 10^9/l$) described by Hodgson (1994) were observed in both yards throughout the training period examined. These values might be attributed to individual differences between resting immunological parameters in horses, as neutrophil counts did not significantly change throughout the period examined.

This present finding of unchanged neutrophil counts with training ($p>0.05$) was in accordance with research studies in humans by Hack *et al.* (1992), Hack *et al.* (1994) and Nieman *et al.* (2000) as well as in horses by Rose and Hodgson (1982) and

Padalino *et al.* (2007). However, when considering neutrophil percentage during the training period examined ($p < 0.001$), higher levels were observed in yard two after eight and ten weeks of training than after two weeks of training as well as after eight weeks of training when compared to pre-training levels. Total leukocyte counts did not change significantly as the percentage of lymphocytes in the total leukocyte population decreased, while the percentage of neutrophils increased. Other studies observed increased neutrophil levels with training in both, humans (Yamamoto *et al.*, 2008) and horses (Tyler-McGowan *et al.*, 1999). Changes in neutrophil levels during training reported in other research studies were related to high-intensity training periods, i.e. high-intensity exercise leading to longer-lasting changes in haematological parameters and recurrent exercise bouts before parameters had normalised again, or to muscle damage and inflammation relating to repeated exercise bouts of untrained individuals (Yamamoto *et al.*, 2008). As an increase in neutrophil levels was only reported in one yard in this study, it seems likely that differences in individual training intensity and thereby resulting muscle damage and inflammation in the individual horses might be the reason behind the reported results. Thus, overall results from this study indicate that changes in neutrophil levels do not relate to the effect of training on horses, but mostly to the effect of single or recurrent exercise bouts of varying intensity. This was supported by research studies examining the effect of acute exercise on neutrophil counts. Increases in neutrophils have been reported in several human studies (Hack *et al.*, 1992; Hack *et al.*, 1994; Nieman *et al.*, 1997; Nieman, 2000; Pyne *et al.*, 2000), and in equine research studies (Robson *et al.*, 2003). These changes were largely attributed to increases in plasma cortisol following exercise.

Lymphocytes

As with the other haematological parameters examined, lymphocyte counts below the normal range ($2.0\text{-}5.5 \times 10^9/l$) described by Hodgson (1994) were observed in this study, which, in the absence of clinical symptoms, were attributed to differences between individual horses.

Similar to neutrophil counts, lymphocyte counts in this study did not fluctuate significantly throughout the training period examined ($p > 0.05$). This finding agreed with results of studies in humans (Hack *et al.*, 1994; Gleeson *et al.*, 1995; Nieman *et*

al., 2000) and horses (Rose and Hodgson, 1982; Padalino *et al.*, 2007). Whereas lymphocyte percentage in horses from yard one did not vary throughout training, lymphocyte percentage in yard two peaked after two weeks of training, but had decreased after week eight and ten of training ($p < 0.001$). This finding was supported by a non-significant reduction in lymphocyte counts observed in the present study. A significant decrease in lymphocyte levels during chronic exercise training was also reported in other studies on human athletes (Nehlsen-Cannarella *et al.*, 1991) and horses (Tyler-McGowan *et al.*, 1999). Changes in neutrophil and lymphocyte percentages observed in yard two resembled a 'stress leukogram' associated with neutrophilia, lymphopenia and eosinopenia (Tyler-McGowan *et al.*, 1999). In general, these changes in lymphocyte count or percentage can be attributed to changes in stress hormone and cytokine concentrations, body temperature changes, increases in blood flow, lymphocyte apoptosis and dehydration occurring as a response to acute exercise (Nieman, 1997; Nieman *et al.*, 2000). Result of this study can possibly be associated with the rise of cortisol as a response to high-intensity or long-term submaximal exercise inducing leukocytosis, while inhibiting entry of lymphocytes into the circulation and facilitating the re trafficking of them to peripheral tissues. This would be in contrast to the effects of catecholamines, which have been shown to induce mild leukocytosis and rapid lymphocytosis as a response to acute endurance exercise (Nehlsen-Cannarella *et al.*, 1991). It thus appears likely that horses in yard two were exposed to significantly more stress possibly resulting from different training intensity causing an 'overreaching' effect of acute exercise bouts (Mackinnon, 2000; Gleeson, 2007) as described beforehand.

Monocytes

Monocyte count and percentage were not found to have been significantly affected by training in this study ($p > 0.05$). This is supported by research findings from studies on humans (Nieman *et al.*, 2000) and horses (Tyler-McGowan *et al.*, 1999; Padalino *et al.*, 2007) which also described unchanged monocyte levels with training. However, a non-significant increase in monocyte count and percentage was observed in both yards with training. Similar to this, Tyler-McGowan *et al.* (1999) reported elevated monocyte levels throughout a 34 week training programme. However, as overall results from this study were non-significant and changes not as prominent as those of

other haematological parameters, it appears reasonable to conclude that monocyte levels are not as strongly influenced by training as some other parameters. Moreover, changes in monocyte levels, similar to those of other haematological parameters, have mainly been linked to the effect of acute exercise causing transient increases in monocyte levels in both, humans (Nieman, 1997; Nieman, 2000) and horses (Robson *et al.*, 2003).

Eosinophils

A marked trend in horses in both yards towards a declining eosinophil count and percentage could be observed, however, changes were only significant in one yard (Y1: $p < 0.01$; Y2: $p > 0.05$). Rose and Hodgson (1982) observed a decrease in eosinophil levels during training, which was linked to a parasite burden of the horses at the beginning of the training period examined, whereas Tyler-McGowan *et al.* (1999) observed a decrease in eosinophil counts throughout the training period, which was thought to have indicated increasing levels of training stress. Taking differential leukocyte levels into consideration, it can be concluded that horses in this study suffered from some degree of exercise-induced stress, which appeared to have been more prominent in yard two. It should also be noted that absolute eosinopenia occurred in this study at all times of sampling. Tyler-McGowan *et al.* (1999) linked absolute eosinopenia to clinical signs of illness. However, closer monitoring of the health of the horses may have been beneficial to judge the effect of absolute eosinopenia observed in this study.

Effect of Training on Plasma Biochemistry

Total Protein

A trend towards declining total protein levels over the time period examined was observed in horses in both yards ($p < 0.01$). However, minimum values, mean and median remained within the normal range (55-75 g/l) described by Hodgson (1994) at all times of sampling. Prior to the beginning of training maximum values above the normal range were observed. As alterations in total protein have been mainly attributed to fluid shift out of the plasma and fluid loss (Hodgson, 1994; Poracova *et al.*, 1998), these values could potentially have been an indicator of dehydration, most likely relating to external influences such as weather or excitement due to the new environment. Rose *et al.* (1983) as well as Robson *et al.* (2003) found that elevations in total protein concentration were linked to fluid loss and fluid shift out of the plasma resulting from acute exercise. Rose and Hodgson (1982) observed no changes in total protein concentration during a 12 week training programme. Furthermore, McKeever *et al.* (1987) reported no changes in resting total protein concentration during training, although a decrease was observed at the end of a recovery period following the 15-day exercise training period. Also, Tyler-McGowan *et al.* (1999) and Padalino *et al.* (2007) reported no differences in horses undergoing hypothesized overtraining and those undergoing less intensive training, indicating that training itself had no significant effect on total protein concentration. Contrasting research findings reported in the present study were hence thought to have been the result of external factors and the previously described 'overreaching' effect of acute exercise bouts on resting biochemical parameters. It was suggested that training did not likely affect this biochemical parameter.

Albumin

Similar to total protein measurements a significant reduction in albumin levels in horses in yard two during training was shown ($p < 0.05$). However, albumin levels remained within the normal range (26-38 g/l) as described by Hodgson (1994) at all times of sampling and, as such, the observed decrease in albumin levels was not considered as clinically relevant. Rose and Hodgson (1982) reported no significant changes in albumin levels throughout a twelve week training programme, whereas

Tyler-McGowan *et al.* (1999) demonstrated an increase in albumin throughout the entire training programme. Also, Padalino *et al.* (2007) did not observe any differences in albumin levels between hypothetically overtrained and less intensively trained horses. On the other hand, McKeever *et al.* (1987) observed an increased plasma albumin concentration after one week of exercise training with levels remaining elevated for the duration of the study. During and following an endurance race increased albumin levels associated with fluid loss were reported by Rose *et al.* (1983). Taking results from this study into consideration with findings from other studies it is hence suggested that changes in plasma albumin are not associated with training, but with acute exercise or changes in external factors such as weather conditions as, like with all other protein measurements, information about the hydration status of the individual is provided. Alternatively, changes in protein measurements have also been linked to infection, inflammation, or a variety of pathologic states (Hodgson, 1994; Poracova *et al.*, 1998). To accurately link changes in protein measurements observed to factors other than hydration status of the horse, closer monitoring of the health of each individual animal would have been necessary.

Globulin

Mean and median globulin levels in the sample population examined were reported to lie above the normal range (20-35 g/l) described by Hodgson (1994), particularly prior to the beginning of training. Maximum values above the normal range were observed at all times of sampling. However, a significant decrease in globulin levels was observed in horses in both yards ($p < 0.05$). Given elevated globulin levels prior to the commencement of training, this indicated a normalisation of globulin levels throughout training. Similar to findings reported in the present study, Tyler-McGowan *et al.* (1999) reported high globulin concentrations prior to training and after the initial training phase of training, which, presumably, caused elevated serum protein values. On the other hand, Padalino *et al.* (2007) suggested a link between increasing globulin levels and overtraining in combination with alteration in other haematological and biochemical parameters. McKeever *et al.* (1987) reported no changes in plasma globulin concentration during training, but plasma globulin concentration had decreased at the end of the recovery period following a 15-day training programme. Changes in globulin concentration are thought to reflect

inflammation and disease in most cases, although the hydration status of the horse can also impact on concentration of globulins (Kingston, 2004).

Muscle Enzymes – Aspartate Transaminase (AST)

Both elevated levels as well as low levels of AST were observed at various sampling times in either yard, relative to the normal range (150-400 U/l) described by Hodgson (1994). Increased levels of AST are thought to be indicators of muscular dysfunction (Rose *et al.*, 1983; Tyler-McGowan *et al.*, 1999; Padalino *et al.*, 2007; Yamamoto *et al.*, 2008), or even distress (Padalino *et al.*, 2007). Once elevated AST levels can be detected for longer periods than CK due to the prolonged half life of AST (Cardinet *et al.*, 1967: in Harris *et al.*, 1998) and could, hence, have caused the detection of elevated AST levels at rest resulting from the effect of acute exercise bouts taking place during a training programme. Similar to findings by Rose and Hodgson (1982), no changes in AST levels were observed in horses in yard two throughout the training period examined in this study ($p>0.05$). On the other hand, AST levels of horses in yard one were significantly reduced after ten weeks of training indicating a normalisation of AST levels as training progressed ($p<0.05$). Increases in AST levels in human athletes during training described by Yamamoto *et al.* (2008) were associated with muscle damage and inflammation as a result of initial training, which was similar to findings by Harris *et al.* (1998), who reported increases in AST during the initial training phase of thoroughbred horses. Elevated AST levels were also described as markers for overtraining by Tyler-McGowan *et al.* (1999) and Padalino *et al.* (2007). Variations of AST levels throughout training were therefore thought to be related to the initial response to training or overtraining.

Muscle Enzymes – Creatine Kinase (CK)

Similar to AST levels, maximum CK levels above the normal range (100-300 U/l) as described by Hodgson (1994) were observed in individual horses throughout training. Elevated CK levels also appeared to be associated with muscle damage, mainly resulting from acute exercise bouts, in humans and horses (Rose *et al.*, 1983; Yamamoto *et al.*, 2008). However, no changes in overall CK levels during training were observed in horses from yard two in this study ($p>0.05$), which was supported by similar findings from Rose and Hodgson (1982) as well as Tyler-McGowan *et al.* (1999). However, CK levels in horses in yard one had decreased during training

possibly indicating a normalisation of muscle enzyme status with the progression of training ($p>0.05$). Padalino *et al.* (2007) further found elevated CK levels in overtrained horses. Changes in CK levels were therefore also linked to initial response to exercise as well as to overtraining.

Gamma-Glutamyl Transferase (GGT)

Although no changes in GGT, an indicator of liver function, were observed in either this study ($p>0.05$) or in a study by Rose and Hodgson (1982), GGT levels above the normal range (10-40 u/l) were detected in horses from yard one. Elevated GGT levels during training were also observed by Tyler-McGowan *et al.* (1999). In conjunction with elevated total bilirubin levels, which were also observed in horses from yard one, some degree of hepatic dysfunction could have contributed to this result (Rose *et al.*, 1983). The significant increase in GGT during training and overtraining as described by Tyler-McGowan *et al.* (1999) and Padalino *et al.* (2007) was also suggested to have been a sign of hepatic dysfunction associated with training load.

Total Bilirubin

Total bilirubin levels four times above the normal range (36.7-57.6 $\mu\text{mol/l}$) were observed in horses from yard one. In conjunction with elevated GGT levels of horses in yard one, this indicated hepatic dysfunction in some horses. Furthermore, large individual variations of horses in yard one were indicated by a large S.E. Although no significant differences between total bilirubin levels in yard one and yard two were observed, no values as high as in yard one were reported in yard two, indicating an external effect on liver function. For example, plasma free bilirubin levels have been shown to increase six-fold following an 80-90 hour period of fasting in horses (Gronwall and Mia, 1972). Hyperbilirubinemia was also associated with haemolysis of red blood cells, hepatic disease and internal haemorrhage by Duncan *et al.* (1994: in Buechner-Maxwell *et al.*, 2003). As no signs of haemolysis of red blood cells or internal haemorrhage were observed in this study, the underlying reason for elevated bilirubin levels was likely due to hepatic dysfunction as supported by elevated GGT levels in horses from yard one. Training did not appear to affect total bilirubin concentration in yard one in this study ($p>0.05$) or in a study by Rose and Hodgson (1982), while total bilirubin concentration had significantly increased in horses from

yard two after ten weeks of training ($p < 0.05$). Tyler-McGowan *et al.* (1999) also described a significant increase in total bilirubin levels during training. Padalino *et al.* (2007) even suggested total bilirubin levels increased with overtraining. However, varying results, large differences between individual bilirubin levels and differences between yards suggested changes in total bilirubin concentration were due to external influences on hepatic function or possibly training intensity. The latter appeared to be a reasonable explanation for the significant increase in total bilirubin levels reported in yard two, when taking haematological parameters such as the differential leukocyte count into consideration, which are thought to be indicators of a higher training intensity in yard two.

Electrolytes – Sodium (Na), Potassium (K), Chloride (Cl)

Elevated sodium and potassium levels were reported in both yards throughout the time period examined. The mean and median sodium levels observed were above the normal range (134-144 mmol/l) reported by Hodgson (1994), while mean and median potassium levels lay within the accepted normal range (3.2-4.2 mmol/l). Chloride levels fell within the normal range (94-104 mmol/l) except for a slight elevation in yard two after ten weeks of training. Generally, changes in plasma electrolytes have been associated with changes in extracellular fluid balance (sodium) or accumulation of hydrogen ions in active muscle, leading to a reduced reuptake by the fibres and an impairment of the Na^+ , K^+ -ATPase pump (potassium) occurring during high-intensity exercise. Changes in plasma electrolytes during low and moderate intensity exercise have been associated with fluid loss such as perspiration (Rose *et al.*, 1983; Hodgson, 1994). Acute exercise has therefore been linked to changes in plasma electrolytes in horses (Rose *et al.*, 1983). Similar to findings by Rose and Hodgson (1982) potassium levels in yard two ($p < 0.05$) and chloride levels in yard one ($p < 0.001$) did not fluctuate significantly during training. A research study by McKeever *et al.* (1982) reported that plasma sodium concentration had increased after one week of training, but decreased again significantly after the second week of training as well as throughout the recovery period. Sodium content in yard one increased during the initial two weeks of training, but had decreased again significantly after ten weeks of training. On the other hand sodium levels in horses in yard two had increased during the training period examined ($p < 0.01$). It is important to note that sodium levels after ten weeks of training were still elevated in either yard. These changes could only be

explained by extracellular fluid balance changes associated with exercise. Furthermore, McKeever *et al.* (1987) observed decreased plasma potassium concentrations after a 15-day training programme with levels continuing to decrease throughout a recovery period following the training programme. A decrease in potassium observed in yard one throughout training could be linked to potassium leaving muscle fibres bound to phosphate during exercise. As mean and median levels remained within the normal range, this reduction in potassium was not considered clinically significant. On the other hand, chloride levels remained unchanged during training in yard one, while an increase throughout the entire training period was reported in yard two. However, chloride levels lay within the normal range throughout training. Hence, this finding was not thought to have been clinically significant. It should be noted that changes in plasma electrolytes were unlikely to have occurred due to the effect of training as varying results were reported. Research had shown that changes were most likely associated with the 'overreaching' effect of acute exercise bouts to consecutive blood sampling at rest (Mackinnon, 2000; Gleeson, 2007).

Chapter Five

Conclusions and

Recommendations

Conclusions and Recommendations

To the knowledge of the researcher, no other study published to date has examined the effect of introduction to training in racehorses on aspects of the adaptive and innate immune system as well as on plasma haematology and biochemistry with respect to investigating the hypothesis that introduction to training in racehorses is associated with immunosuppression.

Several aspects of the immune system appear to change with training. However, components of the innate system seem to be more susceptible to change than components of the adaptive immune system. Overall, the observed effects on innate immunity lead to rejection of the experimental hypothesis in that introduction to training was not associated with immunosuppression, but rather with stimulation of certain immunological parameters of the innate immune system.

- The most prevalent change in immunological parameters observed was an increase in granulocyte and monocyte oxidative burst capacity during training.
- While T-cell proliferation in response to stimulation with a mitogen *in vitro* did not change with training in racehorses, components of the phagocytic process varied throughout the training period examined.
- However, changes observed in granulocyte and monocyte phagocytosis most likely occurred due to other external factors such as training intensity and duration.

Closer monitoring of the health status as well as antibody titres of the individual horses could provide valuable information on the connection between health and aspects of the immune system measured *in vitro* during future research. It could then be established whether a link between immunosuppression, and, indeed, increases in certain immunological parameters and increased susceptibility or resistance to infectious disease upon introduction to training exists. Both, plasma haematology and biochemistry remained largely uninfluenced by chronic exercise training; however, some variations in these variables with training were observed.

- Significant changes that resembling a typical ‘stress leukogram’ in neutrophil and lymphocyte percentage in one yard only were observed during the training period examined. Changes were thought to indicate that factors such as training intensity affect the effect of exercise on leukocytes.
- Decreasing levels of eosinophil counts and percentage with training were observed, however, changes were only significant in one yard.
- No significant changes in AST, CK, GGT and total bilirubin (one yard) were observed with training, while decreasing levels of AST and CK as well as increasing levels of total bilirubin were associated with differences in factors such as training intensity and duration.
- Elevated levels of GGT and total bilirubin were observed in one yard only throughout the time period examined.
- Elevated levels of plasma globulin were noted throughout the training period examined, but levels appeared to normalise with training.
- Some changes in plasma electrolyte concentrations were observed with training. However, changes were inconsistent and causes were multifactorial.

In spite of numerous and variable effects of introduction to training on immunity in racehorses, research has indicated that most changes, especially those relating to plasma haematology and biochemistry, are associated with the effects of acute rather than chronic exercise. It is thought that changes observed during training programmes can be attributed to an ‘overreaching’ effect resulting from repeated bouts of acute exercise during a training programme that lead to transient changes in the immune system which can carry over to blood sampling at rest during training. It should be noted that exercise intensity and duration as well as training status of the horse appear to affect the duration of the transient changes observed following acute exercise. Hence, even blood samples taken at rest might not represent true resting levels of the immunological parameters measured. It appears likely that horses might be transiently more susceptible to contracting infectious disease for a period following acute exercise. Further research to examine the effect of chronic exercise training on the immune response to acute exercise is necessary to further the understanding of the connection between exercise and infectious disease.

Although the extent of this study did not allow consideration of the possible mechanisms underlying changes in immunity as well as haematology and biochemistry, it is reasonable to infer that changes in immunity and plasma haematology were linked to changes in the endocrine system such as variations in plasma cortisol and catecholamines. Clinical consequences of alterations in the immune response during training are not yet known, however, managers of horses should take into consideration that the immune system of a horse may be affected by acute and chronic exercise.

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Appendices

Haematology Reference Values

Relevant reference values for the resting haemogram in normal adult thoroughbred horses were obtained from Hodgson and Rose (1994) and are displayed in table A.

Table A Reference Values for the Resting Haemogram in Normal Adult Thoroughbred Horses

<i>Value</i>	<i>Range</i>	<i>Mean</i>
<i>Erythrocytes ($\times 10^{12}/\text{litre}$)</i>	7.0 – 11.0	9
<i>Haemoglobin (g/litre)</i>	110 – 170	140
<i>PCV (litre/litre)</i>	0.32 – 0.46	0.40
<i>MCV (fl)</i>	42 – 47	44
<i>MCHC (g/litre)</i>	330 – 380	350
<i>MCH (pg)</i>	14.0 – 17.0	15.5
<i>Platelets ($\times 10^9/\text{litre}$)*</i>	141 - 191	166
<i>Leukocytes ($\times 10^9/\text{litre}$)</i>	6.0 – 11.0	8.5
<i>Neutrophils ($\times 10^9/\text{litre}$)</i>	2.5 – 6.5	4.5
<i>Lymphocytes ($\times 10^9/\text{litre}$)</i>	2.0 – 5.5	3.5
<i>Monocytes ($\times 10^9/\text{litre}$)</i>	0.2 – 0.8	0.5
<i>Eosinophils ($\times 10^9/\text{litre}$)</i>	0.1 – 0.4	0.2

Table taken from Hodgson and Rose (1994)

*Data taken from Irish Equine Centre

Biochemistry Reference Values

Relevant reference values for plasma biochemical measurements in mature performance horses were obtained from Hodgson and Rose (1994) and are displayed in table B. Where data could not be attained from this source reference values as suggested by the Irish Equine Centre were used.

Table B Normal Ranges for Plasma Biochemical Measurements in Mature Performance Horses

<i>Measurement</i>	<i>Normal Range (SI units)</i>	<i>Normal (traditional units)</i>	<i>Range</i>
<i>Total Protein</i>	55 – 75 g/l	5.5 – 7.5 g/dl	
<i>Albumin</i>	26 – 38 g/l	2.6 – 3.8 g/dl	
<i>Globulin</i>	20 – 35 g/l	2.0 – 3.5 g/dl	
<i>AST</i>	150 – 400 iu/l	150 – 400 iu/l	
<i>CK</i>	100 – 300 iu/l	100 – 300 iu/l	
<i>GGT</i>	10 – 40 iu/l	10 – 40 iu/l	
<i>T. Bilirubin*</i>	36.7 – 57.6 µmol/l*	n/a	
<i>Sodium</i>	134 – 144 mmol/l	134 – 144 mEq/l	
<i>Potassium</i>	3.2 – 4.2 mmol/l	3.2 – 4.2 mEq/l	
<i>Chloride</i>	94 – 104 mmol/l	94 – 104 mEq/l	

Table taken from Hodgson and Rose (1994)

*Data taken from Irish Equine Centre