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VITAMIN E REQUIREMENTS OF ADULT DOMESTIC CATS (*FELIS CATUS*) FED DIETS CONTAINING HIGH LEVELS OF FISH OIL

A thesis presented in partial fulfilment of the requirement for the degree of Master of Nutritional Sciences at Massey University, Palmerston North, New Zealand

Yuben Wu

1999
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>α-TO*</td>
<td>α-Tocopherol radical</td>
</tr>
<tr>
<td>α-TOH</td>
<td>α-Tocopherol</td>
</tr>
<tr>
<td>1^O_2</td>
<td>Active oxygen</td>
</tr>
<tr>
<td>3^C*</td>
<td>Triplet excited carotenoid</td>
</tr>
<tr>
<td>3^H-TdR</td>
<td>Tritiated thymidine</td>
</tr>
<tr>
<td>3^O_2</td>
<td>State oxygen</td>
</tr>
<tr>
<td>AAFCO</td>
<td>Association of America Feed Control Officials</td>
</tr>
<tr>
<td>Ascorbate*</td>
<td>Ascorbate radical</td>
</tr>
<tr>
<td>Brdu</td>
<td>5-Bromo-2'-deoxyuridine</td>
</tr>
<tr>
<td>Caro</td>
<td>Carotenoids</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>Con A</td>
<td>Concanavalin A</td>
</tr>
<tr>
<td>CPM</td>
<td>Counts per minute</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FRAP</td>
<td>The ferric reducing ability of plasma</td>
</tr>
<tr>
<td>GSH</td>
<td>Reduced glutathione</td>
</tr>
<tr>
<td>GSHPx</td>
<td>Glutathione peroxidase</td>
</tr>
<tr>
<td>GSH_red</td>
<td>Glutathione reductase</td>
</tr>
<tr>
<td>GSSG</td>
<td>Oxidised glutathione</td>
</tr>
<tr>
<td>H_2O_2</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>HO*</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>LO_2*</td>
<td>Lipid hydroperoxide radicals</td>
</tr>
<tr>
<td>LOOH</td>
<td>Lipid hydroperoxides</td>
</tr>
<tr>
<td>LPO</td>
<td>Lipid peroxides</td>
</tr>
<tr>
<td>MCDP</td>
<td>10-N-Methylcarbamoyl-3,7-dimethylamino-10 H-phenothiazine</td>
</tr>
<tr>
<td>NADP</td>
<td>Triphosphopyridine nucleotide</td>
</tr>
<tr>
<td>NO_2*</td>
<td>Nitrogen dioxide</td>
</tr>
<tr>
<td>NRC</td>
<td>National Research Council</td>
</tr>
<tr>
<td>O_2^-</td>
<td>Superoxide anion</td>
</tr>
<tr>
<td>OH*</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>PA_2</td>
<td>Phospholipase A_2</td>
</tr>
<tr>
<td>PHA</td>
<td>Phytohemagglutinin</td>
</tr>
<tr>
<td>PHGSHPx</td>
<td>Phospholipid hydroperoxides glutathione peroxidase</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>PWM</td>
<td>Pokeweed mitogen</td>
</tr>
<tr>
<td>R*</td>
<td>Carbon-centered radical</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell</td>
</tr>
<tr>
<td>ROO*</td>
<td>Fatty acid hydroperoxide radicals</td>
</tr>
<tr>
<td>ROOH</td>
<td>Fatty acid hydroperoxides</td>
</tr>
<tr>
<td>Se</td>
<td>Selenium</td>
</tr>
<tr>
<td>SI</td>
<td>Stimulation index</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>TBA</td>
<td>Thiobarbituric acid</td>
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ABSTRACT

The vitamin E (α-tocopherol) requirement of adult cats fed diets containing high levels of fish oil was investigated. Thirty-two (16 male, 16 female) adult domestic cats (*Felis catus*) were randomly allocated to four groups according to sex and fed one of four experimental diets (A, B, C, and D) for 126 days. The cats were housed in large outdoor pens in groups of 8 cats. Diets A, B, C and D contained approximately 300 g of fish oil per kg diet dry matter and were supplemented to contain 0, 5, 10, and 15 IU DL-α-tocopheryl acetate per g added fish oil per kg diet, respectively. The diets were provided *ad libitum* with water being available at all times. Food intake was measured daily and body weights were measured at weekly intervals. Blood samples were taken from the jugular vein of each cat at bi-weekly intervals during the study. Blood samples were analysed for plasma α-tocopherol, red blood cell H$_2$O$_2$ (4 and 2 %) haemolysis, the ferric reducing ability of plasma, plasma lipid peroxides, plasma triglycerides, alkaline phosphatase and whole blood lymphocyte proliferation.

All cats remained healthy throughout the study except one female cat who was removed after 3 weeks due to poor food intake. The four diets were analysed and found to be free of peroxides. The average daily metabolisable energy intake of the cats on diet A, B, C and D at the end of study were similar and were 289, 261, 256, and 267 kJ·kg$^{-1}$ body weight, respectively. No clinical signs of vitamin E deficiency were observed in any of the cats. The plasma α-tocopherol concentrations of the cats in the four groups at the start of the study were not significantly different between the four groups (mean ± SEM, 3.4 ± 0.2 µg·ml$^{-1}$). When the cats were fed diet A (unsupplemented), the mean plasma α-tocopherol concentration remained relatively low and the RBC 4 % H$_2$O$_2$ haemolysis remained high, while the RBC 2 % H$_2$O$_2$ haemolysis decreased consistently. Plasma lipid peroxides remained relatively low throughout the study. The ferric reducing ability of plasma status was compromised in the cats on the unsupplemented diet. There was no significant (P < 0.05) difference in any of the response parameters measured amongst the cats fed diets B, C and D except for the RBC 4 % H$_2$O$_2$ haemolysis of the cats on diet B which was significantly higher than those on diet C and D at week 4 and week 8, and the LPO value of the cats on diet D which was significantly higher than those of the cats on diet B and C at week 4.
The vitamin E requirement of adult cats fed a high level of fish oil, using the response parameters measured, was estimated to be between 0 and 5 IU of vitamin E per g added fish oil per kg diet. The current recommendation of the Association of American Feed Control Officials (10 IU vitamin E/g fish oil/kg diet) appears to be well in excess. The results from the present study also showed that there was no beneficial effect of dietary vitamin E on whole blood cell proliferation when vitamin E levels were 150% of the recommendations of the Association of American Feed Control Officials. The vitamin E requirement of adult cats to optimise immune response warrants further investigation.
ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

List of Abbreviations ii
Abstract iii
Acknowledgements v
Table of Contents vi
List of Tables ix
List of Figures x

GENERAL INTRODUCTION 1

CHAPTER 1 REVIEW OF LITERATURE 3

1.1 Introduction 3

1.2 Free radicals, lipid peroxidation and various antioxidants within the mammalian body 4
  1.2.1 Free radicals and lipid peroxidation 4
  1.2.2 Antioxidant nutrients and enzymes, and their interaction within the mammalian body 6
    1.2.2.1 Vitamin E (α-tocopherol) 6
    1.2.2.2 Vitamin C (ascorbic acid) and glutathione 6
    1.2.2.3 Uric acid 7
    1.2.2.4 β-Carotene and other related carotenoids 7
    1.2.2.5 Selenium, glutathione peroxidase, superoxide dismutase and catalase 9
    1.2.2.6 Interactions of vitamin E with selenium 10

1.3 Dietary factors affecting vitamin E requirement of mammals 11
  1.3.1 Polyunsaturated fatty acids 11
  1.3.2 Selenium 12
  1.3.3 Sulphur amino acids 13
  1.3.4 Synthetic antioxidants 13
  1.3.5 Vitamin C 14
1.3.6 Vitamin A 14
1.3.7 Zinc 15
1.3.8 Summary 15

1.4 Targeted tissues and signs of vitamin E deficiency 15
1.4.1 Affected tissues and signs of vitamin E deficiency in animals 16
1.4.2 Affected tissues and signs of vitamin E deficiency in cats 18

1.5 Vitamin E requirements of several mammalian species 19

1.6 Methods of assessing vitamin E status 20
1.6.1 Plasma α-tocopherol 21
1.6.2 Red blood cell hydrogen peroxide haemolysis assay 23
1.6.3 Lipid peroxides assays 23
1.6.4 Whole blood lymphocyte proliferation assay 24
1.6.5 The ferric reducing ability of plasma assay 26

1.7 Determination of vitamin E requirements of animals 27
1.8 Determination of vitamin E requirement in cats 28
1.9 Inferences from review of literature 28

CHAPTER 2 MATERIALS AND METHODS 30

2.1 Animals and diets 30
2.2 Blood sample collection and processing 32
2.3 Whole blood and blood plasma assays 33
2.3.1 Chemicals used 33
2.3.2 Plasma vitamin E (α-tocopherol) analysis 34
2.3.3 Red blood cell hydrogen peroxide haemolysis 35
2.3.4 Plasma lipid peroxides 35
2.3.5 Whole blood lymphocyte proliferation 35
2.3.6 The ferric reducing ability of plasma 36

2.4 Chemical analyses 37
2.5 Data analysis 38
CHAPTER 3 RESULTS

3.1 Body weight and metabolisable energy intake 39
3.2 Plasma α-tocopherol 40
3.3 Red blood cell hydrogen peroxide (4 and 2 %) haemolysis 41
3.4 Ferric reducing ability of plasma 42
3.5 Plasma lipid peroxides 44
3.6 In vitro whole blood lymphocyte proliferation with mitogen (CON A, PHA and PWM) and without mitogen 45
3.7 Plasma triglycerides and alkaline phosphatase 48

CHAPTER 4 DISCUSSION 49

CHAPTER 5 CONCLUSION 54

CHAPTER 6 REFERENCES 55
LIST OF TABLES

CHAPTER 1
Table
1 Targeted tissues and vitamin E deficiency symptoms in selected animal species 16
2 The minimum vitamin E requirements for growing and adult animals of several mammalian species 20
3 Plasma α-tocopherol status in healthy animals 21
4 Plasma α-tocopherol status in selected vitamin E deficient animal species 22
5 Vitamin E requirements of selected animal species using different response criteria 27

CHAPTER 2
Table
1 Ingredient composition of the experimental basal diet 30
2 Chemical composition of the four experimental diets 31
3 The fatty acid profile of the four experimental diets 32
4 Calculated fatty acid profile of the four experimental diets 33

CHAPTER 3
Table
1 Statistical significance of selected variables on measured response parameters 39
LIST OF FIGURES

CHAPTER 1

Figure
1 Schematic presentation of lipid peroxidation 5
2 The glutathione system 8
3 Interaction of vitamin E and selenium. 10

CHAPTER 3

Figure
1 Average daily ME intake of the adult cats on the four diets 40
2 Mean (± SEM) plasma α-tocopherol concentration of the adult cats on
the four diets 41
3 Mean (± SEM) red blood cell 4 % H₂O₂ haemolysis of the adult cats on
the four diets 42
4 Mean (± SEM) red blood cell 2 % H₂O₂ haemolysis of the adult cats on
the four diets 43
5 Mean (± SEM) ferric reducing ability of plasma of the adult cats on the
four diets 43
6 Mean (± SEM) plasma lipid peroxide levels of the adult cats on the four
diets 44
7 Mean (± SEM) in vitro stimulation index of cat whole blood lymphocytes
to concanavalin A 46
8 Mean (± SEM) in vitro stimulation index of cat whole blood lymphocytes
to phytohemagglutinin 46
9 Mean (± SEM) in vitro stimulation index of cat whole blood lymphocytes
to pokeweed mitogen 47
10 Mean (± SEM) in vitro counts per minute of unstimulated cat whole blood
duffyocytes 47
11 Mean (± SEM) plasma triglyceride concentration of the adult cats on
the four diets 48
GENERAL INTRODUCTION

The domestic cat (*Felis catus*) is a member of the Felidae family of the order Carnivore and one of the most popular companion animals. Part of their attraction lays in their playful behaviour (Houpt *et al.*, 1988). In recent years researchers have discovered that the relationship between humans and their pets provides numerous physiological and psychological benefits to the owner (Case *et al.*, 1995).

Besides proper health care and medical attention, nutrition is considered to be an important component of the care of cats. Nutritional balance and preferences of diets must be considered when a diet is formulated for cats by an animal nutritionist (Case *et al.*, 1995). It is well known that many cats prefer fish and consequently, numerous cat foods are composed of fish or flavoured with fish (Houpt *et al.*, 1988). However, there have been several reports of vitamin E deficiency in cats as a result of the exclusive feeding of fish and fish based diets (Cordy and Stillinger, 1953; Coffin and Holzworth, 1954; Munson *et al.*, 1958; Griffiths *et al.*, 1960; Watson *et al.*, 1973; Gaskell *et al.*, 1975; Flecknell and Gruffydd-Jones, 1978; Summers *et al.*, 1982; Koutinas *et al.*, 1993; Tidholm, 1996) over the last 50 years.

The vitamin E requirements of other animal species such as humans, rats, pigs, dogs, and guinea pigs have been extensively studied (Van Vleet, 1975; Farrell *et al.*, 1985; Hakkarainen *et al.*, 1986; Jensen *et al.*, 1988a; Mahan, 1991; Meydani *et al.*, 1991; Cho and Choi, 1994; Barja *et al.*, 1996; Wang *et al.*, 1996; Kubo *et al.*, 1997). These studies have demonstrated that the *in vivo* vitamin E requirements are markedly influenced by dietary composition. A high dietary level of polyunsaturated fatty acids increases the requirement for vitamin E as a result of the increased susceptibility of tissues to peroxidation (Duthie, 1993). The dietary vitamin E requirement of cats has been set at 30 IU·kg⁻¹ dry matter: a figure mostly extrapolated from other animal species (NRC, 1986). In order to prevent vitamin E deficiency in cats fed commercially sold, fish based diets, the Association of America Feed Control Officials (AAFCO, 1997) recommends that diets containing fish oil should be supplemented with 10 IU of vitamin E for every g of fish oil per kg diet. AAFCO (1997) failed to provide evidence to substantiate this value and, therefore, the exact vitamin E requirements of cats fed high levels of polyunsaturated fatty acids are still largely unknown.
The main objective of this study was to determine the vitamin E requirement of cats fed high dietary levels of polyunsaturated fatty acids from fish oil. This study was also undertaken to obtain baseline data on α-tocopherol levels in blood plasma of adult cats, which can be used in the diagnosis of vitamin E deficiency.